1. **Introduction**

Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains. The carbohydrate is attached to the protein in co-translational or post-translational modification. This process is known as glycosylation. In proteins that have segments extending extracellularly, the extracellular segments are often glycosylated. Glycoproteins are often important integral membrane proteins, where they play a role in cell-cell interactions. Glycoproteins are also formed in the cytosol. There are two types of glycoproteins i.e. N-glycosylated glycoproteins and O-glycosylated glycoproteins. In N-glycosylation the addition of sugar chains can happen at the amide nitrogen on the side chain of the asparagine. In O-glycosylation the addition of sugar chains can happen on the hydroxyl oxygen on the side chain of hydroxylysine, hydroxyproline, serine or threonine. Eight sugars commonly found in glycoproteins are, β-D-Glucose (Hexose), β-D-Galactose (Hexose), β-D-Mannose, α-L-Fucose, N-Acetylgalactosamine (Aminohexose), N-Acetylglucosamine, N-Acetyleneuraminic acid (Aminononulosonic acid/Sialic acid) and Xylose (Pentose). The sugar group(s) can assist in protein folding or improve proteins stability. Chemically salivary glycoproteins can be broadly divided into two groups.

**Mucous glycoproteins**

a. Mostly O-linked oligosaccharides (Ser/Thr-GalNAc).
b. Higher molecular weight.
c. Greater than 40% carbohydrate
d. Negligible amount of mannose
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**Serous glycoproteins**

a. N-linked oligosaccharides (Asn-GlcNAc)
b. Lower molecular weight
c. Less than 50% carbohydrates
d. Significant amount of mannose

For many years glycoproteins have been a subject of interest. However, it is in the second half of this century that they have aroused the interest of biochemists and biologists from a wide range of fields. This increased interest is partly due to the fact that glycoproteins were discovered to be abundant in living organisms. It is also due to the diverse functions of glycoproteins; glycoproteins appear in nearly every biological process studied.

Many glycoproteins have structural functions. One of many instances is their role as a constituent of the cell wall. Glycoproteins also form connective tissues such as collagen. They are also found in gastrointestinal mucus secretions. Glycoproteins are used as protective agents and lubricants. They are also found abundantly in the blood plasma where they serve many functions.

The diverse function of glycoproteins is a direct result of their structure. These macromolecules are composed of a peptide chain with one or more carbohydrate moieties. There are two broad categories of glycoprotein structure. The carbohydrates are either linked N-
glycosidically or O-glycosidically to their constituent protein. Within these broader categories, there can be fine structural differences which account for the large diversity of functions among glycoproteins. Controlling of glycoproteins is achieved through synthesis and degradation. Those processes are controlled by very specific enzymes. Not so much is yet researched on glycoprotein regulation in general.

**Structure**

Structurally, glycoproteins consist of a polypeptide covalently bonded to a carbohydrate moiety. The carbohydrate can make up anywhere from less than one percent to more than 80 percent of the total protein mass. The saccharide chains, referred to as glycans, can be linked to the polypeptide in two major ways. The first classes of glycoproteins are the O-linked glycans. These usually contain an N-acetylgalactosamine which is attached through a glycosidic bond to the O-terminus of either threonine or serine. The other classes of glycoproteins are the N-linked glycans. These involve a glycosidic bond between N-acetylglucosamine and the N-terminus of an asparagine residue (Mathews, *et al.*, 1990 and Schulz, *et al.*, 1979).

O-linked glycans consist of N-acetylgalactosamine attached to the O-terminus of a threonine or serine residue. N-acetylgalactosamine is simply a galactose molecule with an amine group covalently bonded to the second carbon. This amine group is bonded to a carboxyl group. N-acetylgalactosamine attaches to the carboxyl group
of the amino acid through the hydroxyl group of its anomeric carbon. Another type of O-linked glycan consists of a galactose or a glucosyl-galactose disaccharide linked to the hydroxyl of hydroxylysine. Yet another type of O-linkage involves the binding of arabinose to the hydroxyl of hydroxyproline. In all of the O-linked glycans, there can be a variety of different monosaccharide or polysaccharide chains attached to the sugar that is bonded to the amino acid (Mathews, et al., 1990 and Lennarz, 1980).

The other classes of glycoproteins are N-linked glycans. These molecules consist of an N-acetylglucosamine bonded to the amide nitrogen of an asparagine molecule. N-acetylglucosamine is simply a glucose molecule which is bonded to an amine group. This amine group, in turn, is bonded to a hydroxyl group. The N-acetylglucosamine is bonded to the asparagine through its anomeric carbon. The asparagine must be surrounded by a specific amino acid sequence, or sequon. This sequence is -X-Asn-X-Thr; the X can be any amino acid. A large variety of polysaccharide side chains can be linked to the N-acetylglucosamine. A typical polysaccharide chain is Man2 a(1-6)-Man B(1-4)-GlcNAc B(1-4)-GlcNAc B(1-N) Asn. Adding on to this structure can create many different N-linked glycans (Mathews, et al., 1990 and Lennarz, 1980).

The carbohydrate chains of glycoproteins can play a role in the structure of the polypeptide. For example, in human immunoglobulins, the carbohydrate chain wraps around one of the protein domains.
By doing so it prevents contact of this domain with the neighboring domain. The carbohydrate side chains of a rabbit antibody were removed through glycosidase digestion. The result was that the domain where the carbohydrate had previously been attached could no longer perform its ordinary function. Because an immunoglobulin's function is determined to a large extent by its structure, it can be concluded that removing the carbohydrate affected the structure of the molecule (Lennarz, 1980).

Because carbohydrates and proteins by themselves serve in a vast number of biological functions, it should not be surprising that linking the two together results in a macromolecule with an extremely large number of functions. Because of this and their biologically ubiquitous nature, the best way to go about exploring glycoprotein function is to break it down into categories that are fairly general. According to the function glycoproteins are divided as follows:

**Structural:** Glycoproteins are found throughout matrices. They act as receptors on cell surfaces that bring other cells and proteins (collagen) together giving strength and support to a matrix (Ivatt, 1985).

**Proteoglycan-linking glycoproteins** cross links proteoglycan molecules and are involved in the formation of the ordered structure within cartilage tissue. In nerve tissue glycoproteins are abundant in gray matter and
appear to be associated with synaptosomes, axons, and microsomes. Prothrombin, thrombin, and fibrinogen are all glycoproteins that play an intricate role in the blood clotting mechanism (Gottschalk, 1972).

**Protection:** High molecular weight polymers called mucins are found on internal epithelial surfaces. They form a highly viscous gel that protects epithelium from chemical, physical, and microbial disturbances. Human lacrimal glands produce a glycoprotein which protects the corneal epithelium from desiccation and foreign particles. Human sweat glands secrete glycoproteins which protect the skin from the other excretory products that could harm the skin (Gottschalk, 1972).

**Reproduction:** Glycoproteins found on the surface of spermatozoa appear to increase a sperm cell's attraction for the egg by altering the electrophoretic mobility of the plasma membrane. Actual binding of the sperm cell to the egg is mediated by -linked glycoproteins serving as receptors on the surface of each the two membranes. The zona pellucida is an envelope made of glycoprotein that surrounds the egg and prevents polyspermy from occurring after the first sperm cell has penetrated the egg's plasma membrane (Ivatt, 1985).

**Adhesion:** Glycoproteins serve adhere cells to cells and cells to substratum. Cell-cell adhesion is the basis for the development of functional tissues in the body. The interactions between cells are mediated by the glycoproteins on those cell's surfaces.
In different domains of the body, different glycoproteins act to unite cells. N-CAM is also found on muscle cells indicating a role in the formation of myoneural junctions. With cell-substratum adhesion, glycoproteins serve as cell surface receptors for certain adhesion ligands that mediate and coordinate the interaction of cells. Substrates with the appropriate receptor will bind to the cell related to that receptor. For example, a substrate containing the glycoprotein fibronectin will be recognized and adhered to by fibroblasts. The fibroblasts will then secrete adhesion molecules and continue to spread, producing a pericellular matrix (Ivatt, 1985).

**Hormones:** There are many glycoproteins that function as hormones such as human chorionic gonadotropin (HCG) which is present in human pregnancy urine. Another example is erythropoietin which regulates erythrocyte production (Gottschalk, 1972).

**Enzymes:** Glycoprotein enzymes are of three types. These are oxidoreductases, transferases, and hydrolases (Gottschalk, 1972).

**Carriers:** Glycoproteins can bind to certain molecules and serve as vehicles of transport. They can bind to vitamins, hormones, cations, and other substances.
Inhibitors: Many glycoproteins in blood plasma have shown antiproteolytic activity. For example, the glycoprotein α1-anti-chymotrypsin inhibits chymotrypsin.

Freezing-point depression: Glycoproteins were found in the sera of antarctic fishes to decrease the freezing point due to their apparent interaction with water (Gottschalk, 1972).

Vision: In bovine visual pigment a glycoprotein forms the outer membranes of retinal rods (Gottschalk, 1972).

Immunological: The interaction of blood group substances with antibodies is determined by the glycoproteins on erythrocytes.

A. Mucins

The major components of mucous secretion of salivary glands are mucous glycoproteins i.e. (mucins). Salivary mucins are important contributors to the lubricative and rheological properties of whole saliva and thus play a critical role in physiological processes such as mastication, swallowing and speech (Cohen and Levine, 1989). Mucins are constituents of the biofilm covering both hard and soft tissues in the oral cavity providing protection from mechanical or chemical injury and from microbial infection (Mande, 1987; Scannapieco and Levine, 1993; Tabak, 1995).
Mucins are secreted predominantly by a pair of sublingual glands. The larger submandibular gland is a “mixed” gland containing both serous and mucous acini whereas the smaller sublingual gland is referred to as a “mucous” gland comprised almost entirely of mucous acini (Ten Cate, 1994).

Both the glands originate from budding of buccal epithelium in early development and are functionally classified as exocrine glands. Mucins are also produced to a much lesser extent by minor salivary glands (Labial, buccal and palatine) distributed throughout the submucosa in the oral cavity. Two distinct mucins, named mucous glycoprotein 1 (MG1) and mucous glycoprotein 2 (MG2), have been isolated and partially characterized with respect to their biochemical and functional properties (Levine, et al., 1987; Loomis, et al., 1987; Cohen and Levine, 1989; Scannapieco and Levine, 1993).

MG1 has a higher number of O-linked carbohydrate units than does MG2, and these range in size from 4-16 residues, as compared with oligosaccharides of from 2-7 residues in MG2. Interestingly, MG2 also contains a small number of N-linked units. The O-linked carbohydrate units of MG2 have been structurally defined (Reddy et al., 1985). The protein moiety of MG1 is comprised of several disulfide-linked subunits, while MG2 contains a single peptide chain. The amino acid compositions of the two mucins differ markedly (Loomis et al., 1987b). Of the total amino acids in MG2, THR+SER+PRO+ALA=75% and GLY=1.4%. In MG1, THR+SER+PRO+ALA constitutes 43% of the total, while GLY=8.6%. Biophysical studies also point out differences between these two mucins. MG1 binds the hydrophobic fluorescent probes, 1-anilino-8-napthalenesulfonate (ANS) and N-phenyl-1-naphthylamine, while MG2 does not (Loomis, et al., 1987b).

B. Sialic acid

Sialic acid is a generic term for the N- or O- substituted derivatives of neuraminic acids, a monosaccharide with a nine carbon backbone (Varki, et al., 2008). It is also the name for the most common member of this group, N-acetylneuraminic acid (Neu5Ac or NANA). Sialic acids are found widely distributed in animal tissues and to a lesser extent in other species ranging from plants and fungi to yeast and bacteria, mostly in glycoproteins and gangliosides.
The typical modification of hydroxyl groups of sialic acids is the addition of O-acetyl esters. Early studies on some glycoproteins containing O-glycans such as mucin indicated species specificity in the distribution of sialic acids. For example, 4-O-acylated sialic acid analogues are abundant in equine tissues (Manzi, et al., 1990), 9-O-acetylated analogues in bovine and murine tissues (Haverkamp, et al., 1977), and N-glycolyl analogues in porcine tissues (Varki, 1992).

Modifications of sialic acids also show remarkable tissue-specific and developmentally regulated expression on a variety of systems. The importance of the O-acylated molecules in the generation of the host immune response to melanoma gangliosides was explored (Ravindranath and Morton, 1989). The presence of a novel cyclic sialic acid has also been reported and correlated with selectin ligand activity (Mistuoka, et al., 1999).

The wide occurrence of sialic acids in mammalian tissues has been related to a range of different biological functions (Montreuil, 1980; Reutter, et al., 1982; Schauer, 1982; Schulte and Spicer, 1985; Schulte, et al., 1985). The different sialic acids are ubiquitous components of tissues and biological fluids, where they occur either as monomers or linked to glycomolecules, that is, glycolipids and glycoproteins. The latter can occur as structural components of cell plasma membranes and/or secretory products. In addition, sialic acids are basal constituents of serum glycoproteins as well as gangliosides, which are exclusively located on the cell membrane (Horowitz and pigman 1977, Corfield and Schauer, 1982).
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The most commonly occurring sialic acids are the two N-substituted forms: N-acetylneuraminic acid and N-glycolylneuraminic acid. They can also be O-substituted at the 4, 7, 8 and 9 positions, giving rise to a wide variety of physiological and pathological events (Ledeen and Yu, 1976, Schauer, 1982, 1987; Devine, et al., 1991, Mancini, et al., 1991; Werner, et al., 1991; Dall'Olio, et al., 1992).

Sialic acids are important components of salivary gland mucins (Blix, 1936; Klenk and Lauenstein, 1954). By means of histochemical (Spicer and Warren, 1960; Quintarelli, et al., 1961; Junqueira and Fava De Moraes, 1965) and chemical methods (Spicer and Warren, 1960; Junqueira, et al., 1967) their presence has been detected in most mammalian salivary glands. In rats and mice, a great sialic acid concentration has been shown in sublingual glands, a moderate concentration in the submandibular glands, and a low concentration in the parotid glands (Spicer and Warren, 1960; Fava De Moraes and Nicolau, 1965; Curbelo, et al., 1968).

Functions of sialic acid

The structural diversity of sialic acid is reflected in the variety of its biological functions (Schauer, 1985; Schauer, 1992; Schauer, et al., 1995; Kelm and Schauer, 1997). Due to its size and the hydrophilic character, along with its negative charge, this sugar
can have a simple physicochemical effect on its environment. Its main function, however, is that of specific phenomena related to cellular and molecular recognition.

Due to their negative charge, sialic acids are involved in binding and transport of positively charged molecules (e.g. Ca\(^2+\)) as well as in attraction and repulsion phenomena between cells and molecules. Their exposed terminal position in carbohydrate chains, in addition to their size and negative charge enable them to function as a protective shield for the sub terminal part of the molecule (preventing e.g. glycoproteins from being degraded by proteases) or cell (as is the case for the mucous layer of the respiratory epithelium).

In infectious processes, the colonization of bacteria can be limited by the sialic acid coat covering the host cell surface.

Another interesting phenomenon is the spreading effect that is exerted on sialic acid-containing molecules due to the repulsive forces acting between their negative charges (Müller, 1974). This stabilizes the correct conformation of enzyme or cell membrane (glyco) proteins, for example, and is important for the slimy character and the resulting gliding and protective function of mucous substances, such as on the surface of the eye or on mucous epithelia (Schauer, 1992).
Sialic acids take part in a variety of recognition processes between cells and molecules. Thus, the immune system can distinguish between self and non-self structures according to their sialic acid pattern. The sugar represents an antigenic determinant, for example of blood group substances, and is a necessary component of receptors for many endogenous substances such as hormones and cytokines.

In addition, many pathogenic agents such as toxins (e.g. cholera toxin), viruses (e.g. influenza), bacteria (e.g. *Escherichia coli*, *Helicobacter pylori*) and protozoa (e.g. *Trypanosoma cruzi*) also bind to host cells via sialic acid-containing receptors (Schauer and Kamerling, 1997).

Another important group of sialic acid-recognizing molecules belongs to the so-called lectins. They are usually oligomeric glycoproteins from plants, animals and invertebrates that bind to specific sugar residues.

**C. Fucose**

Fucose is a hexose deoxy sugar with the chemical formula $C_6H_{12}O_5$. It is found to on N-linked glycans on the mammalian, insect and plant cell surface, and is the fundamental sub-unit of the fucoidan polysaccharide. Alpha1-3 linked core fucose is a suspected carbohydrate antigen for IgE-mediated allergy (Daniel and John, 2003). Two structural features distinguish fucose from other six carbon sugars present in mammals:
the lack of hydroxyl group on the carbon at 6th position (C-6) and the L-configuration. It is equivalent to 6-deoxy-L-galactose. In the fucose-containing glycan structures, fucosylated glycans, fucose can exist as a terminal modification or serve as an attachment point for adding other sugars (Daniel and Robert, 1999). In human N-linked glycans, fucose is most commonly linked α-1, 6 to the reducing terminal β-N-acetylglucosamine. However, fucose at the non-reducing termini linked α-1, 2 to galactose forms the H antigen, the substructure of the A and B blood group antigens. Fucose is metabolized by an enzyme called alpha-fucosidase.

L-Fucose (6-deoxy-L-galactose) is a constituent of many glycoproteins and glycolipids synthesized by microorganisms, plants, and animals (Percival, 1962; Buddecke, 1972; McKibbin, et al., 1977). Radioactive L-fucose (Bennett and Leblond, 1970; Bennett and Leblond, 1971; Bennett, et al., 1974) and GDP-L-fucose (Jabbal and Schachter, 1971; Munro and Schachter, 1973; Schachter, 1974; Watkins, 1974) have been used in a variety of systems to study the biosynthesis of fucose-containing macromolecules. Radioactive L-fucose is considered to be an excellent precursor for such studies because it is not converted to other sugars (Shull and Miller, 1960; Coffey, et al., 1964; Sturgess, et al., 1973). Parenterally administered free L-fucose can, however, be extensively oxidized to carbon dioxide by the human body (Segal and Topper, 1960) but not by the rat (Coffey, et al., 1964) and probably not by the mouse (Shull and Miller, 1960). A pathway for L-fucose degradation has been described in microorganisms (Green and Cohen, 1956; Ghalambor and Heath, 1962; Heath and Ghalambor, 1962; Hacking and
Lin, 1977) involving the conversion of L-fucose to L-fuculose-1-phosphate followed by an aldolase-catalyzed cleavage to L-lactaldehyde and dihydroxyacetone phosphate; the latter two compounds can enter the central oxidative pathways of the cell.

**Functions of fucose**

- Fucosylated blood group antigens play an important role in host-microbe interactions.

- One of the best-studied functions of fucose is its role as an essential component of the carbohydrate ligands for the selectin family of cell adhesion receptors (Kansas, 1996; Vestweber and Blanks, 1999).

- There is evidence for involvement of fucosylated glycans in ontogenic events. The Lewis epitope, a (1, 3)-fucosylated glycan also known as the stage-specific embryonic antigen-1 and CD15, is expressed during early embryogenesis (Solter and Knowles, 1978).

- In contrast to its role as a terminal modification of oligosaccharides, fucose may also be found in direct linkage to hydroxyl groups of serine and threonine residues. This glycosylation event, known as O-fucosylation, is carried out by one or more Golgi-resident O-fucosyltransferases (Wang and Spellman, 1998; Wang,* et al.*, 2001).
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that act on specific consensus sequences in epidermal growth factor (EGF)-like modules of cell surface and secreted proteins, including several blood coagulation factors (Harris and Spellman, 1993; Moloney and Haltiwanger, 1999).

✓ Fucosylated glycans have been implicated in the pathogenesis of several human diseases. Two prominent examples of altered glycosylation in cancer involve fucose-containing oligosaccharides. First, expression of A and B blood group antigens is lost in many tumors with concomitant increases in H and Lewis expression, changes that correlate with poor clinical prognosis (Lee, et al., 1991; Miyake, et al., 1992; Kim and Varki, 1997; Orntoft and Vestergaard, 1999). Second, up-regulation of sialyl Lewis and sialyl Lewis has been demonstrated in numerous cancers, and these increases are also associated with advanced tumor grade and poor prognosis.

All acidic mucins whether carboxylalid or sulfated ionize at pH 2.5 to produce anionic groups (COO, SO$_3^-$). Thus the standard alcian blue-pH2.5 stains all acidic mucins. In contrast, mucins that contain carboxylated carbohydrates will stain strongly with AB- 2.5 but not with AB-1. The more acidic sulphonic groups are capable of ionization at pH-1 and stains with AB-1. The carboxyl groups do not ionize at this lower pH as a result the mucins will display neutral characteristics thus alcian blue does not stain neutral mucins.
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The PAS technique is perhaps the most versatile and widely used of the techniques for the demonstration of glycoproteins, carbohydrates and mucins. Unlike the other techniques described thus far, the PAS technique also recognizes neutral mucins. The reactivity of the PAS technique is not based upon the presence of acidic groups among the polysaccharides but instead upon the structure of the monosaccharide units.

The PAS technique is based upon the reactivity of free aldehyde groups within the monosaccharide units with the Schiff reagent to form a bright red magenta end product. The initial reaction in the PAS technique is the oxidation of hydroxyl (OH) groups attached to adjacent carbon atoms. These groups are referred to as 1, 2 glycols. Oxidation of these glycols results in the formation of Schiff reactive aldehyde groups. In most protocols, a 0.5% to 1.0% solution of periodic acid is used as the oxidant. The intensity of the color that develops following treatment with the Schiff re-agent is proportional to the concentration of reactive 1-2 glycol groups within the tissue. The PAS technique is particularly sensitive for the detection of neutral mucins as well as acid mucins that contain significant quantities of sialic acid. In addition to mucins, the PAS technique is also widely used for the detection of glycogen and various glycoproteins. The PAS technique is particularly valuable for the visualization of basement membranes due to the presence of Schiff reactive glycoproteins within these structures.
2. Material and Methods

A. Material

Swiss male albino mice (*Mus musculus* Linn) were used for the present investigation. The breeding pairs of mice were obtained from Hindustan Antibiotics Ltd. Pune. The animal ethics committee approved the protocol of animal experiments. The mice were allowed to bred and reared in the air-conditioned animal house of the department. They were fed with Amrut Rat/Mice feed, which was obtained from Pranav Agro Industries Ltd. Sangli and water was given *ad Libitum*.

To assess the structure and function of sublingual glands in the absence of submandibular glands in male mice, the effect of sialoadenectomy on glycoprotein content in male mice was examined. For this the animals were operated at the age of 20 days. They were grouped into following two groups;

1. Control Group
2. Sialoadenectomised Group

The operated mice were maintained in animal house with proper care up to the age of 3 months and thereafter were sacrificed, sublingual glands was removed and used for further procedure.
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B. Methods

a. Estimation of Sialic Acid (Warren 1959):

For estimation of sialic acid sublingual glands were homogenized in 0.1N H$_2$SO$_4$ (1mg/ml) and heated at 80°C for one hour to release bound sialic acids. Aliquots were assayed in triplicates by thiobarbituric acid methods (Warren, 1959). The optical density was determined at 549 nm against blank.

b. Estimation of Fucose (Dische and Shettles 1948):

For estimation of fucose sublingual glands were weighed and homogenized in distilled water (2 mg/ml distilled water). The homogenate was centrifuged at 5000 rpm for 10 minutes at 10°C. The absorbance was determined at 400 nm and 427 nm adjusting colorimeter to zero with blank.

c. Histochemical Demonstration of Glycoproteins:

Sublingual glands were fixed in 2% calcium acetate formaldehyde for 24 hours. The glands were washed in running tap water for 24 hours, dehydrated through alcoholic grades, cleared in xylene and embedded in paraffin. The sections of 7µ thickness subjected to the following staining techniques for histochemical localization of glycoproteins and were counter stained with hematoxyline.
i. Alcian blue (AB) at pH 1.0 (Mowry, 1956)

ii. Alcian blue (AB) at pH 2.5 (Mowry, 1956)

iii. Periodic Acid–Schiff Reaction (PAS): (Mc Manus, 1964; Hotchkiss, 1948)

iv. PAS-Sodium borohydride technique for O-acetylated and non acetylated sialic acid (Culling, et al, 1976)

3. Results

a. Sialic acid content in sublingual gland

The sialic acid content was estimated by using N-acetylneuraminic acid as standard. It was expressed as µg sialic acid / mg protein. The sialic acid content in sublingual gland of control mice was $0.00141 \pm 0.00008$ and in sialoadenectomised mice it was increased to $0.00297 \pm 0.00007$. The increase was highly significant ($P<0.0001$) (Table 6).

b. Fucose content in sublingual gland

Fucose content in the sublingual gland of control and sialoadenectomised mice is given in table 7. The fucose content is expressed in terms of µg fucose/ mg protein. It was observed that there was significant increase in fucose content of sublingual gland after sialoadenectomy. The fucose content in control mice sublingual gland was $0.046 \pm 0.000045$ and in sialoadenectomised mice it was increased to $0.09078 \pm 0.000022$ and the increase was highly significant ($P<0.0001$).
c. Changes in sublingual gland glycoproteins histochemically.

Histochemical localization of glycoproteins in the sublingual gland of mice from both the groups i.e. control and sialoadenectomised were studied by the AB-pH 1 (Plate X and XI- fig. 1, 2), AB-pH 2.5 (Plate XII and XIII- fig. 1, 2), PAS (Plate XIV and XV- fig. 1, 2) and Thionin Schiff (Plate XVI and XVII- fig. 1, 2) techniques.

Plate X and XI (Fig. 1) showed histochemical demonstration of glycoproteins from sublingual gland of control mice. The sublingual gland showed normal structure containing mucous acinar (MA) cells stained positively with AB-pH 1. Mucous acinar showed homogenous blue colouration with some secretions found in the lumen. Mostly the sulphated glycoproteins are stained with AB-pH 1. The serous demilunes (SD) were AB-pH 1 negative with narrow lining of AB.

The sublingual gland of sialoadenectomised mice showed similar structure as that of control. The acinar cells appeared to be smaller but were intensely stained with dark blue colour and intensity of AB-pH 1 reactivity was increased considerably. The interlobular (IED) and intralobular (IAD) ducts were increased in size but showed absolutely zero reactivity with AB-pH 1. (Plate X and XI Fig. 2).
Plate XII and XIII (Fig. 1) show glycoprotein staining of alcian blue, which at pH 2.5 selectively stains the acidic mucosubstances of mucus secreting cells of sublingual gland from control mice. The sublingual gland of control mice showed dark blue coloured homogenously stained mucous acinar cells similarly like AB- pH 1 staining. The SD, IED and IAD also showed AB-pH 2.5 negative reactivity but a narrow granular lining of AB +ve material as like in AB-pH 1 staining.

The MA of sublingual gland from sialoadenectomised mice was reduced in size but there intensity of AB reactivity was increased considerably. Some acinar cells deposited the blue colour material darker than that of control group. The duct size was increased but showed AB- pH 2.5 negative reactivity (Plate XII and XIII Fig. 2).

Plate XIV and XV (Fig. 1) showed histochemical demonstration of glycoproteins by PAS staining from sublingual gland of control mice. The sublingual gland contained large number of magenta coloured well formed secretory mucous acini showing PAS positive secretory material. The SD, IED and IAD could not show any reaction to PAS staining.

The mucous acini from sublingual gland sialoadenectomised mice had increased in number and almost all cells were positive for PAS and darkly stained with magenta colour and considerably increased in the PAS reactivity but cells were reduced in size.
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The IED and IAD had extensively increased tubular walls and increased in size were having PAS negative reactivity (Plate XIV and XV Fig. 2).

PAS-sodium borohydride method is used to demonstrate the O-acylated sialomucins. Plate no. XVI and XVII Fig. 1 shows the O-acylated sialomucins stain light pink colour in secretary acinar membrane of sublingual gland of control group.

Plate no. XVI and XVII Fig. 2. demonstrates O-acylated staining reactivity in the sublingual gland of sialoadenectomised mice. The sialoadenectomised mice sublingual gland showed remarkable increase in staining for O-acylated sialic acids, dark magenta colour found in acinar membrane of the cell.
4. Discussion

The salivary gland contains mainly glycoproteins complexes including sulphated hexoses, fucoses and sialic acids. They may be neutral or acidic depending upon the number of positive and negative changes in the molecular entity, may be due to the presence of sulphated sialic acids or uronic acid moieties in it (Spicer, *et al.*, 1965).

The glandular secretion of oral cavity are highly specialized fluids that provide lubrication, prevent mechanical damage, protect efficiently against viral and bacterial infections and promote the clearance of external pollutants (Warner, *et al.*, 1982). Glycoproteins of the mucous secretion of the salivary glands are high molecular weight substances responsible for viscous nature of the produced mucous. The physical properities and biological functions of these substances are determined by their high carbohydrate content (Carlsson, 1968; Baig and Aminoff, 1972; Pigman and Moschera, 1973; Carlsson, 1977).

Physiologically stressful conditions like diseases, surgery, radiotherapy and pharmacotherapy might be strain reserve capacity, hindering salivary gland ability to compensate for increased metabolic demands and results ultimately into functional compromise (Everse, *et al.*, 1994).
In the present investigation, mice were sialoadenectomised. The sialoadenectomy brings about increase in sublingual gland size and number of mucous acinar cells from sublingual gland. The sialic acid and fucose content were increased significantly from sublingual gland of sialoadenectomised mice as compared to control.

The sialic acids in the mucous acinar cells have been shown to major constituent of secreted mucous glycoproteins (Tabak, et al., 1982). Sialic acid comprises N or O acid derivatives of carbon sugar neuraminic acid. The sialic acids are present in tissues like erythrocytes, platelets, salivary glands, throat, stomach, colon, cartilage (Sillanaukee, et al., 1999). The negative charge of sialic acid constitutes to cell-to-cell repulsion (anti-adhesion effect), functional stability, and survival of glycoproteins in blood circulation and cell-to-cell matrix interaction. Due to shedding effect, sialylated glycan protect part of glycoprotein from proteolytic attacks. Gokmen, (2000) have suggested that either shedding or secreting of sialic acid from the cell or cell membrane surface may be responsible for an increased sialic acid concentration. Increased sialylation of serum proteins may increase sialic acids (Flynn, et al., 1999) or reduction in desialylation of plasma glycoproteins increases sialic acid (Morell, et al., 1971). As a result of cell-to-cell repulsion and secretion of sialic acids from cells may have increased sialic acid content in sialoadenectomised mice sublingual glands.
Fucose is one of the eight essential sugars the body requires for optimal function of cell to cell communication. In any of the saccharides, the L form is only one recognized by the body, while the D form is a synthetic analogue. When taken orally fucose readily absorbed from the small intestine and incorporated either directly or after metabolism into glycoproteins. Unabsorbed fucose is metabolized by friendly intestinal bacteria.

In human, fucose is excreted mainly in the urine at a rate of approximately 17mg/min. Nursing mothers also eliminate fucose from the breast milk. During the later stage of pregnancy, excretion of fucose in the urine is markedly increased, which is consistent with fetal development and the transfer of immunity to the newborn.

L-fucose occurs in mammalian tissues as component of oligosaccharides linked to O-glycosidically to protein (e.g. mucin of human blood group glycoproteins), of oligosaccharide linked N-glycosidically to protein (e.g. immunoglobulins), of free oligosaccharides found in urine and milk and finally of glycosphingolipids (Percival, 1962; Buddechke, 1972; Spiro, 1973; Hakomori, et al., 1977; Marcus and Schwating, 1976; Gardas andkoscielak, 1974; Slomiany, 1977; Mckibbin, et al., 1977). L- fucose occurs in mammalian glycoconjugates linked α 1,2 to D-galactose, α 1,3 to D-glucose and N- acetyl-D-glucosamine, α 1,4 to D- glucose and N acetyl- D-glucosamine, and α-1,6 to N-acetyl- D- glucosamine. The sugar is somewhat unique among mammalian monosaccharide since it is the only major deoxy-sugar found in glycoconjugates and occurs as the L-isomer in contrast to
the other monosacharides which all occur as the D-isomer. Also, like sialic acid, L-fucose usually occurs in a terminal non-reducing portion in mammalian glycoconjugates.

L-fucose can be hydrolyzed from glycoconjugates by α-L-fucosidase which are widely distributed in mammalian tissues (Leavy and McAllan, 1961; Troost, et al., 1976, Chester, et al., 1977, Dawson and Tsay, 1977; Brungraber, 1978). Free L-fucose can thus occur in the mammalian organisms either from the diet or from the action of α-L-fucosidase on glycoconjugates. The rate of free L-fucose has been studied in whole animals (rats, mice, rabbits and humans) and in controlled cell lines such as HeLa cells. Starved mice given injection of L-fucose did not produce an increased level of liver glycogen, indicating that the entry of L-fucose into the glycolysis pathway was relatively slow (Shull and Miller, 1960).

Fucose is a powerful immune modulator. It is distributed in macrophages, which are critically important to immune function. There have been numerous well-documented benefits for its immune function, especially that of an overactive immune system, the cause of autoimmune disorders. Fucose is showing promise in its ability to normalize immune function. Fucose is particularly active in inflammatory diseases and has the ability to suppress such allergic skin reactions as contact dermatitis. The saccharides guards against respiratory tract infections and inhibits allergic reactions.
Chapter V: Effect of sialoadenectomy on glycoprotein

It is now known that fucose glycoconjugates (glycoproteins and glycolipids) are an essential part of eliminating or reversing such disease processes as cancer, inflammation, and immunity. Thus increase in fucose content reveals its role in reversing the process like inflammation, immunity etc.

Histochemical localization of glycoproteins in the sublingual gland of mice was studied by AB pH-1, AB pH-2.5, PAS and PAS –Sodium borohydride for the presence of sulphated mucins, acidic mucins, neutral mucins and O-acylated sialomucins respectively. In sialoadenectomised mice, there was increase in staining intensity of T.S. of sublingual gland of AB pH-1, AB pH-2.5 and PAS was observed. Alcian blue at pH-2.5 stains acidic glycoproteins. Acidic glycoproteins also include carboxymucins and sulphated glycoproteins. The sulphated glycoproteins specially show affinity towards the alcian blue pH-1. Presence of neutral glycoproteins can be revealed histochemically by staining the section with PAS technique. PAS staining reactivity indicates the presence of glycogen and neutral mucosubstances. There was increase in O-acylated sialomucins, which was demonstrated histochemically by PAS-Sodium borohydride. The increase in O-acylated sialomucins confirms the biochemical results of increase in sialic acid content. The ducts of sublingual glands stained poorly by all above techniques, showing absence of glycoproteins in various ducts.
Spicer and Warren (1960) showed presence of acid mucopolysaccharides which have been characterized as sialo and sulphomucins in mucous acini. Curran and Kennedy (1955), Jennings and Florey (1956), described that sublingual gland is rich in sulphated glycoproteins.

The mucins and the properties that they import to saliva appear to be crucial to the presence of moisture retentive barrier of high film strength at the interface of soft tissues and the outer environment. This barrier is fundamental to protection of the sensitive of the oral mucosa as it prevents desiccation, can reduce permeability to potential toxins and lubricates thus preventing physical damage (Proctor and Carpenter, 1998). The moistening and lubricating properities of glycoproteins are believed to minimize the sensation of mucosal dryness. Thus, increase in glycoproteins histochemically proves increase in secretion of sublingual gland indicating that in the absence of submandibular gland sublingual gland tries to compensate submandibular glands function.