1. Introduction

Saliva, an exocrine secretion of the salivary glands, consists of water, electrolytes, enzymes, immunoglobulin, mucosal glycoproteins and numerous antimicrobial proteins, growth factors and regulatory peptides. These components aid in the sensation of taste, digestion of starch and lipids and are responsible in maintenance of oral health by protecting tissues with lubrication and buffering properties, contributing to the physical-chemical integrity of tooth enamel, and preventing adhesion of and colonization by microorganisms.

The importance of salivate oral health is best illustrated in those who have chronic xerostomia. They experience difficulty in eating and swallowing and even speaking and may experience a bad taste, ‘burning’ mucosa, widespread mucosal and carious lesions associated with candidal and bacterial infection (Daniels and Talal, 1987). Saliva performs a number of functions which are crucial to the maintenance of oral homeostasis. Some of these functions such as the moistening of food before swallowing or the removal of food residues and debris from mouth could in theory be fulfilled by the presence of water or any other fluid in the mouth. However, saliva has special physical and biochemical properties which results from its composition and enables it to fulfill a number of other functions. Most of these functions are dependent to a large extent upon the protein components of saliva.
The acinar cells of the salivary gland secrete the salivary fluid as well as most of the salivary proteins and the ductal cells that secrete some protein and modify the ionic composition of the saliva as they convey it to the mouth. Proteins in salivary secretions and whole saliva affect hard and soft tissues in the oral environment in a variety of beneficial ways. The physiological importance of the organic and inorganic components in saliva is illustrated dramatically in situations where saliva production is impaired as in autoimmune diseases such as Sjogren’s syndrome, systemic lupus erythematosus and scleroderma (Fox, 1997; Sreebny, 2000; Al-Hashimi, 2001). A reduction in saliva production also occurs in patients taking some prescription medications (Navazesh, et al., 1996) and in patients receiving radiation treatment for head and neck cancer (Valdez, et al., 1993). It is also well recognized that diminished salivary flow leads to difficulty in physiological processes such as mastication, swallowing and speech (Tabak, 1995) and can affect the quality of life causing problems related to retention of dentures, infection with oral bacteria and yeast, development and progression of dental caries and alterations in taste (Fox, 1997; Sreebny, 2000). Clearly if oral health is dependent upon salivary proteins then it is also dependent upon the mechanisms which control the synthesis and release of salivary proteins.

Saliva contains a wide variety of secreted proteins, including: α-amylase, an enzyme involved in digestion of starch; lysozyme, peroxidase, immunoglobulins (IgA) and many additional proteins and/or antiviral properties; and mucins, which are multifunctional glycoproteins involved in mechanical protection and prevention of dehydration of the oral epithelia,
as well as in lubrication for solid food and trapping of microorganisms (Young and Cook, 1996). As already indicated, most of the proteins in saliva are secreted by the acinar cells.

Salivary proteins exhibit vectorial transport from the rough endoplasmic reticulum, where they are synthesized, through a succession of membrane-bounded compartments including the Golgi complex, condensing vacuoles and secretory granules (Von Zastrow and Castle, 1987). The secretory granules migrate to particular locations within the cell close to the apical membrane prior to the release of their contents into the acinar lumen.

In the three major salivary glands, parotid, submandibular and sublingual, exocytotic protein secretion is primarily controlled by the autonomic nervous system; sympathetic stimulation elicits protein release from parotid and submandibular gland acini and parasympathetic stimulation elicits protein release from sublingual gland acini as well as some release from parotid acini (Quisell and Tabak, 1989; Spearman and Butcher, 1989).

Saliva contains a large number of proteins that participate in the protection of the oral tissues, for instance lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinin and mucins (Nieuw Amerongen and Veerman, 2002). In addition, several peptides with bacteria killing activity have been identified. These include histatins, defensins and the only human cathelicidin, LL-37 (table a). Because all of these proteins and peptides have a broad spectrum of antimicrobial activity there seems to be a considerable overlap in functionality. This may account for the observation that
susceptibility to oral diseases can apparently not be related to the concentration of a single component (Rudney et al., 1999).

Table a. Antimicrobial proteins in glandular saliva (Nieuw Amerongen, et al., 2004)

<table>
<thead>
<tr>
<th>Salivary glycoprotein</th>
<th>Tissue of origin</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC5B (mucin MG1)</td>
<td>All mucous salivary glands</td>
<td>5-20</td>
</tr>
<tr>
<td>MUC7 (mucin MG2)</td>
<td>All mucous salivary glands</td>
<td>5-20</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>B lymphocytes: in all salivary glands</td>
<td>5-15</td>
</tr>
<tr>
<td>Proline-rich glycoprotein</td>
<td>Parotid</td>
<td>1-10</td>
</tr>
<tr>
<td>Cystatins</td>
<td>Submandibular&gt;sublingual</td>
<td>10</td>
</tr>
<tr>
<td>Histatins</td>
<td>Parotid and submandibular</td>
<td>5</td>
</tr>
<tr>
<td>EP-GP (= GCDFP15, SABP, PIP)</td>
<td>Submandibular, sublingual</td>
<td>1-2</td>
</tr>
<tr>
<td>Agglutinin (= DMBT1, gp340)</td>
<td>Parotid&gt;submandibular&gt;sublingual</td>
<td>1-2</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Sublingual&gt;submandibular, parotid</td>
<td>1-2</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>All salivary glands: mucous&gt;serous</td>
<td>1-2</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Parotid&gt; submandibular</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cathelicidin (hCAP18, LL37)</td>
<td>Salivary glands, neutrophils</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Defensins</td>
<td>Salivary glands, epithelial cells, neutrophils</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
## Table b. Antimicrobial proteins in glandular saliva (Nieuw Amerongen, et al., 2004)

<table>
<thead>
<tr>
<th>Salivary proteins</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinin</td>
<td>Aggregation of bacteria</td>
</tr>
<tr>
<td>Cathelicidin (LL37)</td>
<td>Broad-spectrum killing bacteria</td>
</tr>
<tr>
<td>Cystatins/ VEGh</td>
<td>Protease inhibitor</td>
</tr>
<tr>
<td>Defensins</td>
<td>Broad-spectrum killing bacteria</td>
</tr>
<tr>
<td>EP-GP</td>
<td>unknown</td>
</tr>
<tr>
<td>Histatins</td>
<td>Broad-spectrum killing bacteria</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Inactivation and aggregation of bacteria</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Growth inhibition</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Growth inhibition</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Killing</td>
</tr>
<tr>
<td>MUC5B (mucin MG1)</td>
<td>Proton-diffusion barrier in pellicle</td>
</tr>
<tr>
<td>MUC7 (mucin MG2)</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Proline-rich glycoprotein</td>
<td>Unknown: aggregation?</td>
</tr>
<tr>
<td>Proline-rich proteins (aPRPs)</td>
<td>Adherence</td>
</tr>
<tr>
<td>Proline-rich proteins (bPRPs)</td>
<td>Unknown: membrane disturbing?</td>
</tr>
<tr>
<td>Satherin</td>
<td>Adherence</td>
</tr>
</tbody>
</table>
Chapter IV: Effect of sialoadenectomy on amylase and protein

Mucins constitute another important class of salivary glycoproteins. In unstimulated whole saliva they are the major components, making up 20–30% of the total protein. Two types of genetically different salivary mucins can be distinguished (Levine, et al., 1987; Loomis, et al., 1987): MG1, high-molecular-weight mucin (Mr 10–30 MDa), encoded by the MUC5B gene, now designated MUC5B (Thornton, et al., 1999), and the low-molecular weight MG2 (Mr F130 kDa), the translation product of the MUC7 gene, now designated MUC7 (Bobek, et al., 1993). Characteristic of mucins is the abundance of carbohydrate side chains which are covalently attached to the polypeptide backbones, forcing the molecule into an extended conformation. On a weight basis, the carbohydrates comprise 60% (for MUC7) to 80% (for MUC5B) of the molecule. The large dimensions and elongated form of MUC5B, in combination with the presence of a hydrophilic sugar coat, are responsible for the characteristic viscoelastic character of MUC5B-containing solutions (van der Reijden et al., 1993). MUC5B is synthesized exclusively in mucous acinar cells of all (sero) mucous salivary glands (Nieuw Amerongen, et al., 1995; Veerman, et al., 2003). MUC5B is a constituent of the protein layers that form on dental enamel after prolonged incubation with saliva, and is indispensable for the proton-barrier function of these so-called pellicles (Nieuw Amerongen, et al., 1987). Because of its hydrophilic properties, MUC5B-containing pellicles lubricate the dental surfaces, protecting them against mechanical wear.

<table>
<thead>
<tr>
<th>Band</th>
<th>Protein</th>
<th>Molecular weight (kDa)</th>
<th>Observed</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactoferrin</td>
<td>75</td>
<td>76.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Peroxidase</td>
<td>75</td>
<td>72-78</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Amylase</td>
<td>60</td>
<td>55-60</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Carbonic anhydrase</td>
<td>38</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Proline rich glycoprotein</td>
<td>35</td>
<td>38.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Glycosylated PRPs</td>
<td>28</td>
<td>20-30</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Glycosylated PRPs</td>
<td>20</td>
<td>10-20</td>
<td></td>
</tr>
</tbody>
</table>

The low-molecular-weight mucin MUC7 differs from MUC5B in structure, localization and function. MUC7 is a single monomeric protein, decorated with short oligosaccharide side chains, which are two or three residues long. MUC7 is synthesized in serous acinar and demilune cells of the (sero) mucous glands (Veerman, et al., 1997, 2003) and is detectable in all (sero) mucous glandular saliva (Bolscher, et al., 1999). In contrast to MUC5B, MUC7 binds a wide variety of bacterial species, including *S. mutans* (Liu, et al., 2000). Both mucins have been implicated in the protection against viruses (Bergey, et al., 1993a, b; Bolscher, et al., 2002).
Salivary alpha amylase

Salivary alpha- amylase (α-1, 4-α-D- glucan 4-glucanohydrolase; EC 3.2.1.1) is one of the most important enzymes in saliva. The enzyme was first described in saliva by Leuchs in 1831 (Zakowski and Bruns, 1985). It consists of two families of isoenzymes, of which one set is glycosylated and the other contains no carbohydrate. The molecular weight of the glycosylated form is about 57,000; that of the non-glycosylated form are about 54,000. Salivary alpha-amylase accounts for 40% to 50% of the total salivary gland produced protein, most of enzyme being synthesized in the parotid gland (80% total) (Zakowski and Bruns, 1985; Makinen, 1989). It is a calcium-containing metalloenzymes that hydrolyzes the α-1, 4 linkages of starch to glucose and maltose. It is known to be mainly involved in the initiation of the digestion of starch in the oral cavity. However, salivary alpha-amylase has also been shown to have an important bacterial interactive function (Scannapieco, et al., 1993).

Alpha-amylase is one of the major protein components of saliva. The main function of salivary amylase is the enzymatic digestion of carbohydrates, but it is also important for mucosal immunity in the oral cavity, as it inhibits the adherence and growth of bacteria (Scannapieco, et al., 1993; Bosch, et al., 2003). Among other proteins, alpha-amylase is synthesized and secreted by acinar cells, which make up more than 80% of the cells in the major salivary glands (Castle and Castle, 1998).
Chapter IV: Effect of sialoadenectomy on amylase and protein

Secretory pathways of salivary alpha amylase

Acinar cells are innervated by both the sympathetic and the parasympathetic branches of the autonomic nervous system (Emmelin, 1987). Autonomic nerves are adjacent to both acinar and ductal cells. The afferent pathways for taste are via the facial and glossopharyngeal nerves to a solitary nucleus in the medulla. There is also input from higher centers in response to smell, sight, etc. The parasympathetic efferent pathways for the sublingual and submandibular glands are from the facial nerve via the submandibular ganglion; for the parotid gland they are from the glossopharyngeal nerve via the otic ganglion. The sympathetic postganglionic pathways are from the cervical ganglion of the sympathetic chain.

Neurotransmitters are the first messengers in the communication pathway between nerves and secretion. Neurotransmitters exert their activity at the cell membrane; they communicate with intracellular second messengers that have direct control of secretory processes (Smith, 1996). Released in response to secretory stimuli, they bind to specific receptor proteins on the basolateral membrane, causing acute elevation of intracellular Ca. This results in large-scale fluid and electrolyte transport, and modest exocytosis of stored protein. Norepinephrine from sympathetic neurons binds to both alpha- and beta-adrenergic receptors on the acinar cell. Alpha-receptor activation is linked to elevation of intracellular Ca, while beta-receptor activation causes elevation of intracellular cyclic adenosine monophosphate (cAMP), which is linked to the secretion of salivary proteins that are stored in membrane-bound secretory granules (Baum, 1993; Castle and Castle, 1998).
The secretory process of salivary proteins may be divided into the three stages: (1) synthesis, (2) packaging and storage, and (3) release. Each of these stages is regulated by phosphorylation of target proteins brought about by a protein kinase such as cyclic AMP-dependent protein kinase (protein kinase A) (Smith, 1996).

**Pathways of alpha-amylase secretion**

In acinar cells, release of salivary components is under control of neuronal stimuli. Classic neurotransmitters and specific bioactive peptides serve as the main stimuli for salivary alpha-amylase secretion.

Batzri and co-workers indicated the specific involvement of the autonomic nervous system in the secretion process of salivary alpha-amylase. In studies on slices of the parotid gland in rats, they found that beta-adrenergic receptors caused secretion of salivary alpha-amylase (Batzri and Selinger, 1973; Batzri, *et al.*, 1973; Selinger, *et al.*, 1973). Anderson *et al.*, (1984) examined differential contributions of the two branches of the autonomic nervous system to salivary alpha-amylase secretion. They found that sympathetic stimulation in unconscious rats led to the secretion of parotid saliva characterized by low salivary flow rate and high total protein and amylase concentrations. In contrast, parasympathetic stimulation induced a rich flow of saliva with low protein content, with mean concentrations of salivary alpha-amylase approximately 1% of those measured in
sympathetically stimulated saliva. Asking (1985) compare sympathetic and parasympathetic stimulation of the parotid gland in the rat both separately and concomitantly. Anderson et al., (1984) showed that sympathetic stimulation caused a low flow of saliva containing amylase in very high concentration, whereas parasympathetic stimulation produced saliva with a low concentration of amylase. After combined sympathetic and parasympathetic stimulation, however, salivary alpha-amylase secretion was much higher than the sum of the two separate stimulation concentrations. Using the beta-1-antagonist pafenolol, Asking (1985) showed that the higher salivary alpha-amylase secretion due to sympathetic stimulation superimposed on parasympathetic background stimulation was elicited via beta-1-adrenoceptors. In a subsequent study (Asking and Gjorstrup, 1987), these findings were replicated in that sympathetic and parasympathetic stimulation led to equal salivary alpha-amylase concentrations and the combination of the two drastically increased salivary alpha-amylase concentrations. The authors found that salivary alpha-amylase secreted on sympathetic activation consisted of pre-formed amylase, stored in granules, that was not replaced during secretory activity under these conditions.

Ongoing sympathetic stimulation led to an attenuation of salivary alpha-amylase concentration. Asking and Proctor (1989) studied the effects of prolonged parasympathetic nerve stimulation in rat parotid glands on alpha-amylase content in saliva and glands. The concentration and total output of salivary alpha-amylase were measured throughout a stimulatory period of 120 min, as were glandular concentrations of salivary alpha-amylase.
The results suggest that amylase stores are much more rapidly replenished by synthesis during parasympathetic than during sympathetic activity, whereas sympathetic nerve excitation causes a pronounced loss of amylase-containing acinar granules. No such loss could be detected on parasympathetic stimulation, although the output of amylase was about the same as during sympathetic stimulation.

Skov Olsen et al., (1988) found that stimulation of the beta-adrenergic receptors in rats increased the concentration of salivary alpha-amylase by a factor of 30, while stimulation of the alpha-adrenergic receptors increased the concentration of salivary alpha-amylase by a factor of 10. Experiments with several substances showed that salivary alpha-amylase secretion was mainly contributed by beta-1-adrenergic mechanisms. Busch et al., (2002) investigated the differences in release of salivary alpha-amylase by the parotid and the submandibular gland in rats. They found that submandibular salivary alpha-amylase did not respond with an increase to the administration of isoproterenol, a beta-adrenergic agonist, whereas parotid salivary alpha-amylase did. This effect was inhibited by the selective beta-1-antagonist atenolol, but not by the beta-2-antagonist butoxamine (Busch, et al., 2002).

Speirs et al., (1974) provoked a sympathetic response either by immersing subjects up to the waist in cold water (4-5°C) or by administering isoprenaline and propranolol (both are beta-adrenergic blockers).
Exposure to cold water and isoprenaline raised salivary alpha-amylase concentrations in the parotid gland, whereas propranolol led to a reduction of salivary alpha-amylase concentrations. These results offered first evidence for the sympathetic control of salivary alpha-amylase secretion in humans (Speirs, *et al*., 1974). As shown in a number of the animal studies described above, stimulation of beta-adrenergic receptors modulates the synthesis and the release of salivary alpha-amylase.

Laurikainen *et al*., (1988) studied the effects of timolol maleate, a widely used beta-blocking agent, on the quantity and quality of saliva secretion controlled by beta-adrenoceptors in healthy subjects. Alpha-amylase concentrations in parotid saliva decreased significantly after drug intake, thus corroborating similar findings from rat studies. Nederfors and coworkers investigated the effects of therapeutic doses of the selective beta-1-antagonist atenolol and the non-selective beta-antagonist propranolol on stimulated glandular saliva. Atenolol, but not propranolol, resulted in a decrease of parotid amylase in the morning and in a decrease of submandibular/sublingual amylase both in the morning and at lunch time (Nederfors, *et al*., 1994). The authors were able to replicate their findings in a well-controlled study on human hypertensive subjects with the selective beta-1- antagonist metoprolol (Nederfors and Dahlof, 1996).
Thus, these studies demonstrate the importance of beta-adrenergic mechanisms of salivary alpha-amylase secretion in humans, as already shown in rats. Ehlert et al., (2006) proposed that salivary alpha-amylase increases might reflect the interaction of stress dependent sympathetic and parasympathetic stimulation via central nervous noradrenergic input.

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 protein content, amylase 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.

**B. Methods**

**a. Estimation of Amylase activity (Jayaraman, 2000):**

For estimation of amylase activity sublingual glands were weighed and homogenized in distilled water (1mg/ml distilled water). The homogenates were centrifuged at 3000 rpm for 10 minutes at 10°C. Supernatants were used for estimation of amylase activity. The optical densities were converted to µg of maltose from the standard graph. Amylase activity in terms of µg maltose per mg salivary proteins was calculated.

**b. Estimation of Protein (Lowry, *et al.*, 1951):**

Homogenization of the sublingual gland was carried out using refrigerated glass mortar and pestle. Sublingual gland was crushed at the bottom of the mortar by instantaneous freezing and gradual thawing with cold distilled water (0.1 mg/ml distilled water). The perfectly uniform homogenates were centrifuged at 10°C at 3000 rpm for 10 minutes. The supernatant was used for estimation of proteins. The Protein concentration from sample was calculated by using standard graph of protein.
Chapter IV: Effect of sialoadenectomy on amylase and protein


10 % Polyacrylamide gel electrophoresis was performed by using 1.5M Tris (Hydroxymethyl-amino ethane) buffer as separation gel buffer and 0.5M Tris stacking gel buffer, 0.025M (pH 8.3) Tris buffer as electrode buffer. The sample dye contains 1ml of supernatant + 1ml of sample buffer. After electrophoresis, gel were removed, and stained in 1% Comassie blue at 60°C for 2-3 hrs and then destained with several wash of 50% methanol + 10% acetic acid + 40% distill water. The gels were washed with 7% acetic acid and photograph of gel were made to record the observation.


10 % Polyacrylamide gel electrophoresis was performed by using 1.5M Tris (Hydroxymethyl-amino ethane) buffer as separation gel buffer and 0.5M Tris stacking gel buffer, 0.025M (pH 8.3) Tris buffer as electrode buffer. The sample dye contains 1ml of supernatant + 1ml of sample buffer. After electrophoresis, gel were removed, and stained with PAS method. The gels were washed with 7% acetic acid and photograph of gel were made to record the observation.
3. Results

a. Body Weight

Effect of sialoadenectomy on body weight in grams is depicted in table 2. The body weight of control mice was 41 ± 2.2361 and in sialoadenectomised mice it was decreased to 32 ± 2.7386 and the decrease was highly significant (P<0.0001).

b. Sublingual gland weight

The table 3 shows the sublingual gland weight in mg from both control and sialoadenectomised mice. In sialoadenectomised mice the sublingual gland weight was increased significantly. In control mice the sublingual gland weight was 18.8 ± 1.7899 and in sialoadenectomised mice it was increased to 32.4 ± 2.51 and the increase was highly significant (P< 0.0001).

c. Amylase activity from sublingual gland

Amylase activity in µg /mg protein was estimated from sublingual gland by using starch as substrate is described in table 4. The amylase activity in sublingual gland of control mice was 13.526 ± 0.0011 and in sialoadenectomised mice it was increased to 16.91 ± 0.0007 and the increase was highly significant (P<0.0001).
d. **Protein content in sublingual glands**

Protein content from sublingual gland from both control and sialoadenectomised mice is depicted in table 5. The protein concentration is expressed in terms of mg protein / mg tissue. The protein content in sublingual gland of control mice was 0.09458 ± 0.00008 and in sialoadenectomised mice it was increased to 0.1485 ± 0.00008. It was observed that there was highly significant increase (P< 0.0001) in protein content of sublingual gland after sialoadenectomy.

e. **Electrophoretic separation of protein from sublingual glands**

Plate no. VIII shows the electrophoretic separation of proteins from sublingual gland of control and sialoadenectomised mice was revealed by using SDS-polyacrylamide gel electrophoresis. Samples were applied at the cathode and the flow of migration was from cathode to anode. The gels were stained with Comassie brilliant blue-R250. The 10% SDS-PAGE was used to separate proteins from both groups. The electrogram contains three lanes. Lane 1 is of standard protein marker (Broad range) consisting of myosin (2, 05, 000 da), phosphorylase B (97, 400 da), bovine serum albumin (66, 000 da), ovalbumin (43, 000 da), carbonic anhydrase (29,000 da), soybean trypsin inhibitor (20,100 da), lysozyme (14,300da), aprotinin (6, 500 da) and insulin (3, 500 da).
Chapter IV: Effect of sialoadenectomy on amylase and protein

The lane 2 shows separation of protein from control sublingual gland and lane 3 shows proteins from sialoadenectomised sublingual gland. The lane 2 and 3 both shows major and minor proteins separated from sublingual glands of both groups.

The electrophoretic separation of protein from both group showed MG 2 having molecular weight 205 kDa, lactoferrin and peroxidase which are having molecular weight 75 kda were separated between 97 to 66 kDa. The amylase having molecular weight 58 kDa was separated between 66 to 43 kDa. The proline rich glycoproteins were separated between 43 to 29 kDa. The minor proteins like glycosylated PRPs were separated between 29 to 14 kDa. Also some major and minor proteins from sublingual gland were present between these proteins.

Thus the electrophoretic separation of proteins from sublingual gland of sialoadenectomised mice show similar banding pattern like control mice. The staining intensity was same in both groups. But in sialoadenectomised mice there was appearance of two new bands (marked by arrow in Lane 3), the one band was observed in amylase region and other was found in glycosylated PRPs region. Thus it confirms the biochemical results that concentration of amylase has been increased in the sublingual gland of sialoadenectomised mice.
Chapter IV: Effect of sialoadenectomy on amylase and protein

f. Electrophoretic separation of glycoprotein from sublingual glands

Plate no. IX shows electrophoretic separation of mucin and non-mucin glycoproteins from sublingual gland of control and sialoadenectomised mice by using SDS-PAGE. The electrogram contains three lanes. Lane 1 is of standard protein marker (Broad range) consisting of phosphorylase B (97, 400 da), bovine serum albumin (66, 000 da), ovalbumin (43, 000 da), carbonic anhydrase (29,000 da), soybean trypsin inhibitor (20,100 da) and aprotinin (6, 500 da). Lane 2 shows separation of glycoproteins from sublingual gland of control mice and lane 3 shows separation of glycoproteins from sialoadenectomised mice.

The electrophoresis of glycoprotein from sublingual gland showed similar separation of band pattern in both control and sialoadenectomised mice. The sublingual gland mucins and non-mucins were determined on the basis of their molecular weights. The mucins MG1 and MG2 having higher molecular weight appeared at top of the gel. MG1 has an apparent molecular weight of >1000 kDa and appears as a tight PAS reactive band in stacking gel. MG2 has molecular weight ranging between 170 to 190 kDa and comprises largest PAS reactive band in separating gel. Non-mucin glycoproteins from approximately 97 kDa to 43 kDa were designated as band I to VIII. The staining intensity of bands II, IV, V, VI, VII and VIII was increased in lane 3 as compared to lane 2.
4. Discussion

The effect of sialoadenectomised on the sublingual gland was examined. In that the effect of body weight, sublingual gland weight and protein content and amylase activity from sublingual gland was studied.

The sialoadenectomised mice showed decrease in body weight as compared to control. The sialoadenectomised mice deposited in their bodies a smaller amount of body substance than control mice. It was, may be due to removal of submandibular gland, which results in an impairment of the digestive and /or absorptive processes. Thereby, making less food available for energy and storage. But Haldi in 1963 reported that there was no appreciable difference in the caloric value of the total fecal output of the sialoadenectomised and control mice, indicating that the lesser weight gain of the sialoadenectomised animals was not due to impairment in digestion and absorption.

Increase in the sublingual gland weight in sialoadenectomised mice may be due to increase in the acini or acinar cells. But previously no body has tried to study the sublingual gland weight and other parameters like protein, glycoproteins etc. in sialoadenectomised mice.

In the present investigation it was found that there is increase in the amylase activity and protein concentration in sublingual gland of sialoadenectomised mice as compared to control.
Chapter IV: Effect of sialoadenectomy on amylase and protein

Amylase is considered to be a good indicator of proper functioning of salivary gland (Enberg, et al., 2001) as it shows alterations. Secretion of amylase, a major digestive enzyme of saliva, is regulated both by the sympathetic and parasympathetic nervous systems (Schneyer and Hall, 1966; Yamamoto and Kojima, 1969) and increase in amylase secretion is especially caused by sympathetic nerve stimulation, the β-adrenoceptors being involved in this mechanism (Kojima, 1967).

Increase in salivary amylase have been reported in response to other psychologically stressful conditions; such as experience of medical procedures (Yamaguchi, et al., 2006a), adverse musical stimuli in men (Nater, et al., 2006a), mothers watching their children being exposed to a stressful task (Granger, et al., 2006), the cold presser test (West, et al., 2006; van Stegeren, et al., 2008), achievement and interpersonal stress (Stroud, et al., 2006), a driving stimulation (Yamaguchi, et al., 2006b), use of noise burst and infant arm restraint in depressive mothers (Shea, et al., 2006), execution of neck/face surgery by medical trainees (Yamakage, et al., 2007), oral academic examination (Schoofs, et al., 2007), a standardized test battery in toddlers (Fortunato, et al., 2008), affective picture viewing (van Stegeren, et al., 2008) and in a peer rejection paradigm in adolescents (Stroud, et al., 2009). The scarcity of non-significant changes suggests that salivary amylase is indeed a highly sensitive parameter reflecting changes caused by psychological stressors.
Several scientists have proved that there is increase in salivary amylase in stress conditions (Morse, et al., 1981; Chatterton, et al., 1996; Bosch, et al., 2003; Rohleder, et al., 2004; Nater, et al., 2005, 2006).

Beyond this a number of studies applying psychological stress protocols have shown that salivary alpha amylase is highly sensitive to stress-related changes. Bosch et al., (1996) measured several salivary parameters including salivary alpha amylase. Whole unstimulated saliva was taken 30min before the examination, 2 weeks later and 6 weeks later. Results indicated an increase in concentration and output of salivary alpha amylase during the stress condition, while the salivary flow rate did not change. In a separate analysis, the authors found that the number and severity of critical life events was related to salivary alpha amylase activity, thus suggesting that everyday stress also contributes to stress dependent changes in salivary parameters (Bosch, et al., 1998). Chatterton et al., (1997) studied subjects preparing for skydiving and found increased salivary alpha amylase prior to jump than in control subjects who did not jump. The highest levels were observed immediately after landing. Using a stressful video game to induce laboratory stress, Skosnik et al., (2000) found a significant increase in salivary alpha amylase after the 15 min stressor. Bosch et al., (2003) measured whole unstimulated saliva before, during and after an active memory task, passive watching of a gruesome video and control conditions. Salivary alpha amylase output differed significantly between the three conditions, with highest levels being measured during passive video task.
A decrease in salivary alpha amylase output was found in the active memory task, suggesting that the salivary alpha amylase response depend on the nature of the stressor and the active or passive coping capabilities of the subjects. Takai et al., (2004, 2007) also used a stressful and a relaxing video to induce stress and rest conditions and directly compared the effects of both on salivary cortisol and alpha-amylase responses in whole unstimulated saliva. The stressful video induced marked increase in both cortisol and salivary alpha amylase, whereas the relaxing video produced no changes in salivary cortisol, but significant decrease in salivary alpha amylase concentrations. Noto et al., (2005) examined salivary alpha amylase during mental arithmetic task and observed significant increase in salivary alpha amylase.

Thus increase in amylase in sialoadenectomised mice suggest that there is stressful condition in sublingual gland in absence of submandibular gland, which leads to increase in secretion of various enzymes.

Salivary secretions of fluid and proteins is regulated by efferent parasympathetic and sympathetic autonomic nerves that innervate salivary glands and once these nerves have been sectioned secretion ceases almost entirely (Proctor and Carpenter, 1998). A minority of salivary glands are additionally capable of secreting saliva in the absence of impulses from nerves, a phenomenon referred to as spontaneous secretion (Emmelin, 1981). The pattern of innervation of different salivary glands within and between species varies greatly, particularly with respect to the sympathetic innervation and this is reflected in the different fluid and protein secretory responses that
can be obtained by electrically stimulating these nerves (Garrett, 1987). The main protein-secreting cells in salivary and other exocrine glands are the acinar cells which contain large numbers of protein storage granules.

Stimulation of the sympathetic nerves leads to a profound exocytosis of storage granules from the protein storing acinar cells and secretion of saliva rich in protein. The sympathetic stimuli evoking exocytosis of storage granules are meditated by three adrenoceptors on acinar cells and intracellular coupling of stimulus to secretion involves rise in cAMP and the activity of protein kinase A (Quissell, 1992; Baum, 1993). Stimulation of the parasympathetic nerves in general leads to secretion of a copious saliva containing lower concentrations of protein (Garrett and Thulin, 1975).

Ductal cells also have a well recognized role in modulating the ionic concentration of saliva but are also able to secrete proteins. In man and cat, the proteolytic enzyme kallikrein has been localized in small apical secretory granules of ductal cells (Garrett, et al., 1982), while in rats and mice the ductal cells have developed into major protein storing cells, the granular duct cells (Barka, 1980).

The results showed that the protein content in sublingual gland of sialoadenectomised mice was increased significantly as compared to control. The increase in protein content has also been shown by electrophoresis of protein. The proteins in both groups show same banding patterns but in sialoadenectomised mice there is appearance of two new bands, one in amylase region and other in glycosylated PRPs region. The increase in protein was also confirmed by electrophoresis of glycoproteins.
There was increase in staining intensity of II, V, VI, VII and VIII band in sublingual gland of sialoadenectomised mice as compared to control.

The salivary glycoproteins may have multiple functions and that some of their biological properties are dependent on their carbohydrate structures (Levine, et al., 1987). Ishii and Nakagawa (2000) demonstrated alterations in salivary secretion due to surgical procedures in mice. Simple surgical procedures like abdominal skin incision, the development of a flap and suturing increase salivary protein concentrations. Ishii and Nakagawa (2000) also suggested that surgical stress enhanced the secretion of salivary proteins, which is mainly due to α-adrenergic receptor stimulation.

Thus, increase in amylase activity, protein and glycoprotein content biochemically and appearance of one new band in between 66kDa to 43kDa in region of amylase electrophoretically in sublingual gland of sialoadenectomised mice proves that there may be stressful condition in sublingual gland in absence of submandibular gland. This condition leads to increase in secretion of different proteins, glycoproteins and enzymes in sublingual gland to compensate the functions of submandibular gland in its absence.
1. Introduction
   A. Mucins
   B. Sialic acid
   C. Fucose

2. Material and Methods
   a. Estimation of sialic acid from sublingual gland
   b. Estimation of fucose from sublingual gland
   c. Histochemical demonstration of glycoproteins
      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

4. Discussion