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. Aquatic vertebrates, barring the cyclostomes which possess a large so called 'salivary gland' of unknown function, do not possess such oral glands. With emergence of terrestrial forms the mouth could no longer be kept moist with ambient water, and hence, the oral glands appear to rise to the occasion of keeping the oral cavity from getting dried. The secretions of gland aid in moistening the oral cavity and in capturing the prey. Many reptiles possess sublingual glands and 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, but in all cases the homologies are decided on the basis of the openings of their 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 alveolii are known as end piece cells (Young and Van Lannep, 1978). The salivary glands consist of three cell types: serous, mucous and seromucinous cells. The first type has small granules containing amylase and some amount of mucopolysaccharides. The second, contains droplets of mucus and acid mucopolysaccharides. Seromucinous cells contain both acidic and neutral mucopolysaccharides. The parotid is largely a serous gland, the sublingual is chiefly of mucous type, and the submandibular a mixed gland. The serous cells of these glands tend to be at the terminal positions of the acini, forming crescents or demilunes. The system of ducts of salivary glands comprises intercalated, striated/granular and excretory ducts. The structural arrangement of acini is compatible with function of primary salivary secretion, the latter then known to be modified by processes of absorption and secretion during its passage through the various regions of the sytem of ducts. The phenomenon has been shown to be functionally comparable to that of nephrons, at least as far as the flux of elctrolytes and water (Winston et al., 1989) 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) gland has both serous and mucous cells in roughly equal proportions. Owing to its mixed nature, the submandibular gland has 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 viscosity and lubricating property. Blood group substances represent an important component of salivary glycoproteins.

It is also known that salivary glands elaborate epithelial growth factor (EGF) and nerve growth factor (NGF)(Hoshino and Lin, 1968; Thoenen and Barde, 1980; Gresik and Barka, 1983). Mammals are usually in the habit of licking their wounds and these factors may possibly aid in enhancing the healing of wounds (Harper, 1988). According to Byyny et al. (1974) the release of EGF in blood plasma is controlled by sympathetic nervous system.

As early as 1940, Lacassagne demonstrated sex-dependent differences in the histological structure of mice submandibular glands. He had shown that the portion of whole gland represented by granular tubules is appreciably greater in the male than in the female mice. Later, it was shown that there also exists a sex-dependent functional difference in case of protease activity it being higher in submandibular glands of male mice than that of female (Junqueira et al., 1949). Administration of testosterone and dihydroxytestosterone to female mice have been reported to increase EGF in submandibular gland (Barthe et al., 1974; Kasayama et al., 1989). Buillard and Delsuc (1953), based on histological studies, have shown that the submandibular glands of female mice assume appearance of male type after receiving testosterone. However, in rat salivary glands such sex-dependent morphological differences are not prominent, nevertheless, sex-related physiological differences have been observed (Schneyer et al., 1972). Further, in case of rat parotid gland the secretion of elctrolytes is known to show sex-dimorphism; wherein the concentration of electrolytes in parotid saliva of male is higher than that in female.

Occurrence of various steroid metabolites in the saliva indicates involvement of salivary glands in metabolising steroid hormones (Seaton and Fahmy, 1979; Walker et al., 1981; Poland and Rubin, 1982; Read et al., 1984). Supraphysiological uptake and retention of testosterone enanthate and testosterone cyclohexane (Schuermeyer et al., 1984) by the salivary glands suggests possession

of steroid receptors, which bind the hormones free fraction present in blood plasma. Relative potencies of testosterone, testosterone propionate and other related steroids in restoring some of the morphological and functional characteristics of submandibular gland, have been investigated in castrated mice Minetti et al. (1985). It was shown by these authors that testosterone propionate was most effective with regard to stimulation of proteolytic capacity. Further, they suggested that there might be some receptor-independent mechanisms which exert trophic metabolic effects on murine submandibular glands. Lacassagne and Chamarro (1953) have reported atrophy of submandibular gland of mice after hypophysectomy and its reversal on administration of androgenic hormones. Though various aspects of hormonal regulation of salivary glands are established, it can be said that the details about exact roles played by hormones on the underlying enzymic processes have not been elucidated adequately. It was, therefore, thought desirable to carry out some more work in this direction in order to acquire further understanding of such influences.

It would not be out of place to mention here that earlier work in this laboratory (Ambadkar and Gangaramani, 1976; Ambadkar and Vyas, 1975) under experimental regimes as employed here, has proved that either androgen deprivation or its administration leads to alterations in metabolic processes of different other tissues at very short intervals. Recent 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 rate (MCR) of sex hormones is 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 matter of a few minutes to a few hours) in case of a few important biochemical processes (Weiner et al., 1970; Ambadkar and Gangaramani, 1976; Booth, 1977). 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 yester years. Previous work on male rats in this laboratory has also proved that in less than 48 hr of gonadectomy the circulating androgenic level becomes negligible, hence, replacement therapy was carried out on 48 hr castrates. Only sparse information is available regarding influences of deprivation and of exogenous administration of androgens on the overall pattern of metabolism of submandibular glands of male rats. Hence, experiments were carried out on male albino rats. Deprivation of androgens was brought about through bilateral orchidectomy. Replacement therapy was achieved by administering testosterone propionate (TP) to 48 hr castrates. It was observed that deprivation of androgens brought about transitory variable alterations in the metabolites like glycogen, total lipids, total cholesterol and ascorbic acid. These changes were accompanied by corresponding variations in different enzymes like glycogen synthetase (GS), glycogen phosphorylase (GP), c.AMP-specific phosphodiesterase (PDE), succinate dehydrogenase (SDH), total as well as

Na^+-K^+-ATPase. Results of such changes are dealt with and discussed in Chapters I and II.

In view of variations observed in the submandibular gland due to castration and replacement therapy, it was thought necessary to look into the rapid effects of exogenous administration of TP. Observations were made for the parameters listed earlier, after 1, 2 and 4 hr of TP administration to normal intact rats. The findings are presented and discussed in Chapter VI.

Houssay and Harfin (1954) had shown that administration of 17β-estradiol to castrated and castrated-adrenalectomized male mice for a period of a month caused hypertrophy of submandibular gland. Adrenalectomy reportedly was seen not to have interfered with the effect of castration. It was observed by Berkman and Kronman (1970) that administration of testosterone to spayed females led to alterations in submandibular glands which were comparable to those of the normal male mice. Prosser and Hartman (1983) demonstrated elevation of glucose concentration in saliva of women during pregnency and in non-lactating women preceeding peak mucus days. They concluded that such an increase may result from an increase in glycogen turnover under the influences of estrogens. In the light of these observations, it was thought desirable to study the effect of estradiol administration to intact as well as castrated males for having a wide-based understanding of the problem. Rapid effects of administration of estradiol-17β to normal as well as

castrated male rats on various metabolites and concerned enzymes mentioned earlier and reported and discussed in Chapter VII.

The innervation of salivary glands comprises parasympathetic and sympathetic nerve fibres (Starling and Evans, 1962; 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-type. In cats, parasympathetic stimulation produces copious watery secretion of saliva, whereas sympathetic stimulation produces viscous saliva containing greater proportion of solids; from the submandibular gland (Bell, Davidson and Smith, 1967).

Literature is available on the effects of administration of parasympathetic and sympathetic agonists as well as antagonists on the salivary glands and their secretion in mammals (Schneyer and Schneyer, 1967; Schneyer et al., 1972). Numerous neuroactive agents have been employed to elucidate functioning of salivary glands. Administration of parasympathetic agonist - pilocarpine and acetylcholine - have been shown to reduce the ductal uptake of potassium in the submandibular glands of rat and increase its concentration in secretion (Burgen, 1956; Siegel, 1966). Parasympathetic stimulation is also known to increase acid phosphatase activity of

submandibular glands (Garrett and Kidd, 1977). Administration of atropine, a parasympatholytic agent, produces no secretion but increases the metabolic turnover of the glands. Experiments on dispersed rat parotid acinar cells by Manogs et al. (1975a) have demonstrated that cholinergic substances cause an efflux of potassium. This effect was shown by them to be blocked by atropine.

Sympathetic stimulation regulates the flow of saliva and that it is predominantly effected through the β-adrenergic receptors (Moreno et al., 1984). Studies by Peterson (1981) with isolated fragments of rat parotid and submandibular glands have demonstrated that adrenergic agonists regulate protein content of the secretion via a partially Ca^{++}-dependent route involving β-receptors and c.AMP. Studies on rat parotid by Mangos et al. (1975b)have also shown that administration of isoproterenol (a β-adrenergic agonist) and epinephrine stimulate amylase secretion in the cell suspension medium and enhance the formation of intracellular c.AMP. Further they have shown that this effect was blocked by propranolol (a β-blocker) but not phentolamine (an α-blocker).

It would be relevant here to take notice of earlier work of Novi and Baserga (1972), who have exmined the causes underlying isoproterenol (IPR) induced hypertrophy of salivary gland. These authors had demonstrated that synthesis of r.RNA preceeded that of DNA required for cellular proliferation, thereby suggesting that protein synthesis occurs earlier under the influence of a

β-adrenoagonist (IPR) on salivary cells. As it is a general fact that actual manifestation of protein synthesis occurs after 8 hr, however, the present work is concerned with metabolic alteration occurring at still earlier intervals after administration of neuroactive agents. In rats and mice, enlargement of salivary gland is produced by administering analogs of epinephrine (Selye et al., 1961; Grant, 1961). The enlarging effects brought about by IPR and aludrine - β-adrenergic agonists - are dose-dependent (Seifert, 1966; Abe et al.,1980) and are due to hypertrophy as well as hyperplasia of the gland (Schneyer, 1962; Pohto and Paasonen, 1964). Thus due to multiplicity of functions served by the salivary glands and the variety of physiological processes involved therein, salivary glands have received adequate popularity in the research field.

All the aforesaid studies demonstrate the involvement of adrenergic receptor functions in the metabolism of salivary glands and compositon of saliva. Most of the work reported deals with chronic effects of the drugs. Although, several studies in this field have reported on varied influences of neuroactive agents on the salivary glands, information regarding acute effects on various metabolic aspects are almost lacking. Hence, to have an insight of the acute effects of the administration of β-adrenergic drugs, a study was carried out to investigate alterations induced in the metabolic patterns of submandibular glands within an hour of administration of isoproterenol IPR (a β-agonist) and propranolol PPN (a β-antagonist) in three different intraperitoneal doses viz. -

15, 25 and 35 mg/Kg b.w. and 25, 35 and 45 mg/Kg b.w., respectively, to adult male albino rats. Experiments were carried out after 5, 10 and 60 min of drug administration. Metabolites like glycogen, total lipids, total cholesterol and ascorbic acid contents were studied only at 60 min interval, while enzyme activities of glycogen synthetase (GS), phosphorylase (PS), c.AMP phosphodiesterase (PDE), Na^+-K^+-ATPase, total ATPase, SDH and aldolase were assayed after 5, 10 and 60 min. The results are dealt with in Chapters III, IV and V. The data obtained vividly revealed the antagonistic influences of propranolol and isoproterenol; as far as the metabolic patterns of submandibular glands of rats are concerned.

In animal tissues, sialic acids (SA) occur as constituent of mucoproteins, mucolipids and lipoprotein-carbohydrate complexes. Ubiquitous distribution of sialomucoproteins in animal surface secretions and excretions indicates a protective rather than structural function. Sialomucoproteins are regular components of viscous mucins covering the epithelial surfaces in direct contact with surroundings. SA contents in various tissues have been known to alter under the influence of hormones. Deprivation of androgens was reported to reduce SA content in spermatozoa, luminal plasma of epididymides and vasa deferentia of hamster (Bose and Prasad, 1975) and epididymides and Cowper's gland of rats (Bose et al., 1977) and in dog epididymides (Dixit, 1976).

Based on the above information it was thought pertinent to see whether the SA content of submandibular gland of rat is influenced by neuroendocrine manipulations. Hence, total submandibular SA content was estimated after androgen deprivation, exogenous administration and replacement with TP, and also after estradiol 17-β administration to 48 hr castrates as well as normal intact males. The effects of IPR and PPN administration to intact male rats on SA content of the submandibular gland were also studied to gain an understanding about possible role of β-receptors on this important parameter. Details about such influences are discussed in Chapter VII.

Interest in the study of physiology and biochemistry of salivary glands was stimulated owing to its involvement in various diseases. Enlargement of salivary glands is found in large number of diseases (Rauch, 1959; Thoma and Goldman, 1960; Seifert, 1964 & 1966). In diagnosis special consideration is given to sialdentis, salivary gland tumours and disturbances of secretion (Dyschylia). Dyschylia occurs mainly due to sialadenosis and sialolithiasis. Sialadenosis is a frequent non-inflammatory condition of salivary glands, occurring usually due to disturbances of glandular metabolism as well as secretion of saliva. The condition is normally not associated with pain, and involves bilateral enlargement of the glands, especially the parotid (Seifert, 1964a, b, c & 1966). Sialadenosis is frequently concomitant with pluriglandular disorders such as diabetes mellitus, hypofunction of gonads, Kwashiorkor syndrome,

cirrhosis of liver and chronic alcoholism. The present findigs also suggest that functioning of the submandibular glands may well be conditionally linked in some as yet unsuspected/unrecognized manner with the status of other endocrine organs. Possible implications of clinical significance have been highlighted at appropriate places in the Chapters that follow.