CHAPTER ONE

INTRODUCTION

Amongst the various glandular structures, the salivary glands are the most interesting and complicated group of secretory organs since they have been attributed many diverse functions; just like pancreas they produce digestive enzymes and like the kidney they also function in the withdrawal of the plasma proteins, not only this but their secretory functions, especially quantitative differences in the enzyme secreted by them, are very interesting and related to differences in the sex hormones in the males and females and levels of these hormones in the circulating body fluid. From biological point of view, perhaps even more important function of saliva secreted from salivary glands, is to cleanse and protect the surface of the masticating apparatus. Saliva also plays a role in speech by its dimulescent action and in sensory physiology of gustation. In addition to above functions of salivary glands, there are a number of polypeptides synthesized mainly in the submandibular glands, which are concerned with (i) Growth and differentiation (ii) Homeostasis (iii) Intra-cellular regulation (iv) Digestion (Barka, 1980). Because of multifarious functions performed by their secretions and varieties of physiological processes in which these glands are involved, salivary glands have gained a modest popularity in the field of biological research. The dimensions of this field are indeed multiplicated, some idea of which can be had by cursory glance through a series of articles in the

handbook of physiology edited by Blair West et al. (1967) and recent reviews, papers and monographs in the last two decades (Rauch, 1959; Jenkins, 1960; Pe rson, 1960; Ussing et al, 1960; Burgen and Emmelin, 1961; Kerr, 1961; Jakowska, 1963, 1970; Schneyer and Schneyer, 1967; Leeson, 1967; Angeletti et al., 1968; Baserga et al., 1969; Botelho et al., 1969; Andrews and Bullock, 1972; Emmelin et al., 1972; Pinkstaff, 1972, 1980; Emmelin and Gjorstrup, 1973, 1974, 1976; Levitskii and Barabash, 1974; Glucksmann, 1974; Gresik and Mac Rae, 1975; Dunn and Wilson, 1975; Cutler and Choudhry, 1973; Bullock et al., 1975; Schwab et al., 1976; Bhathena et al., 1977; Chretien, 1977; Lawrence et al., 1977, Kelly et al., 1977; Gresik et al., 1978; Simson et al., 1978; Smith et al., 1979; and Barka, 1980).

i) MORPHOLOGY OF SALIVARY GLANDS

A. Occurrence

Glands which are situated at the anterior portion of the digestive tract and which empty their secretions into the anterior portion of the digestive tract, are known as salivary glands. These glands are present in many groups of invertebrates including annelid worms, the molluscs (with exception of bivalves), and the arthropods.

In the vertebrates most of the salivary glands are of ectodermal origin. Fishes generally lack any organ which can be

called as salivary gland, but presence of some goblet mucus secreting cells all over the tongue is reported in the fishes (Khan, 1968; Nalavade, 1974). In the amphibia these are situated at the terminal portion of the mouth (Francis, 1961) and are mainly of mucous type. Reptiles possess gland of serous and mucous type.

The comparative morphology and microanatomy of the avian salivary glands have been studied by many investigators for more than a century (Tiedmann, 1810-1814; Meckel, 1829; Batelli and Giacomini, 1889; Giacomini, 1890; Cholodkowsky, 1892; Hoelting, 1912; Greschik, 1913; Antony, 1920; Bock, 1961; Foelix, 1970).

In the mammals, salivary glands are well developed and in general their structure varies with the way of the life and feeding habits of the animals. In the aquatic mammals, where fluid is hardly needed, they are somewhat vestigeal. The ruminants, which require large amount of watery fluid to mix, often with relatively dry food, have very well developed glands and their structure may also vary considerably with the age of the individual (Andrew, 1949).

The monotremes, echidna and at least some of the marsupials show three pairs of salivary glands. In the echidna, submaxillary gland is enormous and the parotid gland is small (Andrew, 1949). In certain edentates including ant-eaters, which

have very long tongue for taking their prey, the submaxillary gland has a bladder-like receptacle to hold saliva in readiness (Andrew, 1949). The ungulates have parotid gland which is some four times larger than the size of the submaxillary. The secretion mixed with food to form the "Cud" is highly watery one. Among the rodents, those species which are exclusively herbivorous, have large parotid glands. In the rat the parotid and submaxillary are practically of equal size (Andrew, 1949). In carnivors, parotid may consist of mucous alveoli, which is true of dog (Andrew, 1949). The chiroptera have large parotid gland in fruit eating bats but small in the insectivorous forms (De-Santo, 1962). In the parotid gland the acinar cells are seromucoid in nature and secretion of the gland is rich in mucopolysaccharides, acid and alkaline phosphatases, easterase and possibly a protease (De-Santo, 1962).

In general, in mammals the salivary glands are usually divided into two groups - major and minor. There are three large paired glands: the parotid, submandibular (submaxillary) and sublingual glands are major salivary glands. However, there are numerous smaller glands, throughout the buccal mucosa which also contribute towards the formation of saliva, these are minor glands known as labial, buccal and palatal glands. There are also the anterior lingual glands and glands in the mucous membrane of the tongue. Minor glands are named according to

their anatomical locations.

B. Gross Microscopic Structure

1) Submaxillary Glands

These glands lie in contact with the inner surface of the body of the mandible and their main ducts open on the floor of the oral cavity. They are compound alveolar or tubular glands. Although of mixed type, majority of their secretory units are of the serous variety, mucous units are usually capped by serous demilunes. The submaxillary gland has well defined capsules and fairly predominant duct system (Davies and Davies, 1962). The most proximal unit of the submaxillary gland duct system is the acinus, usually composed of large polygonal cells grouped around a small lumen. These cells secrete the initial saliva including water, electrolytes and organic molecules such as amylase. The acinar cells are surrounded by myoepithelial cells rest upon the basement membrane of acinar cells contain an actinomycin and have motile extension. The next segment of the duct is the intercalated duct which is lined by additional myoepithelial cells (Leeson, 1956; Tamarin, 1966; Tandler, 1965; Garret and Parson, 1973; Cutler and Chaudhry, 1973) contraction of myoepithelial cells serves to expel formed saliva from acinus into intercalated duct and from intercalated duct to next portion. The intercalated duct soon widens to become the striated duct, lined by columnar epithelial cells.

In some glands, e.g., rats and mice submaxillary, the most proximal portion of the striated duct is further distinguished by the presence of numerous granules and it is convoluted and hence is often separately designated as the convoluted granular tubules or granulated duct. Until recently the nature of these tubules was not recognized. They differentiate from the existing portions of the intralobular duct system during postnatal development (Jacoby, 1959, Jacoby and Leeson, 1959; Sreebny et al., 1955) and exhibit sex differences (Arvy and Gabe, 1950; Junqueira et al., 1949). Great number of biologically active polypetides have been purified from the salivary glands. Generally these polypeptides are androgen dependent, but not affected by sex genotype and are located in the granular convoluted tubule cells in the gland. They are secreted into the saliva but are also found in the blood circulation. These are involved in Growth and Differentiation, Homeostasis, Regulation and Digestion. The duct system continues to converge distally as granular tubules extend to form striated ducts, which, in turn leads into a lesser number of small excretory ducts. Finally, the small excretory ducts merge into one main excretory duct that leads from main glandular mass to the oral cavity, where it terminates (Leeson, 1967).

Myoepithelial cells have been acknowledged as a component of salivary glands (Tamarin, 1966; Shear, 1966; Harrop and

Mackey, 1968; Tandler et al., 1970), as well as other reports on the morphology of myoepithelial cells can be found in the articles of Young and Van Lennep (1977). Myoepithelial cells are the components of the mammalian exocrine glands (Leeson, 1960; Ellis, 1965; Tannenbaum et al., 1969). In all sites, where these cells have been observed, they are located on the epithelial side of the basal lamina and as their name implies, they share morphological features of both epithelial cells and smooth muscle cells (Ellis, 1965, Archer and Kao, 1968). These cells are generally stellate shaped (Zimmermann, 1927; Tamarin, 1966). Tamarin has also reported a spindle-like shape when they are associated with the intercalated ducts of rat submandibular gland; however Tandler (1965, 1978) has reported spindle-like shape for those cells associated with secretory end pieces. These cells consist of a cell body from which many processes extend. The arrangement of the processes in relation to the cell body has been well demonstrated in the rat submandibular gland by Tamarin (1966). The contraction of myoepithelial cells causes a transitory increase in the rate of delivery of saliva to the oral cavity, the principal function of the cells seems to prevent distension of the end pieces during secretion. This is achieved partly by providing support for the end piece and partly by a widening and shortening of the intercalated ducts, which has the effect of lowering the outflow resistance (Young and Van Lennep, 1977).

2) Sublingual Glands

These salivary glands are uncapsulated. They are well forward, near the mid line, below the mucous membrane of the floor of the mouth, and their secretion is emptied by several ducts that open along line behind the opening of submandibular ducts. In the rat there is a close anatomical relationship between submaxillary and sublingual glands (Leeson, 1967). They differ from submaxillary in the fact that they are pale in colour and the majority of their alveoli are of mucous type. Their microscopic appearance is different in different parts of the gland. Acini are mostly of the mucous type. In some areas, only mucous secreting units with serous demilunes may be found. The sublingual glands are more variable and more complicated both in gross morphology and histology. The sublingual glands of cat and dog (carnivore) differ from most of the sublingual glands in that they contain large number of purely serous acini. Boerner-Patzelt (1955), in hamster and Shackleford and Klapper (1962b), in guinea pig have reported that the gland consists entirely of mucous acini, whereas in rats and mice demilunes are frequent. Intercalated and striated intralobular ducts are poor in most of the species. As compared to intercalated ducts of parotid they are very short and many times acini directly open into striated intralobular ducts. In cat and dog sublingual gland intercalated ducts are not present. Intralobular ducts are

followed by the several extralobular ducts which open along the line behind the opening of submandibular duct.

3) Parotid Glands

These are the largest of the three pairs of the salivary glands proper. These glands appear diffused and each lies packed in the space between the mastoid process, ramus of the mandible and overflows on the space below the zygomatic arch, plunges through the buccinator muscles to open into the oral cavity opposite to the molar teeth in the man. The gland is enclosed in a well defined fibrous connective tissue capsule (Ham, 1965). The parotid gland consists of pyramidal shaped cells, intercalated ducts, striated ducts and main excretory duct in man. The duct system is complex. The intercalated ducts in general are long and are divided into proximal, distal portions on the basis of presence of secretory granules in the cytoplasm of the cells in the proximal part (Kurtz, 1964). The intercalated ducts connect with a well developed system of striated ducts, which in turn open into main excretory duct.

ii) SECRETION AND ITS CONTROL

Secretion and its products are more or less complex molecules that are built up, accumulated in the cytoplasm and later extruded to the outside of the cell. The best known

examples are hormones, enzymes, mucus, saliva and poison.

Junqueira and Hirsh (1956) defined that secretion is a chain process, which includes two processes, viz., (i) ingestion, which means penetration of raw material into the cell, such as amino acids, sugars, fatty acids, water, ions etc. and (ii) synthesis in which more or less complex molecules are built up. Concentrated and stored in granules, vacuoles and crystals etc. which are visible.

Secretion of saliva from the salivary glands is under the nervous as well as hormonal control (i) Initiation and maintenance of secretion by the salivary glands is almost exclusively dependent on parasympathetic and sympathetic nerves. The parasympathetic system is stronger stimulus for secretion; its fibers are distributed to all salivary glands. These nerves are cholinergic and their released transmitter, acetylcholine, either directly stimulates receptors on secretory cells or stimulates intrinsic cholinergic nerves that release additional quanta of acetylcholine. Each acinar cell has five to ten axons converging on its receptors. These receptors are bound to the cell membrane and may be identical with the membrane-bound enzyme, guanylate cyclase, which manufactures cyclic GMP within the cell. The electrical response of the membrane to acetylcholine is rapid depolarization. Parasympathetic nerve stimulation not only starts and

maintains secretion within the acinar cells and intercalated ducts but also activates transport events in duct cells that change the initial secretion into a final saliva. Parasympathetic nerves also evoke contractions of myoepithelial elements that are located near the acini and ducts, these contractions help to squeeze saliva from the acini and ducts into the mouth.

Sympathetic nerves pass to the salivary acini and, on stimulation, evoke a secretory response. These nerves release catecholamines (Norepinephrine, epinephrine and dopamine) that effect two receptors on the secretory cell membrane, namely the α and β adrenergic receptors. Sympathetic nervous changes in salivary secretion seem to result mainly from activation of the β-adrenergic receptors. Sympathetic stimulation causes a biphasic change in blood flow. The earliest vascular response is a transient decrease in flow. Sympathetic stimulation and local release of catecholamines acting on β-adrenergic receptors also change the composition of saliva, increase metabolism and growth of the gland and prompt contraction of the myoepithelial cells around the acini and intercalated ducts.

The dual autonomic regulation of these glands is unusual because the parasympathetic and sympathetic systems both stimulate secretory, metabolic, trophic, muscular and circulatory functions in similar directions (Jacobson, 1977).

ii) Effect of hormones, especially sex hormones, on the salivary glands of mammals probably comprises a more general phenomenon. On the basis of effect of these hormones sex differences have been shown and described in these glands in several orders. These differences have been observed in rodents such as mouse (Lacassagne, 1940a), rat (Shafer and Muhler, 1955), hamster (Shackleford and Klapper, 1962a), desert rat (Shackleford and Schneyer, 1964), and in primates such as the Indri monkey (Girod, 1964).

Lacassagne (1940a); Junqueira et al. (1949) and Raynaud (1960) showed the effect of androgen on the tubular segment of the submaxillary gland and they have proved that hyper development of a tubular segment and its cellular characteristics in male are dependent on the presence of testosterone. But injection of estrogen into mice of both sexes did not effect on the salivary glands, but chronic injections of estrogen into adult male mice caused hormonal castration with subsequent tubular atrophy and acinar enlargement (Lacassagne, 1940a,b,c; Raynaud, 1960). Furthermore, it was established that the androgen hormone modified exoenzyme content of the submaxillary gland, amylase (Raynaud and Rebeyrotte, 1949a,b,c) and protease (Junqueira et al., 1949) being more abundant in male than the famale. Hormonal regulation of submandibular salivary gland morphology and antigenicity in rats have been reported by White and Mudd (1975). They

observed that by ovariectomy per cent distribution of tubletes was depressed in mature female rats, but was restored by administration of estrogen and/or progesterone. The per cent distribution of acini of aubmandibular glands of young rats was slightly increased by estrogen.

Sex hormones are not only the hormones that influence the submaxillary gland. In 1940 Lacassagne and Chamarro showed that, in mice of both the sexes within several days of hypophysectomy, tubular involution occurred which was more pronounced than that resulting from castration in the male. Regeneration of these tubules can occur after testosterone injection, but regeneration was incomplete. Later on it was found that for the complete regeneration of tubules adrenal hormone and thyroid hormones are also essential. The earliest observations on the role of thyroid hormone in the morphology and maintenance of secretory tubules were made in the rat (Leblond and Grad, 1948, Grad and Leblond, 1949). The results of thyroidectomy in the mouse, studied by Arvy and Gabe (1950) and Raynaud (1960), confirmed in the rat. It was, therefore, proposed (Raynaud, 1960) that thyroxine is responsible for tubular development in both the sexes until puberty; afterward, it maintains tubular integrity in the female, whereas in the male, testosterone further stimulates tubular hypertrophy; thyroxine is nonetheless necessary in the adult male for maintenance of normal tubular cell activity and seems to be

involved in the synthesis of secretory products. Finally, the action of thyroxine on the submaxillary gland is dependent on the presence of adrenocortical hormones. Adrenalectomy plus castration resulted in the same complete tubular atrophy as hypophysectomy. Thyroxine was found to be active in the presence of cortisone or corticosterone (Raynaud, 1955; 1956, 1957, 1960).

Thus, it has been established that the synergistic action of sexual, thyroid and corticoid hormones is necessary for full development and maintenance of the tubular segment of the submaxillary gland. However, in spite of this complicated hormonal control, testosterone seems to play the most important role in the stimulation of tubular cells (Chretien, 1977).

iii) SALIVARY GLAND SEXUAL DIMORPHISM

The credit of the discovery of sexual dimorphism of salivary glands goes to Lacassagne (1940a) who described morphological difference in male and female mouse submandibular glands. Lacassagne's observations later on confirmed by a number of investigators histologically, histochemically and biochemically (Fekete, 1941; Lacassagne and Causse, 1941; Leblond and Grad, 1948; Grad and Leblond, 1949; Junqueira et al., 1949; Junqueira and Rabinovitch, 1954; Raynaud, 1960, 1964; Gresik, 1966; Caramia, 1966; Andrews and Bullock, 1972;

Smith and Frommer, 1972a,b; Nakamura et al., 1974; Floridi et al., 1976; Marcante et al., 1977 and Doonon et al., 1978).

Since 1940 sexual dimorphism of salivary glands has been observed in other mammalian species also including rats (Lacassagne, 1940b; Raynaud, 1964; Pillai, 1974), bovine (Bergrahm, 1961), gerbils (Abouharb, 1955; Mastraccio, 1972), Syrian hamsters (Shackleford and Klapper, 1962a). Europian hedgehogs (Borghi,1963), desert rats (Shackleford and Klapper, 1962b), crab eating monkeys (Girad, 1964), rabbits (Spicer and Duvenci, 1964), men (Mandel et al., 1964), agoutis (Hetem, 1967) Kangaroo rats (Flon et al., 1970), miniature pig (Pinkstaff, 1972), Wistar rats (Mudd and White, 1975) and large white Essex Pigs (Booth et al., 1973). The only report of sexual dimorphism in sublingual glands is in the Syrian hamster (Spicer and Duvenci, 1964). Fava-de-Moraes et al. (1966) have reported sexual dimorphism in parotid glands in two species of pinnipedia, the Patagonian or South American sea lion and the southern fur seal.

The sexual dimorphism of salivary glands described by the above investigators is not only at morphological level but also histological, histochemical and biochemical levels. Lacassagne and others (Lacassagne, 1940a,b,c; Gresik, 1966; Caramia, 1966; Gresik and MacRae, 1975) demonstrated that number of convoluted granular tubules was more in the

submaxillary glands of male than that of the female. The number of granules from the granular tubules, the diameter of granular ducts and height of the cells forming duct in males were different from those of the females described by Gresik (1966), Caramia (1966) and Andrews and Bullock (1972) in mice, Abouharb (1955) and Mastraccio (1972) in gerbils and Raynaud (1964) in musk shrew. Bergrahm (1961) has reported that the striated ducts of the bovine male were of greater diameter than those of the females. Mudd and White (1975) have reported that ratio of an acinar tissue to the granular duct was 1:1 in male wistar rats, but in female, ratio of the acinar tissue to the granular duct was 2:1.

Sexual dimorphism of salivary glands of some species may not be morphologically apparent, but some of these sex differences have been shown by biochemical and histochemical methods. Kronman (1963a) observed a more intense reaction for tryptophan in the secretory end-pieces of male hamster than that seen in the glands from the female hamster. In another study Kronman (1963b) reported higher acid phosphatase activity in acinar cells of sublingual, submandibular glands of female hamster than in the same glands of the male hamster. Pillai (1974) reported intense staining reaction for esterase in the convoluted granular tubules and striated ducts of male rat submandibular gland than that of the female rats. But she observed higher acid phosphatase and β-glucuronidase activities

in acinar cells of submandibular gland of female rat than in the same gland of male rat. Shackleford and Klapper (1962a) have reported stronger staining of submandibular gland acini by alcian blue in female hamster than the male. Girod (1964) also reported similar reaction as described by Shackleford and Klapper in the crab eating monkeys. Pinkstaff (1972) observed alcian blue staining of seromucous demilunes in the submandibular glands of both male and female miniature pigs (Pitmann - Moore Strain), however, the demilunes of the glands from the male pigs were much more intensely alcian blue positive than female, whereas mucous tubules were more alcian blue-positive in the female glands than male glands. Booth et al. (1973) have reported a somewhat different staining pattern in the submandibular glands of large White Essex pigs. The mucous acini of female pig submandibular gland were strongly alcian blue-positive, whereas mucous acini of male pig glands were faintly to moderately positive with the same stain. They also reported much higher concentration of serous cells in the male pig submandibular glands and they have remarked on their virtually unstained appearance. But Smith and Frommer (1972a,b) have shown that in the submandibular gland of chilian rodent acinar cells of male glands are intensely stained with periodic acid schiff technique whereas those of the females are moderately positive. In their quantitative histochemistry Sato et al. (1977) showed higher

concentration of periodic acid schiff positive groups in males than in the femeles.

There are a few examples of sexual dimorphism related by the use of the biochemical research methods. Junqueira et al., (1949) measured the protease activity in the mouse submandibular gland homogenates, in the male gland protease activity is more than the female gland. Bhoola et al. (1973) demonstrated higher level of the activity of trypsin and renin in male mouse submandibular glands than in the female mouse glands. Nakamura et al. (1974) have reported sex-differences in glucose-6-phosphatase dehydrogenase activity which was higher 72.8 ± 7.3 milliunits/milligram protein for males and 38.8 ± 2.8 for females. Marcante et al. (1977) showed sex linked metabolic features in the culture cell derived from male and female C^3H mice submandibular glands where the oxygen consumption that was 38% in the female deprived cells. Doonon et al. (1978) described a sexual dimorphism of mouse submandibular gland with respect to activities of three enzymes N-acetyl-β-D-glucosaminidase, α-D-mannosidase and esterase. The activities of all three enzymes were higher in the extracts of male mouse submandibular glands.

All biochemical studies on sexual dimorphism of salivary glands have not been performed only on mice. Hetem (1967) demonstrated an isoenzyme of esterase in the submandibular

glands of female agouti but not in male. Booth (1972) extracted testosterone and α-5 dihydrotestosterone from submandibular glands of swine. Pillai (1974) described sexual dimorphism of rat submandibular gland with respect to activities of three lysosomal enzyme β-glucuronidase, acid phosphatase and esterase. An enzyme esterase was higher in extracts of male submandibular glands, but biochemically there is no difference in the enzyme levels of β-glucuronidase and acid phosphatase from the homogenates of male and female submandibular glands.

During the past few decades, a number of biologically active polypetides, or factors, have been isolated from saliva and are claimed to be present in the submandibular glands. The richest source for most of these factors proved to be the submandibular gland of male mouse. There are two polypetides which are extensively studied; they are: (i) NGF (Nerve Growth Factor) which promotes growth and differentiation of nerve and its concentration is greater in the submandibular glands of male than the female mice (Hendry, 1972; Hirata and Orth, 1979; Johnson et al., 1971; Murphy et al., 1979, and (ii) EGF (Epidermal Growth Factor) which regulates epidermal growth and keratinization in the immature mouse. Sexual dimorphism is also reflected in EGF content in the mice; its concentration is higher in the submandibular gland of male mice than the female mice (Byyny, et al., 1972; Frati et al., 1976; Murphy et al., 1979.

REASON THAT LED TO THE PRESENT INVESTIGATION

Biologists who are not engaged in active research of salivary glands know that salivary glands secrete saliva containing amylase for the digestion of starchy material and keep mouth cavity wet to prevent dehydration of the cells lining the mouth cavity. But besides these functions there are a number of functions carried out by the salivary glands such as (i) to protect mouth cavity from infection, (ii) growth, regulation and homeostasis are also controlled by these glands, (iii) hormone-like protein is secreted by parotid salivary gland, (iv) metabolism of hormone-like androgen may partly be carried out in the submaxillary gland. Earlier anatomist often referred to the kidney as "viscus elegantissimus, the most elegant organ". But now salivary glands are used to be referred to as elegant organs. This phrase might have been appreciated by reader if they have made a cursory glance on the review of salivary glands and will accept that more and more research in depth is essential in salivary glands.

One of the most important facts regarding the salivary glands is that these are well developed in mammals, but they are diverse. Among the particular group also there is no similarity; they differ from species to species and in between the male and female of the same species also, in their gross anatomy, histology, histochemical architecture, histochemical

staining, physiology and biochemistry.

Even though there is considerable literature pertaining to the various aspects of salivary gland diversity, investigators have been unable to reach any firm conclusion as to why such a difference occurs. It has been suggested that evolution, ecology and feeding habits may all play roles in salivary gland diversity (Junqueira and Fava de Moraes, 1965).

A three striped squirrel (Funambulus palmarum L.) is regarded as important rodent in the evolution of rodent but yet the study of its salivary glands is not so far carried out. So, it was felt that information gained by study of the salivary glands of this animal would be meaningful in our understanding of rodent salivary gland biology.

V) PLAN OF PROPOSED WORK

Keeping in view the above mentioned reason and the amount of work done on the sexual dimorphism of salivary gland, it was decided to work out the histology and enzymorphology of submandibular, sublingual and parotid glands of male and female squirrel Funambulus palmarum L.

a) Choice of the Animal

While selecting the subject for research, care was taken

to select such a mammal wherein no work has been carried out on histology and histochemistry in the salivary glands with respect to sexual dimorphism. The squirrel Funambulus palmarum L., the animal of natural habitat was found suitable for the present investigation. For the investigation animals were collected from natural habitat. Immediately after trapping they were killed for fixation. Their maturity was decided with the help of vaginal smears and condition of ovary in the female and in the male, presence of sperms in the testis.

b) Choice of the Techniques

Since the present investigation aims at a detailed study of the structure of salivary glands, Haematoxylin-eosin technique was used for the study of general structure. To study the nature of ductal system Iron haematoxylin technique was employed and histochemistry of esterase were carried out by using 5-bromoindoxyl acetate as substrate. In the salivary glands secretory units are called as acini; the terminal portion of secretory system. These acini are of different nature. To study the nature of these acini, glands were stained for periodic acid schiff technique and alcian blue pH 2.5 technique. Secretory units are surrounded by myoepithelial cells. These cells could not be revealed with haematoxylin-eosin technique or any other technique employed above. But they could stain for alkaline phosphatase. So myoepithelial

cells were stained for alkaline phosphatase using naphthol AS-MX phosphate as substrate.

In the present investigation, the staining timings were kept constant throughout the work for particular techniques and differences, if any, in the intensity of staining were taken as reflection of differences in the nature of different secretory units.

c) Critical Evaluation of the Observations

The results obtained in the present investigation on salivary glands were analysed critically in relation to:

(A) 1) Histology of submandibular gland. Male submandibular gland compared with female submandibular gland,

2) Nature of secretory acini,

3) Number of granular ducts,

4) Granules in the granular ducts,

5) Myoepithelial cell: their nature and distribution.

(B) 1) Histology of sublingual gland. Male sublingual gland compared with female sublingual gland,

2) Nature of secretory acini,

3) Nature and distribution of myoepithelial cells.

(C) 1) Histology of parotid gland. Male parotid gland compared with female parotid gland,

2) Nature of secretory acini,

3) Nature and distribution of myoepithelial cells.

d) Presentation of the Thesis

It was decided to divide the present thesis into six chapters, the first being on the introduction, it gives brief review on the Morphology of salivary glands, secretion and control of salivary gland secretion, sexual dimorphism in the salivary glands, reason that stimulated to undertake the present investigation and plan of proposed work. The second chapter stands for material and methods employed for the present investigation. Observation and discussion on the sexual dimorphism of the submandibular glands of male and female are presented in Chapter Three. Chapters Four and Five include the observations and discussions on the sexual dimorphism of sublingual and parotid glands of male and female squirrels respectively. The Chapter Six is devoted to summary and concluding remarks. A complete bibliography of the literature cited in the various chapters of the present thesis is given at the end of the thesis.