CHAPTER I

GENERAL INTRODUCTION
The lysosomes are essential constituents of most mammalian cells. Lysosomes being heterogeneous in structure, are difficult to define properly by cytochemical methods. The biochemical studies of de Duve and collaborator have strong impetus to the development of cytochemical procedures (1,2).

Biochemically defined, lysosomes are cytoplasmic organelles containing a variety of hydrolases most of which have maximal activities at acid pH and which display latency if the particles are properly isolated. The latency reflects the impermeability of membranes delimiting the lysosomes to enzymes and substrates. There are two types of lysosomes: (a) Primary lysosome is defined as membrane limiting structure containing newly synthesized acid hydrolases which have not reached their ultimate substrates. (b) Secondary lysosome or digestive body contains not only the specific acid
hydrolases but also the substrates either taken up from the environment or sequestered from the cell's own protoplasm. In general, it is the secondary lysosomes which are demonstrated in the most cells because of their larger size, cytochemical activity and characteristic densities.

In the cells endowed with sufficient endoplasmic reticulum (ER), it is likely that lysosomal hydrolases are synthesized on membrane-associated polysomes and gain access to cisternae of ER through which they are transported to other areas of the cell. Figure 1 indicates the view of Novikoff (3) that lysosomal hydrolases may go directly from ER to autophagic vacuoles of two kinds, referred as AV\textsubscript{1} and AV\textsubscript{2} and that ER itself is involved in microautophagy. However it is also believed that they originate from GERL. GERL is a specialized region of smooth ER found in some cell types. It is situated at the inner aspect of Golgi stack. GERL seems to form three types of lysosomes: residual bodies or dense bodies (DB), type 2 - autophagic vacuoles (AV\textsubscript{2}) and coated vesicles (CV) which probably acts as primary lysosomes.

Hickman and Neufeld had suggested that many lysosomal enzymes share a common "recognition marker" for
Figure 1. Origin and roles of lysosomes.

Abbreviation: 1-4, successive elements of Golgi "stack" 10L, primary lysosomes, AV1, type 1 autophagic vacuole; AV2, type 2 autophagic vacuole; c, crinophagy; DG, dense body; DV, digestive vacuole; ER, Endoplasmic reticulum; GE, GERL; MA, microautophagy; MP, microperoxisomes; MVB, multivesicular bodies; P, Peroxisomes, Ph, phagocytic vacuole; Pi, pinocytic vacuole; RB, residual body; and SG, Secretory granule.

their uptake by fibroblast and that the marker was absent or defective in I-cell fibroblasts. It was subsequently recognized as mannose-6-phosphate (Man 6-P) (4). The receptors for Man 6-P on mammalian cell membranes provide an intracellular transport system for the delivery of the enzymes to lysosomes. I-cell disease, Mucolipidosis - type II, where several lysosomal enzymes were deficient. The evidence of Man 6-P as recognition marker for lysosomal enzymes had been reported by Kaplan et al (5). They showed the uptake of β-Glucuronidase by human fibroblast was inhibited by Man 6-P. However, the observations implicating Man 6-P recognition in pinocytosis of β-Glucuronidase were also observed with several other enzymes (6,7).

Two pathways to transport acid hydrolases to lysosomes were proposed (a) Secretion-recapture pathway (8). (b) Intracellular pathway for receptor-mediated segregation of lysosomal enzymes (9).

The observations with the I-cell disease fibroblasts led to the suggestion that enzymes are normally secreted into the extracellular medium and delivered to lysosomes by receptor mediated recapture (8). This is known as secretion-recapture pathway. It was shown that
Man 6-P inhibited the uptake of exogenous enzymes. It became possible to estimate fractions of enzymes those were first secreted into the medium and then recaptured by normal fibroblasts. In such conditions Man 6-P which blocked enzyme pinocytosis, trapped the secreted enzyme outside and led to depletion of intracellular enzyme level. There are discrepancies of the results obtained in different laboratories carrying the similar type of experiment (9-11).

Von Figura and Weber (11) had suggested an alternative to secretion-recapture hypothesis. They proposed that lysosomal enzymes were normally delivered to lysosomes by vesicles that bring receptor bound enzymes first to plasma membrane, when the receptor bound enzymes were quickly internalized without ever dissociating from the cell surface receptors. Since the major part of the lysosomal enzyme cycles via the cell surface receptors in receptor-bound form, only a minor fraction of lysosomal enzymes would normally be released into the extracellular space.

According to intracellular pathway of receptor mediated enzyme the acid hydrolases were synthesised in the ER and then received phosphomannosyl recognition marker(9).
The marker enabled them to bind to intracellular membrane receptor, which were collected into specialized vesicles of the Golgi complex or GERL and bud off as primary lysosomes. The site of origin of lysosomes was similar to that proposed by de Duve and Wattiaux (12), but required Man 6-P as recognition marker on the enzyme for its receptor on the intracellular membrane. These ligands and receptors provided a mechanism for segregation of newly synthesized lysosomal enzyme from other products of ER that are destined for secretion into the extracellular medium.

Sly and Stahl proposed that both pathways depend on the recognition marker of the enzyme and its phosphomannosyl receptor but the major pathway was the intracellular one. Secretion of the enzyme into the medium and its return by absorptive pinocytosis (recaptures) was suggested to be a less important pathway (13). Recently Gabel et al had shown that an intracellular pathway independent of Man 6-P present in some cells for the delivery of acid hydrolases to lysosomes (14).

Lysosomes are called the digestive bodies of the cells. Several reviews on the functional aspects have been
published (12,15). Lysosomes were involved in (a) autophagy
(b) heterophagy (c) orinophagy (d) absorption of nonprotein
substances (e) accumulation of drugs and cationic substances.
The characteristic feature of autophagy was that it was the
endogenous material (organelle or molecules) that was
degraded by lysosomal hydrolases (fig.2). The process
appeared to be ubiquitous in eukaryotic cells. The process
of autophagy could be described as the autophagic vacuole
having obtained a battery of lysosomal hydrolases became
a secondary lysosome and the macromolecules digested within
the autophagic vacuole were endogenous. Like all secondary
lysosomes autophagic vacuoles may give rise to dense
bodies. The widely investigated autophagic vacuoles were
those induced in hepatocytes by glucagon (16).

The relationship of lysosomes to exogenous material
intercorporated in the heterophagic vacuole was demonstrated
in many cell types (17-19). Exogenous material may enter
cells "in gulps" either by phagocytosis or pinocytosis.
Endocytic vacuoles become digestive vacuoles when they
receive acid hydrolases. This involve merger with either
primary lysosomes or residual bodies, some of which are
often called dense bodies. The undigestible substances
Figure 2. Lysosomal apparatus: A schematic view of various functions of lysosomes as outlined by de Duve and Wattiaux (12).
take the form of electron opaque grains, membranous
whorls and as these residues accumulate the residual
bodies enlarge. The first evidence that absorbed
foreign proteins are located in the particles having
the properties of lysosomes originated from tissue
fractionation experiment by Straus and Oliver (20).

de Duve introduced the term crinophagy to explain
the role of lysosomes in the disposal of secretory
granules (21). According to Farquhar and coworkers (22)
secretory granules originated from the innermost element
of Golgi apparatus. Pelletier and Novikoff (23)
described, that in some pituitary cell types secretory
granules originate from GERL. In contrast to autophagy,
crinophagy involves direct fusion between a secretory
granule and a lysosome, the material to be digested
never comes into contact with the cytoplasm.

Allen (24) has observed by light microscopic
studies that a large number of carbohydrates and other
non-protein substances, if administered in large doses
cause renal tubular changes and these changes referred
to as osmotic nephrosis is of mainly experimental and
pathological interest and involve lysosomes. Studies
with sucrose (25) dextran (26) and mannitol (27) revealed that the large bodies developed during osmotic nephrosis acquired the administered substances by endocytosis from the tubular lumen.

It was earlier known that different drugs can be used for vital staining of the cell, but the intracellular localization of dyes as well as drugs, carcinogenic hydrocarbons and heavy metals are in the lysosomes of living cells (28). The binding of these substances to lysosomes was suggested by Koenig (28) to depend upon salt type linkages. It is noteworthy that no evidence exists that lysosomes accumulate cationic substances through heterophagy or autophagocytosis. Thus the drug accumulating ability of the lysosomes appears to be an essentially different lysosomal function. The biochemical significance of this function is not fully understood.

The hydrolytic enzyme usually exert their action inside the cell, in the vacuolar system of the cytoplasm either on exogenous material engulfed by endocytosis or endogenous material segregated by autophagy. However, lysosomal enzymes may also be excreted by exocytosis and be active outside the cell
in the degradation of some extracellular components (fig. 2).

The proper physiological functioning of the lysosomal system namely intralysosomal digestion should lead to products that are able to escape from lysosomal membranes. The nondigestible materials that cannot pass through this membrane would indeed accumulate in lysosomes causing a pathological overloading leading to the swelling of these organelles.

Lysosomal enzymes can digest proteins extensively in vivo and in vitro through the synergistic action of a series of endo- and exopeptidases that release only dipeptides and free amino acids (29, 30).

The majority of glycosidases are of lysosomal origin, which are involved in the hydrolysis of carbohydrate linkages at acid pH. In view of the subtle differences in structures that distinguish sugars from one another, it is not surprising that the specificity of the glycosidases is often less than absolute. When an enzyme has not been available in pure state, it has been possible to examine its specificity indirectly by use of inhibitory structural analogues of potential substrates. The p-nitrophenyl and 4-methylumbelliferyl-glycosides are the few convenient artificial substrates
that are used.

It was observed that large phaseluent vacuoles similar to secondary lysosomes were formed after addition to some disaccharides or oligosaccharides in the culture medium of mouse peritoneal macrophage which were resistant to macrophage lysates. These vacuoles later disappeared after addition of the required glycosidases in the culture medium (31). Lysosomal membrane was shown to be permeable to monosaccharides (32). An enzyme \( \alpha \)-glucosidase was also reported in the liver lysosomes which can hydrolyse \( \alpha \)-1, 4-glycosidic linkages of maltose and glycogen (33,34) and \( \alpha \)-1, 6-glycosidic linkage of isomaltose and dextran (35,36). Glycogen could be completely digested to Glc by its action. This enzyme is deficient in Type II glycogenesis (Pompe's Disease) characterized by accumulation of glycogen in the lysosomal vacuoles in several tissues (33).

Glycoproteins represents a group of covalent carbohydrate-protein compounds. The digestive activity of highly purified liver lysosomes at acid pH on several glycoproteins have been studied \textit{in vitro} by Aronson and de Duve (37). The first step in the degradation of this
chain involved the removal of the terminal NeuAc by a lysosomal Neuraminidase; this reaction proceeded almost to completion and opened the way to the action of a β-Galactosidase, which removes the galactose at slower rate and then of N-Acetyl-β-glucosaminidase, which released GlcNAc in amount equivalent to those of Gal released. The release of free Man was not detected in this study, suggesting that some resistance to digestion also occurred at or near the branching point in these chains. An α-Mannosidase is known to exist in lysosomes (38,39) and has been found to be deficient in human (40) as well as bovine Mannosidosis (41), an inborn-error of metabolism where Man-rich glycoproteins accumulate in the tissues.

de Duve has reviewed several types of lysosomal pathology (42). One of them is lysosomal overloading which occurs in all inborn lysosomal disorders. An inborn lysosomal disorder is an inborn-error of metabolism which causes the intralysosomal accumulation of the complex molecules those required the missing enzyme for degradation (43). Table 1 shows the deficiency of number of enzymes in some genetic disorders (44).
Table 1. Principal manifestation, stored lipids and enzyme defects in glycolipid storage diseases.

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>SIGNS AND SYMPTOMS</th>
<th>MAJOR LIPID ACCUMULATION</th>
<th>ENZYME DEFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farber's Disease</td>
<td>Hoarseness, Dermatitis, Skeletal Deformation, Mental Retardation</td>
<td>Ceramide, Sphingosine, Fatty Acid</td>
<td>Ceramidase</td>
</tr>
<tr>
<td>Gaucher's Disease</td>
<td>Splen and Liver Enlargement, Erosion of Long Bones and Pelvis, Mental Retardation Only in Infantile Form</td>
<td>Glucocerebroside, Ceramide, Glucose</td>
<td>Glucocerebroside, α-Glucosidase</td>
</tr>
<tr>
<td>Krabbe's Disease</td>
<td>Mental Retardation, Almost Total Absence of Myelin, Globoid Bodies in White Matter of Brain</td>
<td>Galactocerebroside, Galactose</td>
<td>Galactocerebroside, β-Galactosidase</td>
</tr>
<tr>
<td>Metachromatic</td>
<td>Mental Retardation, Psychological Disturbances in Adult Form, Nerves Stain Yellow-Brown with Cresyl Violet Dye</td>
<td>Sulfatide, Galactose-3-Sulfate</td>
<td>Sulfatidase</td>
</tr>
<tr>
<td>Leukodystrophy</td>
<td>Ceramide Lactoside, Slowly Progressing Brain Damage, Liver and Spleen Enlargement</td>
<td>Ceramide Lactoside, Glucose</td>
<td>Neutral β-Galactosidase</td>
</tr>
<tr>
<td>Fabry's Disease</td>
<td>Reddish-Purple Skin Rash, Kidney Failure, Pain in Lower Extremities</td>
<td>Ceramide Trihexoside</td>
<td>Ceramide Trihexoside, α-Galactosidase</td>
</tr>
<tr>
<td>Tay Sachs Disease</td>
<td>Mental Retardation, Red Spot in Retina, Blindness, Muscular Weakness</td>
<td>Ganglioside, Glucose Galactose</td>
<td>Hexosaminidase A</td>
</tr>
<tr>
<td>Tay Sachs Variant</td>
<td>Same as Tay Sachs Disease but Progressing More Rapidly</td>
<td>Globoide (and Ganglioside GM1)</td>
<td>Hexosaminidase A and B</td>
</tr>
<tr>
<td>Generalized Gangliosidosis</td>
<td>Mental Retardation, Liver Enlargement, Skeletal Deformities, About 50 Percent with Red Spot in Retina</td>
<td>Ganglioside, Glucose Galactose</td>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>Fucosidosis</td>
<td>Cerebral Degeneration, Muscle Spasticity, Thick Skin</td>
<td>Fucosidase</td>
<td>α-Fucosidase</td>
</tr>
</tbody>
</table>
The lysosomal enzymes have some properties in common (15) (a) They have acid pH optimum (b) resistant to autolysis (c) most of them are glycoproteins. Reijngound and Targer have used indicator dyes to determine the intralysosomal pH and found the pH upto 1.5 units lower than the surrounding medium (45). However the measurement of pH by indicator dyes was not reliable. Poole and Dhkuma have developed a method based on the pH dependence of the fluorescence excitation spectrum of fluorescein. This substance is introduced into the lysosomes in association with dextran, which served as endocytizable carrier. A pH of the order of 4.7 was observed by this technique in the lysosomes of mouse peritoneal macrophages (46). The lysosomal enzymes seem to share an unusual resistance to autolysis. All the lysosomal enzymes contain oligosaccharide chains which give them a structural similarity. The content of saccharides notably NeuAc, in acid phosphatase seems to be variable giving rise to multiple protein types with similar catalytic activity.
**GLYCOPEPTIDES**

Glycoproteins are conjugated proteins which contain carbohydrate groups attached covalently to polypeptide chain (47). They are usually branched, with relatively low number of sugars and lacking a serially repeating unit. Glycoproteins vary in their composition and size of the heterosaccharide prosthetic groups and protein moiety. Glycoproteins contain a limited number of sugars which include D-Gal, D-Man, D-Glc, L-Fuc, GlcNAc, GalNAc and NeuAc. Few other sugars reported in glycoproteins are L-Ara in nervous tissues and D-Xyl in connective tissues, plants and fungi. The carbohydrate portion of a glycoprotein may vary from 1 to 80% of the weight of the molecule. The essential features of proteoglycans are the presence of serially repeating saccharide units at closely spaced intervals, long polysaccharide chains, the linear structure of prosthetic group and the covalency of polysaccharide protein linkage.

**Linkage:**

The oligosaccharide chains are conjugated through C-1 position of reducing terminal sugar residue.
to peptide portion by two types of primary, covalent-
linkages (48,49).

One type of linkage is O-glycosidic type and it may be subdivided as follows (a) Linkages of L-threonine and L-serine with GalNAc, D-Man, D-Gal and L-Fuc. All of the O-L-seryl and O-L-threonyl glycosidic linkages are alkali labile, and the glycans are detached from the peptide chains by β-elimination reaction provided that the carboxyl group of β-hydroxy amino acid is substituted (50). (b) D-Gal or the disaccharide α- Glc- (1 → 2) β-Gal linked to 5-hydroxy-L-lysine. This linkage is alkali stable (c) The linkage between 4-hydroxy-L-proline and L-Ara is also alkali stable. (d) Unusual linkages having a Gal disaccharide conjugated to thiol group of L-cysteine (51) and the glycosidic linkage between the phenolic group of L-Tyrosine and NeuAc (52).

The second type linkage is between (GlcNAc) and amide group of asparagine. These carbohydrate chains are characterized by two different forms. Linear forms are found in proteoglycans and branched glycans which are more complex type, in glycoproteins (48). Glycoproteins
contain a most common core structure related to their linkage type. O-glycosidic protein core structure is \( \beta\text{-Gal-(1 \rightarrow 3) \alpha\text{-GalNAc (1 \rightarrow 3)- Ser(Thr)} \) while N-glycosidic contains a core structure,

\[
\begin{align*}
4'\text{Man(1 \rightarrow 3)} \\
\beta\text{-Man-(1 \rightarrow 4)-} \beta\text{-GlcNAc-(1 \rightarrow 4)-} \beta\text{-GlcNAc-(1 \rightarrow 4)-}\text{Man(1 \rightarrow 6)}
\end{align*}
\]

N-glycosylproteins may be further classified on the basis of addition of different types of carbohydrate chains on the pentasaccharide core that gives the specific structure to glycoproteins (fig.3).

The first group of glycoproteins in which only Man residues were attached to pentasaccharide core are called high-mannose type (48).

In the second form the pentasaccharide core was associated by a variable number of N-acetyl-lactosamine \( \beta\text{-Gal-(1 \rightarrow 4)-GlcNAc} \) residues and of NeuAc or L-Fuc or both. These structure were called complex type. The various carbohydrate structures that have been found in this group are following: (1) Substitution on the C-2 of the Man-4 and -4' residues of the core by 2 residues of N-acetyl-lactosamine, leading to diantennary glycans (fig.4A). (2) substitution by 3 residues of N-acetyl-
Figure 3. Example of the major classes of asparagine-linked oligosaccharides.
lactosamine, giving triantennary structures either on C-2 and C-4 of Man-4 and on C-2 Man-4' (fig. 4B), (3) substitution by four residues of N-acetyl-lactosamine on C-2 and C-4 of the Man-4 residue and on C-2 and C-6 of the Man-4' residue gives the tetraantennary structure (fig. 4C), (4) substitution on C-4 of the Man-3 residue of the core by a GlcNAc residue (fig. 5). (5) substitution on the C-6 of the GlcNAc-1 residue by a Fuc residue (fig. 5)(48).

Finally the work of Kobata and coworkers (53,54) on ovalbumin glycopeptides gives a third kind of structure of N-glycosidic proteins which is common with high-mannose and complex type. This structure is called as hybrid type of N-glycosidic proteins (fig.3).

However the concept of the common pentasaccharide core and of the oligosaccharide structures common to all of the N-glycoproteins must not be accepted as dogma, as several unorthodox structures have been described in the literature (48).

**GLYCOPROTEIN BIOSYNTHESIS**

It is convenient to consider the biosynthesis of glycoproteins according to linkage type. Several reviews
have been reported which deal with the biosynthesis of Asn-GlcNAc and Ser (Thr)-GalNAc oligosaccharides (55-57). The linkage type is not usually used to classify different glycoproteins because a single glycoprotein molecule may contain more than one type of linkages.

The asparagine linked oligosaccharides of eukaryotic cells-glycoproteins fall into two groups: high-mannose and complex type. It has been reported that high-mannose and complex glycans have a common biosynthetic origin: Glc$_3$Man$_9$GlcNAc$_2$ (58). This species is synthesized while linked to a carrier lipid dolicholphosphate (Dol-P) and then transferred en bloc from lipid carrier to acceptor proteins (59,60).

From the studies with inhibitors and mutant cell lines it has been revealed that Asp-linked oligosaccharides are synthesized through lipid carrier (57). The oligosaccharide is linked to dolichol, an $\alpha$ saturated polyisoprenol carrier lipid via a pyrophosphate group. Dolichol-phosphate which acts as the carrier lipid is also an acceptor of the donor sugar, from GDP-Man and UDP-Glc.

Initiation of the oligosaccharide chain requires the formation of GlcNAc-P-P-Dol and its conversion to
GlcNAc$_2$-P-P-Dol. Here both the GlcNAc residues are donated by UDP-GlcNAc as GlcNAc-P rather than GlcNAc (61, 62). The enzyme involved in the synthesis of trisaccharide lipid Man-$\beta$-GlcNAc$_2$-P-P-Dol has an absolute requirement for GlcNAc$_2$-P-P-Dol and it utilizes GDP-Man, but not Man-P-Dol as Man donor (63). Further $\alpha$-Man residues can be transferred from GDP-( $\alpha$) Man which gives an hepatasaccharide dolichol-phosphate (Man$_5$ GlcNAc$_2$-P-Dol). This was confirmed by Chapman et al. (64) while working with a mutant lymphoma cell line which lacked the ability to synthesize Man-P-Dol. Incubation of membrane preparations with GDP-(H$_3$)-Man gave rise to labelled lipid linked oligosaccharide up to Man$_9$ GlcNAc$_2$ for the parent cell line, but Man$_5$ GlcNAc$_2$ was the largest species synthesized by the mutant. The incubation with (H$_3$)-Man-P-Dol gave rise to oligosaccharide lipids from about Man$_6$ GlcNAc$_2$ to Man$_9$ GlcNAc$_2$ or larger in both cases. It was concluded by the authors that Man-P-Dol is not absolutely required until addition of the sixth Man residue.

Different types of glycosylated species of oligosaccharide lipid have been reported (57). This includes lipid linked Man$_5$ GlcNAc$_2$, Man$_7$ GlcNAc$_2$, Man$_8$ GlcNAc$_2$ and
Man<sub>9</sub>GlcNAc<sub>2</sub>. Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> is the major lipid linked oligosaccharide. Kinetic evidence suggests that Man<sub>5-8</sub>GlcNAc<sub>2</sub> lipids are intermediate in the assembly of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> lipid (65). It is not yet clear whether Glc residues are added before or after transfer of the ninth Man residue to lipid linked Man<sub>8</sub>GlcNAc<sub>2</sub>. Transfer of Glc residues to lipid linked oligosaccharide is not thoroughly characterized. Staneloni et al. have shown that all the three Glc residues can be donated by Glc-P-Dol in rat liver microsomes (66).

The transfer of oligosaccharide from carrier lipid to protein is observed to be cotranslational (67). However there is evidence that oligosaccharide may be transferred after polypeptide chain termination in some cases (68). Under normal conditions protein structure appears to be the major determinant for the glycosylation. It is now well established that Asn in the tripeptide sequence Asn-X-Thr (or Ser) is required for glycosylation where X-can be any of 20 amino acids other than possibly aspartic acid (69). It has been observed in hen oviduct that oligosaccharide transferring was associated primarily with the rough ER (70). Although in other systems a considerable amount of activity is also present in smooth
membrane (71). Kinetic studies, in which the intracellular movement of labelled glycoprotein was followed after a pulse of labelled carbohydrate or amino acid, suggests that the initial oligosaccharide transfer usually occur in the rough ER (59, 72). It has been observed that glucose residues are needed for the transfer of oligosaccharides to endogenous acceptor (73). The oligosaccharides transferring enzyme can discriminate between Glc₃-and Glc₂-ManGlcNAc₂ had been clearly demonstrated in an experiment when a mixture of labelled lipid linked oligosaccharides containing approximately equal amounts of these two species along with smaller nonglucosylated oligosaccharides was added to NIL-S cell-microsomes (74). After the transfer of oligosaccharide from carrier lipid to the protein, it undergoes a series of modification that finally will give different N-linked glycans of mature glycoprotein. During the processing of oligosaccharides, the glycoproteins are transported through several intracellular compartments (57).

It has been demonstrated that immediately after transfer of the precursor glycan Glc₃Man₉GlcNAc₂ to protein, glucose residues are removed (75, 76). There are some smaller pathways in which two mannose residues
are removed from the precursor oligosaccharide that still retains one or more Glc residues (76). It is observed that at least two glucosidase activities are involved in the removal of Glc residues (77). The glucosidase I removes only terminal residue. The second activity (glucosidase II-III) which is inactive towards Glc-Man-GlcNAc but can convert Glc-Man-GlcNAc to Man-GlcNAc has been solubilized and partially purified from rat liver (77). Glucosidase I has been shown to be distinct from glucosidase II-III and the latter activity probably represents a single enzyme.

The second phase of oligosaccharide processing is considered to be removal of one to four $\alpha$-(1$\rightarrow$2) Man residues from protein linked Man$_9$GlcNAc$_2$. If all four of these residues are removed, a Man$_9$GlcNAc$_2$ species containing $\alpha$-(1$\rightarrow$3) and $\alpha$-(1$\rightarrow$6) Man residues remained. Touster and coworkers have solubilized and purified Golgi associated enzyme $\alpha$-mannosidase IA (78). It hydrolyzes $\alpha$-(1$\rightarrow$2) but not $\alpha$-(1$\rightarrow$3) Man residues. It is active on p-nitrophenol $\alpha$-D-Man. Another Golgi $\alpha$-mannosidase ($\alpha$-mannosidase I B) which is inactive on p-nitrophenol $\alpha$-D-mannoside was partially purified by Tabas and Kernfeld (79) with labelled high-mannose
oligosaccharide as substrate. This enzyme activity can convert free \( \text{Man}_{9}\text{GlcNAc}_{2} \) to \( \text{Man}_{5}\text{GlcNAc}_{2} \) species. Although direct proof are still lacking, it seems reasonable, that these two enzymes participate in N-linked oligo-
saccharide processing \textit{in vivo}. Their relative role is not known properly. Now the question arises that what determines whether an N-linked oligosaccharide retains a high-mannose structure or is processed to complex type. Several experiments have been done on N-linked oligo-
saccharides. The results establish that available processing enzymes in host cells may have some effect on the size of complex glycans, but the primary deter-
minant of oligosaccharide structure is the structure of the protein being processed (80, 81).

The first step in the conversion of high-mannose to complex oligosaccharides is catalysed by \text{GlcNAc}
transferase I which adds a single \text{GlcNAc} residue (82, 83, fig.4) to the \( \text{Man}_{5}\text{GlcNAc}_{2} \) structure that remains after removal of the \( \alpha,1,2\)-Man residues from the precursor oligosaccharide. A second enzyme "late Mannosidase" then releases the two terminal Man residues which are not involved in the core. The products of reaction (4) and (5) in (fig.6) are rapidly processed and have not been
Figure 6. Proposed sequence for the processing of asparagine-linked oligosaccharides. The enzymes involved are
1) glucosidase I,
2) glucosidase II,
3) α-mannosidase I,
4) UDP-GlcNAc; α-D-mannoside β 1-2 N-acetyl glucosaminyl transferase I,
5) α-mannosidase II,
6) UDP-GlcNAc, α-D-mannoside β-1-2 N-acetyl glucosaminyl transferase II,
7) GDP-fucose, β-D-N-acetyl glucosaminide fucosyl transferase,
8) UDP-Gal, glycoprotein galactosyl transferase,
9) CMP-SA, glycoprotein sialyltransferase.

N-acetylglucosamine, ○ mannose, ▼ glucose,
● galactose, ◆ sialic acid, and △ fucose.

identified as intermediate in the intact cells. They are however, the predominant glycans on mature rhodopsin (84). The product of GlcNAc transferase I and the late Mannosidase, GlcNAc Man₃GlcNAc₂ is a substrate for at least three enzymes in vitro (a) GlcNAc transferase II, which can convert it to GlcNAc₂Man₃GlcNAc₂ (b) a Fucosyl transferase, which can add α(1→6) linked Fuc to the innermost GlcNAc residue and (c) a Galactosyl transferase, which forms Galβ(1→4)-linkage to terminal nonreducing GlcNAc residues. It should be noted that this scheme is an over-simplification and does not take into account the existence of the alternative linkage, incomplete processing or conversion to other type of complex oligosaccharide. It has been shown that some oligosaccharide structures are having the common features of high-mannose and complex type glycans, which are denoted as hybrid type oligosaccharides (66,67). Recently Schachter et al. have reviewed various kinds of branching during the biosynthesis of asparagine-linked glycoproteins (85).

The high-mannose type glycans contain 6-phospho-mannosyl residues on lysosomal enzymes acts as recognition marker (86). Phosphorylation takes place on the protein bound oligosaccharide rather than on lipid-linked
oligosaccharide precursor, phosphate groups are present in enzymes as phosphomonoester moiety. It has been reported that in the phosphorylation of acid hydrolases N-Acetylglucosaminyl phosphotransferase transfers the α-N-acetylglucosamine 1-phosphate from UDP-GlcNAc to Man residues of high Man oligosaccharide (87,88). Further, the selective removal of GlcNAc residue by α-N-acetylglucosaminyl phosphodiesterase takes place to expose phosphomannosyl group (89,90).

The vesicular stomatitis virus (VSV) glycoprotein G can be used as a model to show the major stages of N-linked glycans processing (91). VSV encoded membrane glycoprotein G follows a route to plasma membrane of infected animal cells, which is indistinguishable from that believed to be taken by most of the cells own surface membrane proteins prior to infection.

The glycoprotein G is synthesized by ribosome bound to ER membrane, where the precursor oligosaccharide is added. Within 10 minutes, the glycoprotein G is transported in Golgi stack, where its oligosaccharides are processed. During the next hour or so, glycoprotein G is gradually delivered to plasma membrane, from which it enters progeny viral particles, as they bud off from
the cell surface. Processing of oligosaccharides in the Golgi proceeds in two major compartments. First, certain sugars especially Man residues are removed in the cis-Golgi region which is proximal to rough-ER. Other sugars such as GlcNAc, Gal, NeuAc and Fuc are added in terminal stage of glycosylation. This process takes place in trans-region of Golgi stack which is distal from rough ER(92,93). Enzyme catalyzing both stages of processing are concentrated 100-fold or more in Golgi fraction of liver and thus can serve as enzyme markers for portions of this organelle (94).

The majority of Thr/Ser-linked glycoproteins are found in epithelial mucins. They are more heterogeneous in structure than N-linked glycoproteins. The Ser (Thr)-GalNAc oligosaccharide chains do not arise from common lipid linked precursor. The monosaccharides are directly incorporated to the protein in a stepwise manner. Ovine and porcine submaxillary mucins have been thoroughly characterized and all the glycosyltransferases required for the assembly of their oligosaccharide prosthetic groups have been reported (55, 56).
The glycosylation of protein in various cell lines were investigated with several inhibitors. There are two types of inhibitors - (1) Analogs of sugars - viz. 2-deoxy glucose (d\text{Glc}), 2-deoxy-2-fluoro-D-Glucose (fluoroglucose) and 2-deoxy-2-fluoro-D-Mannose (fluoromannose) and (2) antibiotics - tunicamycin, bacitracin, amphotericin and similar others. Analysis of the nonglycosylated and partially glycosylated glycoproteins thus resulted helped in the elucidation of various steps in glycosylation reaction (95,96).

Deoxyglucose (d\text{Glc}) exerts its inhibitory effect through derivatives UDP-d\text{Glc}, GDP-d\text{Glc} and d\text{Glc-P-Dol} (97). UDP-d\text{Glc} inhibits the formation of \text{Glc-P-Dol}. GDP-d\text{Glc} inhibited the formation of dolichol linked monosaccharides by trapping of Dol-F as d\text{Glc-P-Dol} leading to the formation of d\text{Glc} (\text{GlcNAc})_{2}\text{-pp-Dol} instead of Man (\text{GlcNAc})_{2}\text{-PP-Dol} (98). The former trisaccharide is a dead end in the assembly pathway, because it can not be elongated by GDP-Man or Man-P-Dol (98). Fluoroglucose and Fluoromannose also act through their nucleotide derivatives (99). Fluoroglucose interferes with mannoseylation reaction. It inhibits the formation of Man-P-Dol and thus blocks the assembly of \text{Glc}_{2}\text{Man}_{9}
(GlcNAc)_2-PP-Dol (100). This inhibition can be reversed (100) or blocked (101) by Man.

Tunicamycin an antibiotic inhibits the formation of GlcNAc-PP-Dol (69). It is a tight binding competitive inhibitor of UDP GlcNAc dolichol-phosphate GlcNAc,1P-transferase (102). Amphotericin inhibits the formation of lipid linked oligosaccharides such as Man-P-Dol, Glc-P-Dol and GlcNAc-PP-Dol. It inactivates several glycosyltransferases that transfer sugar to lipid acceptor (103). Bacitracin inhibits the formation of dolichol phosphate from dolichol phosphate or dolichol diphosphate-linked oligosaccharides (104). The antibiotic forms a complex with polyisoprenoid diphosphates and metal ions.

Another group of compounds have also reported to inhibit the further processing of Glc_3Man_9(GlcNAc)_2 oligosaccharide after its transfer from lipid donor to protein (96). Neogirimycin (5-amino-5-deoxy-D-glucopyranose) and its derivatives and castanospermine (8αβ indolizidine - 1α, 6β, 7α, 8β tetrol) are inhibitors of glucosidase I and therefore interfere with release of the outer glucose residue of newly
transferred oligosaccharide chain (105–107). However, nojirimycin and 1-deoxynojirimycin can also interfere with the synthesis of the lipid-linked oligosaccharide Glc₅Man₉(GlcNAc)₂-PP-Dol (96).

Bromooconduritol (6-bromo-3,4,5-trihydroxy-cyclohex-1-ene) inhibits the release of the innermost glucose residue, thereby preventing the formation of complex oligosaccharides (108).

Swainsonine (8 αβ indolizidine 1,2,8 triol), a toxic plant alkaloid, is a potent inhibitor of Golgi α-D-Mannosidase II and lysosomal α-D-Mannosidase (109). It inhibits the biosynthesis of complex glycoprotein by inhibiting Golgi α-D-Mannosidase II activity. Prolonged ingestion of this plant by grazing animals produces a Mannosidosis type of neurological disorder (110).

1-Deoxymannogalactimycin (1,5-dideoxy-1,5, imine-D-mannitol) is an inhibitor of Golgi α-D-Mannosidase IA and IB (111). It inhibits the conversion of high mannose oligosaccharides to complex glycans.

Inhibition of protein glycosylation has diverse biological effects, depending on the cell-type and glycoprotein affected. However, few important
conclusions were drawn: (1) lack of carbohydrate may increase the susceptibility of proteins to proteolytic break down, (2) carbohydrates may be involved in intracellular transport (96), (3) possible involvement of glycoproteins in differentiation and development (95).

MICROHETEROGEOEITY

Glycosylation of proteins takes place within the cell in post-ribosomal enzymatic steps. Therefore slight alterations in structure of oligosaccharides are not unexpected. For example, the oligosaccharide chain in ovalbumin joined to asparagine can have different structures with Man to GloNac ratio varying from 1.4 to 2.5 (112). A similar case was reported of the oligosaccharide chain linked to asparagine 34 of bovine ribonuclease B containing Man and GloNac. A difference in carbohydrate sequence can occur anywhere in the oligosaccharide sequence. However, since NeuAc is terminal sugar in the most of the glycoproteins the microheterogeneity at the terminus due to the presence or absence of this sugar is most common (113). The variation in structure can be either due to an incomplete biosynthetic sequence or due to post synthetic degradation within the cell or during isolation (114).
FUNCTIONS OF GLYCOPROTEINS

Glycoproteins are abundant in nature. They are common components of cell surfaces and intra cellular constituents. They are found as constituents of lysosomes and among the products exported by the cell. The cell surface glycoproteins have been shown to play an important role in pinocytosis, differentiation, tumorigenesis, intracellular recognition and adhesion, as receptor for enzymes, hormones and viruses and as mediator of immunological specificity. The secreted products function as enzymes, hormones, immunoglobulins

(115). Although glycoproteins are present in great abundance in eukaryotic cells, the biological function of constituent oligosaccharide units remains to be elucidated properly.

It has been observed that desialylated glycoproteins have drastically reduced the survival time in the circulation compared to the native form of the same proteins. This phenomenon was first demonstrated using desialylated ceruloplasmin (116). The penultimate Gal residues of saccharide chains were shown to be cryptic determinants of survival. The survival time was significantly prolonged upon modification of the Gal moiety
by Galactose oxidase or β-Galactosidase or enzymatic replacement of the missing NeuAc residues. The Gal residues served as recognition marker for the rapid uptake and degradation of intact glycoproteins by parenchymal cells of the liver (117).

Gregoriadis et al. have shown the evidence that hepatic lysosomes are the major site of asialoglycoprotein catabolism (118). Le Badie et al. have demonstrated that 5 minutes after injection of (125) - asialofetuin into rat a 23-fold concentration of labelled protein in plasma membrane enriched fraction of the liver, but at 13 minute the radioactivity appeared in lysosomes (119).

Recently Dunn et al. have shown that temperature below 21°C selectively inhibited fusion between pinocytic vesicles and lysosomes during the catabolism of (125) - asialofetuin in perfused rat liver. The cessation of degradation below 20°C was not due to inhibition of proteolytic enzymes since activity in vitro was retained at lower temperature (120).

Receptor-mediated endocytosis does not necessarily mean in lysosomal degradation of asialoglycoproteins. An alternate route has been described in which
ligands escape degradation and are translocated through hepatocytes from serum to bile (121).

In addition to hepatic uptake of asialoglycoproteins, the bone marrow appears to provide a second site of deposition as has been shown in case of transferrin (122). Gal is a recognition marker common to both sites. The nature of bone marrow receptor, its relation to hepatic binding protein and the biological significance of this pathway are not known. Recently, the rabbit hepatic lectin has been isolated and immobilised on Sepharose for examination of its binding sites by a competitive binding assay (123). It was found that the binding site is relatively small, involving primarily the sugar residue at the non-reducing terminus of saccharide chain but extending to at least part of penultimate sugar residue. Methyl glycosides of GalNAc are more potent inhibitors of ligand binding than the glycosides of Gal. The binding site of Sepharose-immobilized rabbit receptor has also been studied using neoglycoprotein to which carbohydrate have been linked covalently (124). Neoglycoproteins containing Mannosides or N-acetylglucosaminides were not
bound by these membranes, but glucosides coupled to albumin showed strong binding. Hence it was concluded that the asialoglycoprotein receptor can not discriminate between the D-Gal and D-Glc configurations (125).

Co-operative effects in vivo have also been suggested. The injection of small doses of asialotransferrin results in an equilibrium between circulation and the receptor on hepatocyte plasma membrane with no significant internalization of membrane bound ligand (126). Thus binding to receptor at plasma membrane and endocytosis may be separate phenomenon. Increasing the amount of injected asialotransferrin led to increased elevation of ligand catabolism. Subsequently, it has been observed that molecules having different carbohydrate structure can act synergistically to induce mutual endocytosis (127).

The binding activity of asialoglycoproteins are recovered in Triton X-100 extracts of crude rabbit liver (128) showing that the integrity of the membrane was not essential for the activity. Hudgin et al. have prepared a water soluble, lipid-free material possessing asialoglycoprotein binding activity by affinity
chromatography on asialoorosomucoid linked to Sepharose (129). The ligand specificity of this preparation is parallel to that of plasma membrane. Approximately 10% of its dry weight was carbohydrate consisting of NeuAc, Gal, Man and GalNAc in a molar ratio of 1:1:2:2. An absolute requirement for calcium and pH optimum between 7 and 9 were noted for the soluble preparation. The integrity of the carbohydrate unit of the receptor molecule appeared to be essential for the functional activity of the binding properties. Treatment of isolated plasma-membrane or purified receptor with Neuraminidase resulted in a loss of binding activity (130). This was the result of recognition and binding by the receptor preparation of its own Gal residues made terminal by NeuAc removal. Stockert et al. have shown that the activity of the protein could be restored after Neuraminidase inactivation, by subsequent exposure to Galactose oxidase or β Galactosidase (131). Paulsen et al. reported that the activity of the Neuraminidase treated receptor was restored through a highly purified Sialyltransferase (132). The asialo-glycoprotein receptor from human liver was isolated and thoroughly characterized. It
showed almost similar properties as rabbit liver
receptor (133).

The initial identification of rat liver plasma
membrane as the major locus of asialoglycoprotein
receptor was subsequently expanded to include membrane
of Golgi complex, the smooth microsomes and lysosomes (117).
These binding proteins were chemically and immunologically
indistinguishable, but the activity of various subcellular
fractions differed markedly in their response to the
detergent Triton X-100. The binding activity of Golgi
complex and smooth microsomes was greatly enhanced
whereas that of lysosomes was decreased and that of
plasma membrane was unaffected. These observations were
interpreted to reflect the effect of detergent in
increasing accessibility to binding sites in Golgi and
microsomal fraction. The inhibitory effect on the lysosome
was ascribed to the detergent induced lysis resulting
the release of hydrolytic enzymes which effectively
destroyed the receptor activity. These findings have
raised the question of topological orientation of the
receptor in membrane from subcellular organelles. This
problem was solved with the availability of the antibodies
to hepatic receptor which effectively blocked binding of the test ligand (I125) asialoorosomucoid. While using these antibodies Tanabe et al. have shown that at least 85% of the binding activity present in smooth microsomes and Golgi complex was oriented towards the lumen of these organelles, consistent with the enhancement of the activity by Triton X-100 but no binding activity was distributed to receptor molecules on the inner surface of the lysosomal membrane (134). Recovery of asialoglycoprotein receptor in microsomes or Golgi complex might be rationalized on the basis of biosynthetic pathway. Its presence in the lysosomes can not be explained even by endocytosis in which receptor would face the interior of the lysosomal compartment, and can be destroyed by hydrolytic enzymes. The receptor protein outlives the ligand whose endocytosis it mediates (134). Taking into account this observation it was postulated that the receptor may reorient in the lysosomal membrane to avoid proteolysis (134). A model membrane also has been proposed to in support of this hypothesis (117).

Certain observations indirectly support the contention that not all the receptor molecules of the cell participate in the ligand metabolism. Treatment
of isolated hepatocytes with Neuraminidase abolishes binding of asialoorosomucoid, but the cells continue to catabolize desialylated ovine submaxillary mucin a ligand of higher affinity for receptors (135, 131). Although the intracellular pool of receptor was unaffected by Neuraminidase treatment, the enzyme treated cells maintained their altered specificity while desialylated mucin continued to be internalized. Similarly, infusion of a single passage of antireceptor IgG was found to block ligand binding by perfused liver for at least 90 minutes (136). Since homogenate of the liver retained greater than 85% of the control binding activity, these results were interpreted to indicate that intracellular receptors were not recruited to cell surface. These results suggest that the plasma-membrane receptors remain segregated from the relatively large internal pool. Telleshaug and Berg have given the Scatchard-plot indicating chloroquin-induced reduction in surface receptor sites (137). They suggested that drug impeded return of receptor to surface, and since the added ligand was absent, constitutive cycling of unoccupied receptor was implied. Oka and Weigal have also suggested that occupied and unoccupied receptors
are internalized at similar rate (138). Other results indicate that surface receptor is sufficient to mediate catabolism of asialoglycoproteins. Mouse L-929 cells that are impotent in this regard have been fused with rat hepatocyte membrane vesicles. This fusion resulted in cells capable of endocytosis and degradation of asialo- orosomucoid under conditions where the introduced receptor apparently did not redistribute to produce an internal pool analogous to that of hepatocytes (139). Some more sugar specific receptors have been purified and characterized (140).

A role of carbohydrate moiety in metabolic stabilization of glycoproteins has been observed (141). But the actual mechanism by which carbohydrate could protect these biologically important molecules against proteolytic degradation is not known. Olden et al. (142) have shown that nonglycosylated fibronectin is more sensitive to protease digestion than the glycosylated protein. Similarly the enzymatic removal of carbohydrate moieties from a variety of glycoproteins increased their sensitivity to proteases (143).

The reported changes in surface carbohydrates during cellular differentiation and neoplastic
transformation suggest their importance in physiology and behaviour of cells. Such changes have long been implicated in malignant transformation (144, 145). The mechanism by which complex carbohydrates at surface of opposing cells would moderate the formation of intracellular adhesion is of fundamental importance. Roseman (146) has suggested that glycosyltransferases on the cell surface are not only be involved in the synthesis of the surface heterosaccharides but also may be able to bind to appropriate acceptor on another cell. This process would result in the adherence of the two cells. Recently Rawala et al. have showed cell adhesion mediated by a purified fucosyltransferase (147). The work of Abercrombie and Ambrose revealed that change of properties of cell surface is responsible for loss of contact inhibition as seen in virally and chemically transformed normal cells. (148). However, there are reports which appear to support the idea that cell adhesion is mediated by interaction of surface carbohydrates and lectins. Rosen et al. have found that univalent antibody fragments, which bind to surface lectin of Pseudomonas palladium inhibit the adhesion of these cells (149). Similarly they demonstrated that lectin specific sugar asialofetuin also blocks cell
adhesion (149). These results were obtained under nonphysiological conditions. May et al. have reported some results which support lectin or lectin carbohydrate involvement in the adhesion of slime mold cells (150).

One of the most challenging problems in developmental biology is the mechanism(s) by which embryonic cells migrate and orient themselves in a highly specific organized fashion. In as far as the normal development depends upon growth, response to signal molecules, adhesiveness, migration and recognition, it is reasonable to assume that glycoproteins play important roles in early embryogenesis. Numerous studies with Eumycymycin suggest that Am- linked glycoproteins are involved in embryonic differentiation. In sea urchins, this antibiotic has no effect on fertilization, cleavage and blastula formation, but arrests the development during the early stage of gastrulation (151). Eumycymycin also inhibits the development of amphibian and mammalian embryos (152-154). These results suggest some as yet unknown role for glycosylation products in differentiation.
It has been experimentally demonstrated that membrane recycling takes place in the cell (155). One proposal is that the membrane system in the cell (plasma membrane, ER, Golgi complex, lysosomes, vesicles etc.) are in dynamic interrelationship with constant membrane flow from one membrane system to another. Olden et al. (115) have reported that lectins and glycoproteins with corresponding carbohydrate "sorting signal" will be copackaged in transport vesicles. This would result in the rapid loss of lectin from its major functional locus within the cell unless it was recycled back to membrane site. There are other reports which showed the recycling of membrane surface lectins (134, 156, 158).

The blood group substances ABH and Lewis (Le\textsuperscript{a} and Le\textsuperscript{b}) are glycoproteins and found in the secretions eg. saliva, gastric juice, and ovarian cyst fluid. Watkins (159) and Ginsburg (160) have reviewed the chemistry of blood group substances. The serological specificities of these molecules are determined by the nature and linkage of the monosaccharide at the nonreducing ends of their
carbohydrate chains. There are two kinds of reducing ends: Type one contains a Gal $\beta (1 \rightarrow 3)$GlcNAc sequence while the second type contains a Gal $\beta (1 \rightarrow 4)$ GlcNAc sequence that form the basis of blood group active structures. A, B and H determinants can be based on either type 1 or type 2 chains whereas Le$^a$ and Le$^b$ active structures in which L-Fuc substituted at 4 position of the GlcNAc are based on type 1 chain. The most important sugars for each specificity are known as immunodominant sugars. The immunodominant sugar for H specificity is Fuc as Fuc $\alpha (1 \rightarrow 2)$ Gal, for A specificity, GalNAc as GalNAc $\alpha (1 \rightarrow 3)$ Gal, of B -specificity Gal as Gal $\alpha (1 \rightarrow 3)$ Gal and for Le$^a$ specificity Fuc as GlcNAc $\downarrow$
\[ \text{Le}^a \] whereas two Fuc molecules are required for Le$^b$ specificity
\[ \text{Le}^b \]

The MN blood group system was found only in erythrocyte membrane and not in serum or secretion (161).
Springer et al. has reported the determinant sugar for M and N antigen (162). The specific sugar for M determinant is two NeuAc residues as:

\[
\text{NeuAc} \xrightarrow{\alpha} \text{Gal} \xrightarrow{\beta} \text{GalNAc}
\]

whereas one Gal residue and one NeuAc residue are required for N specificity

\[
\text{NeuAc} \xrightarrow{\alpha} \text{Gal} \xrightarrow{\beta} \text{Gal} \xrightarrow{\beta} \text{GalNAc}
\]

Antifreeze glycoprotein occurring in the area of several species of Antarctic fish is a unique type of glycoprotein. A disaccharide Gal-\(\beta\)-(1→3)-Gal NAc is attached to each thr residue. The intact glycoprotein has the remarkable property of lowering the freezing point of aqueous solution to the same extent as by an equal weight of sodium chloride (163).

**LECTINS:**

Lectins are carbohydrate binding proteins of plant or animal origin and agglutinate cells containing glycoconjugates of the required specificity on their
Lectins are predominantly present in plants (generally in the seed) and have the property of agglutinating erythrocytes of various animal species (166). Later these types of proteins were isolated from invertebrates and vertebrates (165, 167). Boyd had suggested that both the groups of agglutinin be referred as lectin from the Latin word "Legere" (to pick out or to choose) (168). Some of the lectins isolated and characterized are concanavalin A (Con A), wheat germ agglutinin (WGA) and a galactose binding protein from Ricinus communis RCA₁₅₀, Fuc binding protein from Lotus tetragonolobus, GalNAc and Gal binding lectin from soybean, Man, Glc and GlcNAc binding lectin from Pisum sativum and others (169).

Lectins have the unique property of binding with certain specific sugars and thereby specifically precipitate polysaccharides and glycoproteins. The precipitation is inhibited by sugars as in case of agglutination (170). Taking into consideration these properties lectins find application in blood typing (168), immunosuppression (171) and separation of leucocytes from erythrocytes (172). Moreover lectins serve as
reagents for detection, isolation and characterization of carbohydrate containing macromolecules (173, 174). Enough curiosity has developed at present on probing the nature and distribution of membrane bound, carbohydrate containing structures by using lectins of defined specificity (175, 176).

Interaction between con A and brain lysosomal acid hydrolases and its inhibition by Con A specific sugar led Bishayee and Bachhawat (177) to show the lysosomal acid hydrolases are glycoproteins. The specificity of interaction of Con A with sugars and polysaccharides have been thoroughly investigated by Goldstein and his associates (178, 179). Binding sugar requires O-3, C-4 and C-6 hydroxyl groups of D-Man or D-Glc ring for interaction with ConA, α-Man and α-Glc bind strongly with Con A.

**AFFINITY CHROMATOGRAPHY**

Affinity chromatography utilizes the unique biological specificity inherent in ligand-macromolecule interaction. Here ligand refers to a substrate, product, inhibitor, coenzyme, allosteric effector or any other
molecule that interacts specifically and reversibly with the protein or other macromolecule to be purified (180).

The affinity chromatography is used by covalently attaching the ligand to an insoluble support and packing the support into a chromatographic bed. If a mixture comprising several proteins is applied to the column, only that protein that displays appreciable affinity for the ligand will be retained, others which show no recognition of the insolubilized ligand will pass through the bed unretarded. The specifically adsorbed protein can subsequently be eluted by altering the composition of the solvent, pH or temperature to permit dissociation from the insoluble ligand. This technique has been used for the purification of enzymes, binding, transport and receptor proteins, antibodies, nucleic acids, antigens, and intact cells, polysomes and viruses (180).

The specific interaction between the ligand and its complementary molecule may be exploited in a
number of related affinity technique such as visualization of cell surface and intracellular carbohydrate. Since glycosylated derivatives of horseradish peroxidase and ferritin react with many phytohaemagglutinin, they have been used as cytochemical marker (161).
AIMS AND OBJECTIVES

The glycoconjugates are in dynamic state in biological system and their degradation in the digestive vacuoles of the cell is catalysed by lysosomal enzymes including acid glycosidases. As discussed in the introductory chapter a deficiency of enzymes led to intra-cellular accumulation of undegraded oligosaccharides. In human Mannosidosis, the activity of lysosomal \( \alpha \)-Mannosidase (5.2.1.24) was found to be low in liver, spleen and brain. The \( \alpha \)-Mannosidase activities of neutral and intermediate pH optima localised in Golgi membranes were however normal. High Man rich oligosaccharide in urine of the patients with Mannosidosis has also been reported. A similar disease has been described in bovine and feline species. In all these cases the deficiency of \( \alpha \)-Mannosidase activity is believed to cause accumulation of Man-rich oligosaccharide
which led to further blockage in catabolism. The above background called for a detailed study of the properties of human tissue $\alpha$-Mannosidase.

There is no information on the effect of chemical modification on the biological activity of acid $\alpha$-Mannosidase. The role of free amino groups in the catalytic activity of the enzyme, examined with two reversible amino group modifying reagents, maleic anhydride and citraconic anhydride.

The thesis describes the studies on lysosomal $\alpha$-Mannosidase from normal human placental tissue, an useful hospital waste material.