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I. STRUCTURE, PROCESS OF SECRETION AND FUNCTIONS OF SALIVARY GLANDS

a. Location and Structure :

The salivary glands are the collection of somewhat dissimilar structures in the mouth; they are ectodermal in origin. In most of the mammals there are three pairs of major salivary glands. According to their position, they are referred as the submandibular also called as submaxillary, the parotid and sublingual glands. The glands produce and secrete a common juice, saliva in the mouth.

Glands are consisting of blind end system of microscopic ducts that branch out from grossly visible ducts. One main duct opens into mouth from each salivary gland. The largest salivary structure is paired parotid glands, each is located near the angle of the jaw and ear. The submandibular glands lying on either side of the midline are most conspicuous structures in the ventral cervical region. The major sublingual glands are applied to the anterolateral surface of the submandibular glands. The sublingual glands can be dissected free from the submandibular gland without damage to either.

b. Blood Supply :

The submandibular and sublingual glands receive their blood supply from external maxillary or facial artery, which itself is a branch of external carotid; arising near the angle of mandible. The

parotid is supplied by another branch of the external carotid, the posterior auricular artery (Greene, 1968).

c. Nerve Supply :

In all mammals, nerves from both the divisions of autonomic nervous system, parasympathetic and sympathetic, can be found going to all the three great salivary glands. Initiation and maintenance of the secretion by the salivary glands is almost exclusively dependent on the parasympathetic and sympathetic nerves. These nerves are cholinergic and their released transmitter, acetylcholine, either directly stimulates the receptor on the secretory cells or stimulates the intrinsic cholinergic nerves that release the additional quantum of acetylcholine. Each acinar cell has five to ten axons, converging on its receptors. These receptors are bound to the cell memberane and may be identical with membrane bound enzyme guanylate cyclase; which manufactures cyclic GMP within the cell. The electric response of the membrane to acetylcholine is rapid depolarization. Parasympathetic nerve stimulation not only initiates and maintains secretion within the acinar cells and intercalated ducts but also activates transport events in duct cells that change the initial secretion into the final saliva.

Sympathetic nerves pass to the salivary acini and, on stimulation, evoke secretory response. The nerves release catecholamines (nor epinephrine, epinephrine and dopamine) that

affect two receptors on the secretory cell membrane, namely the α and β-adrenergic receptors. Sympathetic changes in salivary secretion seem to result mainly from the activation of β-adrenergic receptors. (Young and Van Lennep, 1978).

d. Histology of Submandibular Gland :

The submandibular gland has well defined capsules of fibrous connective tissues and fairly predominant duct system. The most proximal unit of submandibular gland is the acinus composed of large pyramidal cells grouped around a small lumen. Each acinus is connected by short tubules and intercalated ducts. The intercalated ducts are followed by granular ducts which are also called as granular convoluted ducts (GCT). Granular ducts have long been interesting because of number of unusual characteristics. These ducts have been considered to be the site of formation of many enzymes. (Chretien and Zajdela, 1965; Smith *et al.,* 1971; Smith Fromer, 1972a, b). The granular ducts are also considered as source of kallikrein (Orstavic *et al.,* 1975; Hojima, *et al.,* 1977) and nonspecific proteases (Shafer *et al.,* 1959; Sreebny and Meyer, 1964; Riekkinen and Niemi, 1968; Bhoola *et al.,* 1973). It has also been suggested that the granular ducts are the source of renin (Bing and Farup, 1965; Bing *et al.,* 1967; Bhoola, *et al.,* 1973; Gutman, *et al.,* 1973) and nerve growth factor (Goldstein and Burdman, 1965; Hendrey and Iverson, 1973; Schwab *et al.,* 1976) epidermal growth factor (Cohen, 1962, Starkey *et al.,*

1976; Turkington *et al.*, 1971; Gresik *et al.*, 1978; Hirata and Orth, 1979, Olsen *et al.*, 1984) and mesodermal growth factor (Weimer and Haraguchi, 1975).

e. Process of Secretion of Organic Product In The Salivary Glands :

Secretory cells have been endowed with double duty of synthesizing protein and glycoproteins not only for internal needs but to carry out functions extracellularly. The secretion of protein or glycoprotein, at least in some degree, may be characteristic feature of all cells. Glandular tissue in higher organisms has become specialized in this function. The sorting out of secretory proteins in the secretory cells is very little studied and described in many reviews (Blobel *et al.*, 1979; Blobel, 1980; Davis and Tai, 1980; Grossman *et al.*, 1983). Incident sequences of events in the course of synthesis, concentration and packing of secretory products are studied in the rabbit parotid gland (Castle *et al.*, 1972, Palade, 1975). In the submandibular glands using same method and the process of glycoprotein synthesis in mucous cells have been investigated by Neutra and Leblond (1966a, b); Bennet *et al.*, (1974).

Proteins are synthesized on the ribosomes and RER and then transported through cisternal lumina of the RER to Golgi systems via transitional vesicles (Castle *et al.*, 1972). In salivary glycoprotein secreting cells, N-acetyl-galactosamine and mannose sugars are added

at the serine or threonine of polypeptide by means of N-acetyl-galactosaminyl transferase (Young and Van Lennep, 1978). Addition of remaining sugars are thought to occur mainly in the Golgi complex and probably also in the transitional vesicles. Studies with radioactive labelled sialic acid and fucose marker's (Bennett and Leblond, 1970; Bennett *et al.,* 1974) have shown that H^3 fucose incorporation into polypeptide chain takes place within the Golgi complex of glycoprotein secreting cells. The membrane of this organelle contains necessary glycosyl transferase (Schachter, 1974). The process of condensation of glycoprotein seems to start in Golgi complex (Jamieson and Palade, 1971). There are several papers emphasizing the possible role of the GERL in the processing and packing of secretory proteins into seeretory granules (Novikoff, 1976; Hand and Oliver, 1977a, b; Novikoff and Novikoff, 1977).

During exocytosis the membrane of the secretory granule fuses with the plasma membrane, thus increase the apparent surface of the plasma membrane. This excess membrane is removed by reabsorption of membrane, which may almost certainly take place by endocytosis (Abrahams and Holtzman, 1973;Geuze and Poort, 1973; Orci *et al.,* 1973).

Excessive secretory granules get digested in lysosomes. The lysosomes presumably first fuse with granules and subsequently digest them (Van Lennep *et al.,* 1977).

The above brief review on the synthesis of secretory material in the salivary gland indicated that –

i) The process of glycoprotein synthesis is mainly accomplished by RER and Golgi complex.

ii) Unsecreted granules and secretory granule membranes are absorbed into the lysosomes thus lysosomes are playing active role in the process of secretion.

f. Functions :

In general salivary glands are subserving four major types of functions :

1. First and perhaps most important function of salivary glands is to secrete saliva which provides lubrication to aid swallowing, the aqueous component of saliva by dissolving solid food makes taste possible. Saliva provides the fluid seal that makes sucking possible.

2. It keeps the buccal cavity clean and moist, moistening of the surfaces of buccal mucosa seems also to be necessary for speech.

3. Salivary glands secrete enzymes, hormones and pharmacologically active components.

   i. amylase which digest substantial part of ingested starch (Junquira *et al.*, 1949),

ii. Saliva contains lysozyme, peroxidase and immunoglobulins which have antibacterial and antiviral properties. (Young and Van Lennep, 1978).

iii. Growth promoting substances, such as epidermal growth factor (EGF), nerve growth factor (NGF) and transforming growth factor (TGF) can be found in large amounts in submandibular glands and saliva and in smaller amounts in sublingual glands but not in parotid saliva (Barka, 1980; Sporn *et al.,* 1982). EGF and NGF were reported to accelerated the rate of wound healing in mice Li *et al.,* 1980; Niall *et al.,* 1982) and humans (Brown *et al.,* 1989). In addition, it has been shown that NGF derived from salivary source can act as chemostatic factor for polymorphonuclear leukocytes and fibroblast like cells *in vivo*, which mimics the cellular response that occurs during normal wound healing (Lawman *et al.,* 1985). Furthermore a study by Bothwell *et al.,* (1979) has established the identity of salivary kallikrein with βNGF endopeptidase, an enzyme involved in the production of NGF from an inactive precursor. TGF isolated from salivary glands has also been shown to increase total protein, DNA and collagen in wound chamber experiments, and this effect was triggered by the addition of EGF (Sporn *et al.,* 1982).

4. The fourth role of salivary gland discerned in fur bearing animals, animal wet their fur with saliva in response to heat stress.

5. Another function is, in some cases, is that of defense and paralysing the prey.

Major Polypeptides in Submandibular Glands :

The chemistry and biology of several submandibular gland factors have been extensively studied, however, significance of presence of those factors in the salivary gland has not been worked out in detail except few like nerve growth factor and epidermal growth factor. The submandibular gland factors are operationally grouped into 1) Growth and differentiation factors, 2) Homeostasis factors, 3) Intracellular regulation factors and 4) Digestion factors. The concentration of these factors in the gland of neonatal animal is very low, with onset of puberty concentration of these factors increases very rapidly. Granular convoluted tubules are the site of formation of these substances. Differentiation and synthesis of proteins in granular convoluted cells is hormone dependent (Barka, 1980).

i. Nerve Growth Factor (NGF) :

Johnson *et al.,* (1971) showed that nerve growth factor is of submandibular gland origin. The structure of NGF is presented by Young *et al.,* (1978). According to them NGF occurs in the submandibular gland in multiple molecular forms; the only stable

form has a molecular weight of 116,000. This stable form is claimed to be secreted in large quantities into the saliva (Aloe and Levi-Montalcini, 1980).

Immunocytochemical staining established unequivocally that NGF is localized in the secretory granules of the GCT cells (Goldstein and Burdman, 1965; Kumar *et al.,* 1972; Schwab *et al.,* 1976; Simson *et al.,* 1978). Levi-Montalcini and Cohen (1960), Caramia (1966) and Ishii and Shooter (1975) suggested that NGF was not only stored but also synthesized in the submandibular gland. The concentration of NGF in the glands of neonatal mice is very low but apparently measurable with the onset of the puberty. NGF concentration increases rapidly (Levi-Montalcini *et al.,* 1960).

Submandibular gland saliva contains high concentrations of NGF and the secretion is mediated primarily by alpha adrenergic mechanisms (Wallace and Partlow, 1976; Wallace *et al.,* 1977; Simson *et al.,* 1978; Hirata and Orth, 1979; Murphy *et al.,* 1980). The plasma NGF level is reduced by sialoadenectomy and after castration (Hendrey and Iverson, 1973), would support a multifocal origin of this factor (Murphy *et al.,* 1980).

ii. Renin :

Cohen and his co-workers (1972) obtained two renin like enzymes from the submandibular glands of male mice in pure and stable forms. The submandibular gland of male mouse contains 20 to

30 fold more renin than the gland of female (Troutschold *et al.*, 1966; Michelakis *et al.*, 1974; Hirata and Orth, 1979). Renin is acidic protease (polypeptide) with a highly restricted substrate specificity. Renin is important in blood pressure regulation, since it catalizes the first step in the renin-angiotensin cascade. Bing *et al.*, (1977) sowed that aggressive behaviour in mice resulted in a vast increase in plasma renin level.

Secretion of renin into saliva is stimulated by alpha adrenergic mechanisms (Manzie *et al.*, 1974; Bing *et al.*, 1977). The assessment of the contribution of submandibular gland to the maintenance of the plasma renin level is complicated by the apparently unavoidable, artifactual long-lasting release of renin from the gland by sialoadenectomy (Bing *et al.*, 1977; Bing and Poulsen, 1976).

iii. Kallikrein :

Kallikrein has been found and isolated form, the submandibular glands of many species man, dog, cow, house, cat, rabbit, mouse, rat, hamster and guinea pig (Werle and Von Roden, 1936; Hopsu-Havu *et al.*, 1967; Bhoola and Dorey 1972; Nustad *et al.*, , 1974; Brandzaeg *et al.*, 1976; Moriwaki *et al.*, 1976; Proud *et al.*, 1977; Fukouka *et al.*, 1979; Lemon *et al.*, 1979; Maltra *et al.*, 1986). Of all tissues the rat submandibular glands contains the highest concentration of kallikrein (Erdos *et al.*, 1968). The presence of Kallikrein in the striated ducts of submandibular in cat is studied by Hojima *et al.*, (1977).

Gerrett (1982) showed kellikrein like activity in human salivary glands. With respect to kellikrein content, the submandibular glands of rat and mouse show no significant sexual differences, and neither castration nor testosterone treatment and significant effect on kallikrein levels (Bhoola *et al.*, 1974; Gecse *et al.*, 1976).

iv. Glucagon :

Glucagon is mainly secreted by the alpha cells of the islets of Langerhans of the pancreas. Glucagon is a small protein and comprises of twenty nine aminoacids. A substance similar to pancreatic glucagon in immunoreactivity is secreted by the gastric, duodenal mucosa (C.C. Chattargee, 1991) and this substance has been named as gut glucagon like immunoreactive material (GLI).

Silverman and Dunbar (1974) first described glucagon in extracts of rat submandibular gland, and showed that such extracts increased blood glucose level in rat, but not in eviscerated rats. They suggested that the submandibular gland participates in the enteroinsulin axis by secreting glucagon, which in turn stimulates insulin secretion. The presence of glucagon in the submandibular glands of several species including the mouse, rat, rabbit, guinea pig, dog and man has been confirmed by Lawrence, *et al.,* (1975, 1976, 1977); Dunbar, *et al.,* (1977), and Hojvat, *et al.,* (1977), Pillai, *et al.,* (2001). According to Kelly, *et al.,* (1977) the glands of male rats

contain about three times more glucagon than the glands of female animals.

v. Insulin :

Insulin is a protein with an isoelectric point of 5.3, consists of two polypeptide chains i.e. A chain and B chain. The three dimensional structure of insulin is related to its biological activity. Both the A chain and B chains are held together by two interchain bisulphite bonds (Gyton, 1981, Talwar *et al.*, 1989). The insulin plays an important role in storing the excess energy substances. The insulin causes fat storage in the adipose tissue. Insulin promotes the utilization of glucose and lowers blood glucose concentration. Insulin also increases the activities of the enzymes that promote glycogen synthesis, including phosphofructokinase. The presence of non suppressible insulin like material is found by RIA and immunoflourescence staining described and reviewed by Barka (1980). Liske and Reber (1976) found by RIA and immunofluorescence staining, the highest concentration of NSILA in rat pancreas and submandibular gland.

vi. Transforming Growth Factor (TGF) :

The transforming growth factors (TGF) were isolated in 1978, as a partially purified activity from culture medium conditioned by Raus sarcoma virus-transformed fibroblast. (Delarco and Todaro, 1978). This partially purified material to normal fibroblasts caused

the reversible appearance of malignant (transformed) phenotype; hence this polypeptide is named as transforming growth factor (TGF). Structurally the TGF was comprised of two proteins, designated as TGF-α and TGF-β. It was postulated that TGF-α functioned in an autocrine manner and other that over expression of this protein might contribute to malignant transformation. Because TGF-α production was found in neoplastic cells (Derynck *et al.*, 1987; Lee *et al.*, 1985).

The human TGE-α gene spans 70-100 kilobases on chromosome 2 and contains 6 exons (Derynck *et al.*, 1984, Marquardt *et al.*, 1984). The 4.5 – 4.8 kilobase TGF-α messenger RNA transcript encodes a 160-aminoacid transmembrane precursor protein that contains the mature 50-aminoacid sequence in the extracellular domain. Aminoacids in the mature TGF-α sequence are 35% identical to EGF. The size of the TGF-α protein were reported t range from 5 to 30 kilodaltons.

The TGF-α expression is clearly not restricted to transformed cells or limited to embryonic tissues (Twardzik, 1985 and Wilcox and Derynck, 1988). Surveys of messenger RNA expression and protein localization indicate a widespread distribution of TGF-α in normal cells and tissues (Yasui *et al.*, 1992; Chou *et al.*, 1994). Expression of TGF-α has been detected in many cells and tissues, including keratinocytes (Coffey *et al.*, 1987) activated macrophages (Rappolee *et al.*, 1988; Madtes *et al.*, 1988) mammary epithelium (Liu *et al.*,

1987 and Smith *et al.*, 1989) and the anterior pituitary (Samsoondar *et al.*, 1986).

TGF-α is best known as a potent growth stimulator, however, a wide variety of other biological effects have been described in nearly all cell types and tissues. Most of the activities of EGF in the gastrointestinal tract will also be observed for TGF-α. TGF-α is more potent than EGF as a stimulator of calcium release from fetal long bones (Stem *et al.*, 1985), arterial blood flow in dogs (Gan *et al.*, 1987), angiogenesis in the hamster cheek pouch assay (Schrieber *et al.*, 1986), cell migration in keratinocytes (Barrandon and Green *et al.*, 1987) and cell membrane ruffling (Mydral *et al.*, 1986).

Evidence exists to implicate both cell biology as well as tissue and organbiology to explain differences in activity between TGF-α and EGF. Cellular consideration occurs primarily at the level of receptor interaction. For example although EGF and TGF-α bind the mammalian EGFr with similar affinity. Winkler *et al.*, (1989) developed anti EGFr antibody that markedly inhibits TGF-α when compared with EGF binding, suggesting that the two peptides bind differently to the same receptor.

vii. Epidermal Growth Factor (EGF) :

The epidermal growth factor (EGF) occurs in the submandibular gland. It was discovered by Cohen in 1962. Cohen purified from the mouse submandibular gland a factor that caused

precocious eyelid opening and early incisor eruption in newborn mice (Byyny *et al.*, 1972). EGF is a polypeptide isolated from the submandibular glands of the mice which exhibits growth stimulating activity on various epidermal and epithelial tissues both *in vivo* and *in vitro*.

EGF is a single polypeptide having aspargine at the NH_2 – terminus, arginine at the COOH-terminus and a pool of 53 amino acid residues (Taylor *et al.*, 1970, Taylor *et al.*, 1974). The EGF contain 6 half-cystinyl residues and no detectable free sulfhydryl groups. It is further characterized by the absence of three amino-acids : lysine, alanine and phenylalanine.

A human EGF immunologically related to mouse EGF, has also been isolated (Cohen *et al.*, 1975; Starkey *et al.*, 1976). EGF stimulates the growth of epithelial cells, fibroblasts and glial cells under various experimental conditions (Cohen, 1960, 1962; Jones, 1966;Turkington, 1969 a.b.; Cohen and Taylor, 1974; Cohen and Savage, 1974; Cohen *et al.*, 1975; Lembach, 1976; Carpenter and Cohen, 1976; Westermark, 1976; Tadara *et al.*, 1976).

A large body of evidence indicates that the EGF is synthesized, stored and secreted by granular convoluted tubule (GCT) cells of the submandibular gland. The concentration of EGF in the gland is closely correlated with the development and differentiation of the GCT under different physiological and experimental conditions.

Turkington, *et al.,* (1971) localized EGF primarily in the basal cytoplasm of all GCT cells by using immunofluorescence. On the basis of light microscopic immunoperoxidase staining, Cohen and Savage (1974) described that EGF is concentrated in the apical secretory granules of the GCT cells. In crude homogenates of mouse submandibular gland EGF occurs as a high molecular weight (74,000) complex, consisting of two moles of EGF and two moles of an EGF-binding protein.

EGF is first detectable in the submandibular gland around the 20^{th} post natal day. EGF was first demonstrable in scattered GCT cells at 20 days of age in male and at 30 days of age in female mice (Gresik and Barka, 1978). The saliva of the male mouse contains far more EGF than the saliva of the female mouse (Hirata and Orth, 1979). After puberty concentration of EGF increases rapidly. Androgen markedly increase submandibular gland EGF content (Byyny *et al.*, 1972), but maximal levels are not reached even at three month of age.

The male mouse submandibular gland contains 1-2 μg/ mg wet weight of EGF. The level of EGF in the gland is androgen dependent. The gland of the female contains about 1/10 or less of that of the male. Castration reduces, while administration of testosterone to female or castrated male increases EGF concentration in the gland (Roberts, 1974; Frati *et al.*, 1976; Ladda *et al.*, 1979; Hirata and Orth, 1979).

EGF is a potent mitotic stimulant for a variety of cell types; it enhances keratization and inhibits gastric acid secretion. It is widely used experimentally, not only in investigations of regulation of cell replication, but also as convenient tool for the analysis of receptor hormone interactions, and receptor mediated endocytosis of hormones.

Attardi *et al.,* (1965) purified mesodermal growth factors which stimulate the growth of mesenchymal cells. This factor displayes protease and esterase like activities and those activities are androgen dependent (Weimer and Haraguchi, 1975).

These biologically active growth factors though synthesized in, and secreted by granular convoluted tubule (GCT) cells of the submandibular gland, their formation is dependent on hormones like androgen, thyroid and probably adrenocorticoid hormones. Extirpation of submandibular gland may lead to an acute shortage of these factors in the plasma, this indicate endocrine like functions of submandibular glands.

II. EFFECT OF EPIDERMAL AND RELATED GROWTH FACTORS ON GASTRO-INTESTINAL TRACT

The cells of gastrointestinal tract have rapid turn over rate. Under normal conditions, cell populations within the gastrointestinal tract are mentioned at dynamic steady state because cell loss through exfoliation of surface cells is balanced by a continuous cells renewal

(Lipkin, 1987). On differentiated precursor or stem cells within crypts of the small intestine and colon and mucous cells of the glandular neck of the gastric mucosa may responsible for continuous cell renewal in gastrointestinal tract (Lipkin, 1987). The balance between cell loss and cell renewal is tightly regulated. If it is not regulated, it can result in atrophy or ulceration, or hyperplasia (Johnson and McCormack, 1994).

Important in this regulation are several growth factors including those of the epidermal growth factor family, the transforming growth factor family and insulin growth factor family. Growth factors are mediators of cell proliferation and/ or differentiation (Jones *et al.*, 1999). Considerable interest has been focussed on the effect of EGF on gastrointestinal tract. In particular it has been suggested that the EGF plays an important role in the pathogenesis of peptic ulcer (Pai. *et al.,* 1998) and in the gastroduodenal tract, accelerate gastric ulcer healing by stimulating cell proliferation and migration and modulate gastric mucosal blood flow (Barnad *et al.,* 1995; Karnes, 1994; Clark *et al.,* 1991; Peppelenbosch *et al.,* 1992; Orsini *et al.,* 1993; Beauchamp *et al.,* 1989; Threadgill *et al.,* 1995). The binding of these peptides to specific transmembrane receptions on the surface of the target cell initiates signal transduction cascades which culminate in the activation of certain genes within nucleus leading to the cell divisions or differentiation (Pai and Tarnawski, 1998). In addition to

their function in cell proliferation and differentiation some growth factors or peptides in the gastrointestinal tract, EGF and TGF-α elicit other independent actions such as inhibition of gastric acid and pepsin secretion (Samloff, 1989; Olsen *et al.,* 1986; Konturek *et al.,* 1988; Olsen *et al.,* 1986; Hui *et al.,* 1993) and stimulating mucus production (Coffey *et al.,* 1995; Cartlidge *et al.,* 1989). In addition several growth factors including EGF and TGF-α and βFGF have been shown to influence cell migration and increase in blood flow (Moore *et al.,* 1980, Zimmermann *et al.,* 1993, Duerr *et al.,* 1995; Conover *et al.,* 1989; Ryan and Costigan, 1993). Moreover administration of EGF in humans and animals exerts a potent suppressive action on acid and pepsin secretion. Among the EGF and TGF, EGF appears to be involved in the maturation of gastrointestinal tract (Rao *et al.,* 1991; Schaudies *et al.,* 1990). The normal expression of EGF in the gastrointestinal tract is restricted to salivary glands, pancreas and Brunner's glands of the proximal duodenum (Poldolsky, 1994). Luminal EGF is most likely to be derived from saliva, duodenal and pancreatic secretion and kidney (Konturek *et al.,* 1989). But recently Playford *et al.,* (1995), reported that EGF is cleared less active forms in gastric acid, thus indicating the importance of duodenal and pancreatic EGF for protection of small intestinal epithelium (Basson 1992). In the small intestine, the highest concentration of TGF-α are found in differentiated. Villus cell compartments (Malden *et al.,*

1989; Thomas *et al.,* 1992) TGF-α is also found in colonic epithelial cells (Suemori *et al.,* 1991).

A growing body of the evidence indicate that EGF is mainly involved in the regulation of gastrointestinal epithelial barrier function. EGF, originally from the mouse submaxillary gland (Hunter, 1995) was shown to modulate maturation of gastrointestinal tract.

EGF has been shown to stimulate DNA synthesis not only in the intestine but also in the gastric mucosa (Dembinski and Johnson, 1985; Arsenault and Menard, 1987; Kuwayama *et al.,* 1994). Recently EGF has been shown to inhibit the appearance of sucrase activity in gastric mucosa (Scheving *et al.,* 1979) suggesting that EGF is able to influence the processing of many digestive enzymes in the entire human gastrointestinal tract.

In addition to reepithilization, repair of deeper injury (erosions) EGF and TGF are mitogenic for progeny cell populations, increased release of gastric mucin, attenute gastric acid secretion and have been shown to stimulate cell migration (MacDonald *et al.,* 1993).

III. EFFECT OF SALIVARY SECRETION ON GASTROINTESTINAL TRACT

The submandibular gland exhibit exocrine as well as endocrine secretion of several peptides called growth factors like epidermal growth factor (EGF), transforming growth factor (TGF), insulin

growth factor and fibroblast growth factors. These factors functions as mediators of cell proliferation and/ or differentiation (Johnson and McCormack, 1994; Podolsky, 1994). The main component of salivary gland is mucus with high buffering capacity. The peptides have also number of effects such as stimulation, cell division, migration and inhibition of gastric acid secretion (Coffey *et al.,* 1995; Cartlidge *et al.,* 1989). Presence of lysozyme in the submandibular gland is also believed to enhance healing by suppressing infection *Helicobactor pylori*, a spiral gram negative bacterium that colonizes mucosa is the predominant cause of chronic gastritis and peptic ulcer diseases in humans (Cover and Blaser, 1996 and Labenz and Borsch, 1994).

The removal of submandibular gland resulted in decrease in mucosal growth (Johnson and Guthrie, 1976; Dembinski and Johnson, 1979; Skinner *et al.,* 1984), mucosal integrity (Skinner and Tepperman, 1981) and protection against various ulcerogens (Skinner and Tepperman, 1981; Olsen *et al.,* 1984; Tepperman *et al.,* 1989). Sialoadenectomy greatly increased susceptibility of the stomach to ulcerogens, which caused about twice as many as mucosal lesions as animals with intact salivary glands. Furthermore exogenous EGF afforted significantly less protection against those acute ulcerogens in sialoadenectomized rat than animals with intact salivary glands. Takeuchi and Johnson (1979) and Konturek *et al.,* (1990) found that sialoadenectomy alone did not cause the formation of gastric lesions

but sialoadenectomy significantly reduced DNA synthesis and increased susceptibility of mucosa to the development of ulcers in response to the stress. No ulcer was observed in sialoadenectomized rats, but lesions area induced by ethanol were significantly higher in sialoadenectomized rats than those with intact salivary glands (Hui *et al.,* 1993).

Skinner *et al.,* (1984) reported that the administration of salivary gland extract containing some EGF like activity as well as EGF itself resulted in an increase in DNA synthesis in the oxytonic mucosa of sialoadenectomized rats. Kanturek, (1990) showed that the sialoadenectomy greatly reduced the content of EGF in the gastric mucosa and this could decrease the cell turn over that might impair the mucosal integrity. Konturek, (1990) further showed that removal of salivary glands resulting in several fold increase in stress ulcerogenecity. It has also been shown that salivary glands contain and secrete PG (Tabor and Tabor, 1984) and EGF has been shown to stimulate mucosal PGE_2 biosynthesis (Chiba *et al.,* 1982). Findings are also that sialoadenectomy reduces mucosal PGE_2 synthesis (Konturek *et al.,* 1990; Tepperman *et al.,* 1989). Muramatsu *et al.,* 1985 proposed that PG generated in the gastric mucosa and EGF released by salivary gland interact on gastric mucosal integrity. The removal of any of these mechanisms may result in mucosal damage and integrity.

Olsen *et al.,* (1986) showed that removal of submandibular glands delayed healing of chronic gastric ulcers. In the rat model sialoadenectomy marked by delays healing of the gastric and duodenal ulcers (Konturek *et al.,* 1991; Wu *et al.,* 1996). There was decrease in the volume of salivary approximately 60% and total output of EGF/ URO in saliva of sialoadenectomized rat (Olsen *et al.,* 1984). According to Olsen and his colleagues (1984) the delay in ulcer healing in sialoadenectomized rat may be due to inadequate saliva in these rats. Saliva has a high buffer capacity that could decrease the acidity of gastric juice and thus may enhance ulcer healing (Helm *et al.,* 1982). Desalivation in the rat is followed by a decreased resistance of the gastric mucosa to damaging agents such as bile salt solution (Skinner and Tepperman, 1981). Salivary mucins might also act protective surface gel and prevent damage from the gastric juice.

In addition peptides that stimulate healing of wound such as epidermal growth factors and others have been isolated from submandibular gland and saliva (Li *et al.,* 1980; Murphy *et al.,* 1979). The submandibular gland exhibit exocrine as well as endocrine secretion of peptides such as EGF (Nexo *et al.,* 1984), but the concentration of salivary EGF/ URO in saliva is considerably higher than in the plasma. The concentration of salivary EGF during stimulation in rats increased by factor 50-100 while no change in the serum concentration was observed (Olsen *et al.,* 1984). This shows

that expression of EGF in the gastrointestinal tract is restricted to salivary glands and Brunner's glands of the proximal part of duodenum (D.K. Podolsky, 1994). Luminal EGF is most likely must have been originated from saliva and duodenal secretion (Kontureck *et al.,* 1989). Recently Playford *et al.,* (1995) reported that EGF is cleaved into less active forms in gastric acid, thus indicating the importance of duodenal and pancreatic EGF for the protection of small intestinal epithelium (Basson *et al.,* 1992). But there is evidence that endogenous luminal EGF is important in ulcer healing process. (Konturek, 1990; Konturek *et al.,* 1992; Konturek *et al.,* 1991). Oral administration of exogenous EGF to sialoadenectomized rats restores healing rate to that of control rats with intact salivary glands (Konturek *et al.*, 1991). The gastrointestinal mucosa has remarkable ability to repair damage. When integrity of the surfacial mucosa is breached, repair is dependent on the ability of epithelial cells to migrate and proliferate process is rapid and *in vivo* can be accomplished with 15 to 60 minutes (Wallace and McKnight, 1990). It appears to be dependent on uninterrupted blood flow (Konturek, 1990; Wallace and Granger, 1996). In this regard several growth factors including EGF and TGF-α have been shown to stimulate cell migration and increase blood flow (Pai and Tarnawski, 1998; Blay and Brown, 1985; Barrandon and Green, 1987; Konturek *et al.,* 1992; Wu *et al.,* 1996).

Hui, *et al.,* (1993) showed no significant effect on the peak and summation blood flow in intact and sialoadenectomized rats under basal condition. However, after treatment with ethanol, sialoadenectomized mice showed reduction in the peak and summation of the blood flow, although difference was not statistically significant.

A study has shown that patients with gastric or duodenal ulcers have a significantly lower salivary EGF compared to healthy subjects suggesting that decreased luminal EGF may predispose these patients to ulcers (Konturek, 1990). Moreover, smoaking has been shown to suppress the release of EGF in both salivary and duodenal secretions and delay ulcer healing (Konturek, 1990).

The above brief review on sialoadenectomy gives an idea that, the gastric mucosa upon exposure to ulcerogenic and/ or necrotizing agents develops. Characteristics morphologic, ultrastructural and functional changes reflecting injury or gastric lesions which includes :

1. Disruption of the unstripped layer and surface hydrophobicity
2. Exfoliation of the surface epithelium
3. Injury of the deeper gastric mucosal layer

All these events results in the formation of mucosal erosions or ulcerations. Formation of mucosal erosions or ulcerations, but the effect of sialoadenectomy on mucosa of duodenum is not described.

There are some reports that EGF is affecting gastrointestinal mucosa, the reviews on EGF describes that the role of EGF in the protection/ integrity of gastrointestinal mucosa. Some reports describes that though EGF is affecting gastrointestinal mucosa it is not necessary that salivary EGF is playing role in this case but also may be EGF originated from Brunner's gland (Kirkegaard *et al.*, 1984; Olsen and Nexo, 1983) and to some extent EGF secreted from pancreas. There are evidences of the presence of EGF like factors in the Brunner's glands.

Therefore, in the present study it was designed to investigate the role of salivary secretion on the integrity of duodenal mucosa. The role of Brunner's gland secretion on the integrity of duodenal mucosa was also investigated in this research.