INTRODUCTION

Among the oral glands in the living vertebrates the salivary glands have received greater attention owing to their structural complexity and functional multiplicity in higher forms. None of the aquatic vertebrates, barring the cyclostomes which possess a large socalled "salivary gland" of unknown function, possess oral glands. With the emergence of terrestrial mode of life the mouth could no longer be kept moist with ambient water, and hence, the oral glands appeared to rise to the occasion of keeping the oral cavity from getting dried. The secretions of these glands aid in moistening the oral cavity and in capturing the prey Many reptiles possess sublingual glands. In the case of *Heloderma*, the only known poisonous lizard, these glands furnish the poison Birds have anterior and posterior groups of oral glands, whose function, in general, is not properly understood. It is in only mammals, except the cetaceans, that the true salivary glands occur, which form the distinctive feature of this group. The salivary glands are usually located in the oral cavity but sometimes may be lodged far back into the neck region. In all cases the homologies are decided on the basis of the openings of the respective ducts The gross morphology of salivary glands is notoriously variable between species In the lower mammalian groups the salivary apparatus comprises of only the submaxillary and sublingual glands, whereas in higher mammals parotids were added later. The parotid gland opens opposite the second upper molar tooth by Wharton's duct. Submaxillary or submandibular gland opens by Stenson's duct anteriorly near the frenulum of the tongue, whereas sublingual glands open through several small ducts on the floor of the mouth.

The salivary glands of mammals are typical tubuloalveolar structures. The cells lining the alveoli are known as end-piece cells (Yo̲ng and Van Lennep, 1978) The salivary glands consist of three cell types: serous; mucous and seromucinous cells The first type has small granules containing amylase and same amount of mucopolysaccharides. The second, contains droplets of mucus and acid mucopolysaccharides. Seromucinous cells contain both acidic and neutral

mucopolysaccarides. The parotid is largely a serous gland, the sublingual is chiefly of mucous type, and the submandibular is a mixed gland The serous cells of this gland tend to be at the terminal positions of the acini, forming crescents or demilunes. The system of ducts of salivary glands comprises of intercalated, striated/granular and excretory ducts. The structural arrangement of acini is compatible with function of secretion of primary saliva. The saliva is known to be modified by processes of absorption and secretion during its passage through the various regions of the system of ducts. The phenomenon has been shown to be functionally comparable to that of nephrons, at least as far as the flux of electrolytes and water (Winsten *et al.*, 1988) is concerned.

The secretion of parotid gland is clear and watery being much less viscous, but richer in amylase, while that of sublingual gland is thick opalescent, sticky and rich in mucin. Submandibular (submaxillary) glands have both serous and mucous cells in roughly equal proportions. Owing to their mixed nature, the submandibular glands have attracted the attention of research scientists.

Secretory functions of salivary glands entail metabolic turnover The mixed saliva emanating from all these glands is viscous, colourless and opalescent fluid with variety of compounds including inorganic ions, organic components like glycoprotein mucin, enzyme ptyalin, amino acids, urea, lipids, citrate etc Some heavy metallic ions like lead and mercury may also appear in saliva occasionally Glycoproteins in saliva include neutral as well as sulfated mucins and those containing only sialic acid components. These give saliva its viscocity and lubricating property. Blood group substances represent an important component of salivary glycoproteins.

The innervation of salivary glands comprises of parasympathetic as well as sympathetic nerve fibers (Best and Taylor, 1985). The functioning of the glands depends on the integrity of both adrenergic as well as cholinergic nerves (Bloom *et al.*, 1981). The parasympathetic innervation is necessary for maintaining the

normal physiological state of the glands. The sympathetic stimulation acts on the salivary glands through the mediation of adrenergic receptors *viz.*- α and β-types Stimulation of submandibular gland of cat parasympathetically produces secretion of copious watery saliva, whereas sympathetic stimulation produces viscous saliva containing greater proportion of solids (Bell, Davidson and Smith, 1972).

It is also known that salivary glands elaborate several biologically active polypeptides such as epidermal growth factor (EGF) (Hoshino and Lin, 1968; Barthe *et al.*, 1974; Roberts, 1974; Thoenen and Barde, 1980; Gresik and Barka, 1983; Tsutsumi *et al.*, 1986; Takai *et al.*, 1986), nerve growth factor (NGF) (Aloe and Levi-Montalcini, 1980; Gresik *et al* , 1980), renin (Bhoola *et al.*, 1973; Gresik *et al.*, 1980), kallikrein (Bhoola *et al.*, 1973), trypsin-like esteroprotease (Angeletti *et al.*, 1967; Takuma *et al.*, 1978). EGF (isolated from male mouse submaxillary glands) was shown not only to stimulate cellular proliferation in mouse mammary epithelium (Taketani and Oka, 1983a & b) but a variety of other mammalian cells (Cohen and Savage, 1974; Carpenter, 1978) Mammals are usually in the habit of licking their wounds and these factors may possibly aid in enhancing the healing of wounds(Huston et al , 1979; Niall et al , 1982; Harper, 1988; Bodner, 1991).

As early as 1940, Lacassagne demonstrated sex-dependent differences in the histological structure of mice submandibular glands The granular ducts, which appear in the submandibular salivary glands of mice at puberty, are more numerous and better developed in the males than in females (Lacassagne, 1940a, Travill, 1966). It was shown that there also exists a sex-dependent functional difference in case of protease activity, it being high in submandibular glands of male mice than that of female (Junqueira *et al.*, 1949). In case of mice the glands of both sexes showed the same cell type during development, but during maturation the glands display a degree of sex-dimorphism (Gresik and Edith, 1975). According to Hosoi *et al* (1978) β-glucuronidase activity in mouse submandibular gland was almost same in both sexes upto 20 days after birth, but with pubertal changes this enzyme activity in males becomes twice as high as that

in females. Computer assisted three-dimensional reconstructions of mice submandibular glands indicated that the female mice possess a more highly branched intercalated duct system, and that, the granular ducts usually terminate within secretory complexes, whereas in males the granular duct typically passes through a secretory complex and forms a prominent cap like structure on the opposite side (Yang *et al.*, 1993). Dimorphism has also been associated with several physiological differences with respect to levels of acid- and alkaline-phosphatase (Junqueira, 1949; Junqueira *et al.*, 1949), amylase (Raynaud and Rebeyrotte, 1949), protease (Sreebny, 1960), iodine (Llach *et al.*, 1960), arginase (Kockakian and Hall, 1955), tryptophan and tyrosine (Junqueira, 1949; Kronman, 1963a & b), β-glucuronidase (Hosio *et al.*, 1978) and oxygen consumption (Wilborn and Fitzgerald, 1964). Buillard and Delsuc (1953), based on histological studies, have shown that the submandibular gland of female mice assume appearance of male type after receiving testosterone. According to Schneyer *et al* (1972), in rat salivary gland such sex-dependent morphological differences are not prominent, nevertheless, sex-related physiological differences do exist

Histology of rat submandibular gland exhibits sexual dimorphism in the ductal system and it shows dependence on the state of sexual maturity (Mudd and White, 1975). Specific androgen and estrogen binding capacities of submandibular salivary glands in mouse and rat have been reported (Verhoeven and Wilson, 1976;Takauma *et al.*, 1977; Verhoeven, 1979;Laine and Tenovuo, 1983, Morrell *et al.*, 1987; Sakabe *et al.*, 1987; Katsukawa *et al.*, 1989) Furthermore, rat salivary glands exhibit a differential distribution of estrogen receptor contents between parotid and submandibular glands (Campbell *et al* , 1990). The presence of estrogen receptors in salivary glands may serve to promote gender-specific differences in submandibular gland.

Influence of sex hormones on composition of saliva has also been observed by several authors as referred to in the following statements. Changes in salivary contents are known to occur during menstrual cycle (Puskulian, 1972; Ben-Ayreh

et al., 1976) and pregnancy (Kullander and Sonesson, 1965) in women. In fact, changes in concentration of glucose (Davis and Balin, 1973; Prosser and Hartman, 1983) and sialic acid in saliva have been suggested as clinical indicators of ovulation in women (Oster and Yang, 1971). Significantly elevated phosphate concentration was observed at mid-cycle in normally ovulating women (Ben-Ayreh *et al.*, 1976). Though various aspects of hormonal influence on components of secreted mixed saliva are known, the same cannot be said regarding the role of hormones on enzymic processess concerned with glandular metabolism and secretory functions adequately. It was therefore, thought desirable to study influence of normal (4-day) estrous cyclicity on submandibular gland metabolism. During each stage of estrous cycle the following parameters were studied - glycogen, lipid, cholesterol and total protein and some of the concerned enzyme activities such as glycogen phosphorylase, succinate dehydrogenase, ATPases and c.AMP-specific phosphodiesterase Simultaneously, plasma glucose levels were also estimated.The findings are presented and discussed in chapter-2.

It would not be out of place to mention here that earlier work in this laboratory (Ambadkar and Vyas, 1975; Ambadkar and Gangaramani, 1976, Desai, 1989; Wagh, 1994), under experimental regimes as employed here, has proved that deprivation of either sex hormones or administration leads to alterations in metabolic processes of submandibular gland as well as other tissues at very short intervals. Work from various other laboratories (Cooke *et al* , 1982, Moger *et al.*, 1982; Moger and Anakwe, 1983; Moger and Murphy, 1983) has amply proved that Metabolic Clearance Rates (MCR) of sex hormones are much faster than expected heretofore In conjunction with this, it has also been realized during last few years that gonadal hormones exert rapid effects (within a matter of few minutes to few hours) in case of a few important biochemical processes (Weiner et al , 1970, Ambadkar and Gangaramani, 1976; Booth, 1977; Ambadkar and Wagh, 1993; Ambadkar and Raval, 1993) Hence, one of the main themes of the present investigation rests on this idea of short-term or rapid effects of gonadal hormones rather than long drawn experimental designs (several days/weeks) as was the

practice during yesteryears. Only sparse information is available regarding influences of deprivation and replacement of ovarian hormones on the overall pattern of metabolism of submandibular glands of female rats. Hence, experiments to unveil possible influences of female sex hormones on metabolic aspects of submandibular salivary glands were carried out. Previous work on male and female rats in this laboratory has proved that in less than 48 H of gonadectomy the circulating gonadal hormone levels become negligible Deprivation of ovarian hormones was brought about through bilateral ovanectomy and effects of ovariectomy were studied after 24, 48 and 72 H. Taking into consideration the observations, that maximum variations due to ovariectomy occurred after 48 H of operation, subsequent work was conducted on 48 H spayed females only 48 H spayed animals were administered with 5, 10 and 15 μg doses of 17β -estradiol (E_2) by way of a single intramuscular injection to each animal. The rapid effects of 17β-estradiol replacement were studied on the submandibular salivary glands after 1,2 and 4 H of hormone administration. Combination replacement therapy was achieved by administering a fixed dose of 2 mg progesterone simultenously with each of the three doses of 17β-estrdiol viz.-5, 10 and 15 μg. It was noticed during E_2 regime that maximum alterations occurred at 2 hourly intervals in most of the cases. So, the rapid effects of combined therapy were observed after 2 H of hormone administration. During each of these experimental conditions the following parameters were studied :- glycogen, total protein and some of the concerned enzymes activities such as glycogen phosphorylase, succinate dehydrogenase, ATPases and c.AMP-specifid phosphodiesterase Simultaneously, plasma glucose levels were also estimated

According to Berkman and Kronman (1970) castration of mice causes reduction in size, diameter and number of granular tubules of the submandibular gland, and these effects could be reversed by testosterone administration. These observations are in conformity with the findings of earlier workers (Lacassagne, 1940; Chaulin-Serviniere, 1942a & b; Shafer and Muhler, 1953; Cassano, 1958) The granules present in convoluted tubular cells of the submandibular gland of

mice decreased after castration and testosterone administration lead to increase in density of granules (Kazuo, 1979). Injection of female sex hormones to male mice scarcely affected the quantity of serous-like granules (Hosoi *et al.*, 1977). According to Tuomela et al. (1990a & b) EGF mRNA concentrations in mouse submandibular gland declined after ovariectomy upto 10 days, which was followed by an increase upto 80 days. On the other hand, it has been reported that long term ovariectomy did not significantly alter the concentration of EGF in submandibular gland of rat (Purushotham *et al.*, 1993). However, sufficient information regarding effects of ovariectomy on metabolism of submandibular gland, in general, is not available. It was, therefore, thought desirable to study short-term effects of ovariectomy on submandibular gland metabolism Observations were made for the parameters listed earlier, after 24, 48 and 72 H of ovariectomy. The findings are presented and discussed in chapter-3

Lacassagne (1940b) had shown that administration of estrone benzoate to male mice resulted in feminization of the gland. As against this, long term administration of diethylstilbesterol and estradiol benzoate to normal female rats has been reported to cause significant decrease in number of granular tubules and their diameter in submandibular gland (Shafer and Muhler, 1953; Liu *et al.*, 1969). On the other hand, prolonged administration of estradiol to gonadectomized mice of both sexes was reported to result in hypertrophy of granular ducts and increase in weight of submandibular gland (Cassano, 1958; Houssay and Harfin, 1973, Curbelo *et al.*, 1974a) However, Raynaud and Rebeyrotte (1949) failed to observe any alterations in the histological structure of the submandibular gland of mice after administration of female sex hormones Implantation of estradiol pellets in female rats were reported to cause changes in cytology of granular duct and that these changes were accompanied by acceleration of protein synthesis (Flynn *et al.*, 1983). It is also known that the rat salivary glands exhibit an estrogen-induced increase in peroxidase activity (Laine and Tenovuo, 1983), analogous to that observed by Lyttle and Jellink (1973) and Lyttle and Desombre (1977) in the estrogen treated uterus. In the light of these observations, it was thought desirable

to study the effects of estradiol administration to 48 H ovariectomized females for understanding of the problem on a wider base . Rapid effects of administration of 17β-estradiol to 48 H ovariectomized female rats on various metabolites and concerned enzymes mentioned earlier are discussed in chapter-4

Liu (1968), working on submandibular gland of rat, has reported that synthetic gestagenic and progestagenic combination dose of mestranol and norethynodrel resulted in reduction in diameter and number of granular tubules. According to Campos *et al.* (1984) administration of medroxy progesterone acetate to female mice brings about increase in weight of submandibular gland and induces enlargement as well as musculinization of granular ducts. Further, they also state that such an influence was observable even in ovariectomized mice. Some progestins also increase the submandibular gland EGF levels in female mice (Barthe *et al* , 1975). Additionally, it is also known that combined treatment with estradiol and progesterone increased the EGF concentration in the submandibular gland of gonadectomized mice (Tuomela *et al.*, 1989). In stimulated whole saliva of women it has been reported that protein, sialic acid, hexasoamine, fucose, hydrogen ion concentration and total electrolyte concentration decreased after administration of oral contraceptive (Norgesterol and ethinyloestradiol) (Magnusson *et al.*, 1975) In the light of these observations it was thought desirable to study the effect of combined 17β-estradiol plus progesterone administration to 48 H spayed rats. Rapid effects of administration of 17β-estradiol plus progesterone on various metabolites and concerned enzymes mentioned earlier are dealt with and discussed in chapter-5.

In animal tissues, sialic acid occurs as an important constituent of mucoproteins, mucolipids and lipoprotein-carbohydrate complexes. Ubiquitous distribution of sialomucoproteins in animal surface secretions and excretions is indicative of rather a protective role of these rather than structural function. Sialomucoproteins are regular components of viscous mucin coverings of those epithelial surfaces which are in direct contact with surroundings. Sialic acid concentrations in various

tissues have been known to alter under the influence of hormones Reports on cyclic variations of sialic acid concentration in various tissues and secretions in female laboratory animals (Jensen, 1967; Rybakova, 1978) as well as women (Oster and Yang, 1972; Moghissi *et al., 1975;* Tenovuo et al., 1983) are available However, adequate information directly dealing with sialic acid concentration of submandibular gland in phase with different stages of the estrous cycle is not available. As against this, some literature is available on effects of gonadectomy and hormones on sialic acid concentration in salivary glands of rodents (Zebrowski, 1972 & 1973; Boyko and Zebrowski, 1972; Curbelo *et al.*, 1974a & b, Desai, 1989). Based on the above information it was thought pertinent to see whether the sialic acid of submandibular gland of rat is influenced by ovarian cyclicity and administration of ovarian hormones Hence, total sialic acid concentration in submandibular gland was estimated during each of the 4 stages of estrous cycle, and after ovariectomy, after replacement with 17β-estradiol and with 17β–estradiol plus progesterone to 48 H ovariectomized females Details about such influences are discussed in chapter-6

Occurrence of various steroid hormones and their metabolites in the saliva indicates involvement of salivary glands in metabolising steroid hormones (Evans and Stewart, 1980; Luisi *et al.*,1981;Walker *et al.*, 1981; Choe *et al*, 1983; Khan-Dawood *et al.*, 1984; Read *et al.*, 1984; Evans, 1986; Lentone *et al.*, 1988, Turkes *et al.*,1989, Adenkulin, 1989, Campbell and Ellison, 1992) Not only this, but estradiol as well as progesterone concentrations in the human saliva have been studied by several workers to gain understanding about the assessment of ovarian functions and for predicting the time of ovulation. Activities of steroid metabolising enzymes *viz.*- 17β, 3α, 3β-hydroxysteroid dehydrogenases in human salivary glands have been studied histochemically by Sirigu *et al.* (1982) However, other workers have assayed steroid metabolising enzymes biochemically (Rosner, 1965 and 1969; Baldi and Charreu, 1972; Charreu *et al.*, 1976; Ferguson and Bannon, 1983;Kyakumoto *et al.*, 1986; Poteat *et al.*, 1986; Furuyama, 1986; Furuyama *et al.*, 1990 and Sawada and Tetsuo, 1993). The works cited so far do not take into

account the variations due to estrous cyclicity in the submandibular gland of females. It was, therefore, thought desirable to study histochemically steroid metabolising enzymes *viz.*-17β, 3α, and 3β-hydroxysteroid dehydrogenase enzyme activities for knowing the possible alterations during different phases of the estrous cycle as far as its steroid metabolizing capacity is concerned Observations on alterations of hydroxysteroid dehydrogenases in submandibular gland of normal 4-day cycling female rats are described in chapter-7.