GENERAL CONSIDERATIONS

The salivary glands of mammals are typical tubuloalveolar structures. The cells lining the alveoli are known as end-piece cells/demilunes (Yang and Van Lennep, 1978). The salivary glands consist of three cell types- serous, mucous and seromucous cells. Submandibular (submaxillary) glands have both serous and mucous cells in roughly equal proportions. The system of ducts of salivary glands comprises of intercalated, striated/granular and excretory ducts. The glandular secretion is known to undergo modification through 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 are concerned (Winsten *et al.*, 1989). The innervation of salivary gland comprises of parasympathetic as well as sympathetic nerve fibers (Best and Taylor, 1985).

As early as 1940(a), Lacassagne demonstrated sex-dependent differences in the histological structure of mice submandibular glands. In case of mice the glands of both sexes exhibit similar cells during development, but during maturation the glands develop sex-dimorphism (Gresik and Edith, 1975). 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 (Kochakian and Hall, 1955), tryptophan and tyrosine (Junqueira, 1949; Kronman, 1963a & b), β-glucuronidase (Hosio *et al.*, 1978) and oxygen consumption (Wilborn and Fitzgerald, 1964). However, in rat salivary glands according to Schneyer *et al.*(1972) sex-dependent morphological differences are not so 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 development 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;Takeuma *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 been observed by several authors (Kullander and Sonesson, 1965; Oster and Yang, 1971; Puskullan, 1972; Davis and Ballin, 1973; Ben-Ayreh *et al.*, *1976;* Prosser and Hartman, 1983). Changes in salivary contents are known to occur during menstrual cycle (Puskullan, 1972; Ben-Ayreh *et al.*, 1976) and pregnancy (Kullander and Sonesson, 1965) in women.

Though various aspects of hormonal influence on components of secreted mixed saliva are known; the same can not be said regarding the role of hormones on enzymic processes concerned with glandular metabolism and secretory functions adequately. It was, therefore, thought desirable to study influence of each of the stages of normal 4-day estrous cycle on submandibular gland metabolism. From the results obtained following observations can be made:-

1) Diestrous stage of the cycle is known to be a quiescent phase and minimal titres of ovarian hormones prevail then (Hori *et al.*, 1968; Brown-Grant *et al.*, 1970; Naftolin *et al.*, 1972; Butcher *et al.*,1974). During this stage the enzymic activities under investigation registered minimal levels. In all probability, lower estrogenic level favoured protein anabolism as was evident from its glandular concentration (24.467 mg/100 mg tissue), and this conforms to the suggestion of Aschkenasy-Lelu and Aschkenasy, 1959).

2) However, when one considers the transition from diestrous to proestrous stage one encounters with rising level of estrogen. This hormonal shift was observed to gear up metabolic machinery for building of glandular glycogen (0.136 mg/100 mg of tissue) and total lipid (9.467 mg/100 mg of tissue) concentrations.

3) As the female rat start coming into estrous condition the sensitive tissues, already primed by estrogen, come under the influence of preovulatory LH surge and increasing influence of progesterone. Under these circumstances it was noted that the c.AMP-PDE activity was at its maximum level resulting in quick lowering of intracellular c.AMP. This is known to enhance the activity of glycogen synthetase (Lamer, 1966; Drumond *et al.*, 1969; Hers *et al.*, 1970; Rindi, 1971). Accordingly, glycogen concentration of submandibular gland was noted to rise despite the fact that glycogen phosphorylase too, exhibited a slight increase. Here enhanced glycogen synthesis of the submandibular gland was probably supported by the higher glycemic level noted during this phase. It can also be said that the hormonal *milieu* in the submandibular gland, in general, was in favour of protein synthesis.

4) Noticeable reduction in glycogen concentration of submandibular gland noted during metestrous stage can be ascribed to abruprt drop in c.AMP-PDE activity leading to adequate availablity of c.AMP facilitating the glycogen phosphorylase enzyme activity. Simultaneously, reduction in glandular protein and lipid concentrations may be considered to indicate predominance of overall catabolic trends during the metestrous stage.

From the above account it can be safely said that cyclic variations in the secretions of ovarian hormones do have subtle influences on the metabolic machinery of this gland. Such changes in the metabolic patterns may be attributed to varying degrees of sensitivity of respective hormone-specific receptors that were demonstrated to be present in this gland (Campbell *et al.*, 1990). If this contention is true then the experimental manipulations of ovarian hormones should get

manifested in alterations of the parameters discussed so far. With this veiw further work was conducted after ovariectomy, 17β–estradiol and 17β–estradiol plus progesterone replacement.

Only sparse information is available regarding influences of deprivation and replacement of ovarian hormones on the overall metabolic pattern of submandibular gland of female rat. Hence, experiments to unveil possible influences of female sex hormones on metabolic aspects of submandibular salivary gland were carried out. 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.

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 gland after 1, 2 and 4 H of hormone administration. 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. Combination replacement therapy was achieved by administering a fixed dose of 2 mg progesterone simultaniously with each of the three doses of 17β-estradiol viz.-5, 10 and 15 μg.

It was noticed that the glycogen phosphorylase enzyme activity was virtually not affected by ovariectomy but the glandular glycogen concentration and c.AMP-PDE activity were enhanced at 24 and 48 H post-ovariectomy intervals. However, by 72 H post-ovariectomy interval these parameters were found to get more or less restored to normal condition. The initial increase in glycogen concentration could be attributed to relative lack of availability of c.AMP and thereby stimulating glycogen synthetase activity, combined with increased uptake of glucose from blood. The latter being possible due to sudden rise in Na^{+}-K^{+} ATPase activity at 24 H and sustained incorporation of blood glucose, taken up initially, into glycogen upto 48 H. The overall inference that could be drawn from recorded data upto 72 H post-ovariectomy is that, initally there is a general acceleration of cellular metabolic activities, but by 72 H there is restoration of most of the parameters to a normal state. This means that ovariectomy immediately alters certain metabolic reactions, but by 72 H most of the glandular metabolic processess get readjusted even in the absence of the gonads.

Maximum influence of different doses of 17β-estradiol was noticeable by 2 H post-injection. However, 15 μg dose was found to be more effective in bringing about better reparative influence, but at 4 H interval. In general, it was apparent that 17β-estradiol administration counteracted the changes due to ovariectomy by favouring a sort of overall catabolic tendency. Such an influence was evident from greater influence on Ca^{++}- and Mg^{++}-activated ATPases than the Na^{+}-K^{+}-ATPase. 17β-estradiol plus progesterone combination regime proved to be quite effective in bringing about reversal of the catabolic pattern of metabolism induced by 17β-estradiol alone in the submandibular gland of 48 H ovariectomized rats. Further, it was noted that 10 μg 17β-estradiol plus 2 mg progesterone combination could bring about restoration of functional state of submandibular gland of ovariectomized females more effectively; particularly at 2 H interval. Another important inference was that the variations in glycemic levels did not depend on the status of either glandular glycogen concentration or phosphorylase enzyme

activity. By way of extention, on the basis of work carried out on the hepatic tissue of female rats under similar experimental conditions (Wagh, 1994), it can be surmized that a synergistic action of the combined replacement regime on liver might have been responsible for glycemic variations.

In the light of the foregoing observations the present author would like to suggest that there do occur certain subtle alterations of metabolic processes in submandibular gland of female rat as early as 48 H after ovariectomy and these could be brought back to a good extent by replacement with combination of 17β-estradiol plus progesterone and not with only 17β-estradiol. As was noted beyond 72 H after ovariectomy by earlier workers the alterations seem to settle down to some patterns which in all probability would lead to those reported in the past. However, it should be pointed out that the time gap between the present observations and those reported after several days of gonadectomy as well as extended replacement regimes would be interesting to investigate for bridging the gap to unveil a coherent picture of physiological adaptations to ovariectomy by female rats.

Sialic acid (SA) occurs as an important and major constituent of mucoproteins, mucolipids and lipoprotein-carbohydrate complexes. Naturally, this is also applicable to the escertion of saliva by the salivary glands. 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 administration of appropriate 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.

The results obtained do exhibit some conformity with previous report of Rybakova (1978) on mucopolysaccharides of rat submandibular gland. According to Oster and Yang (1971), the salivary SA content in normally cycling women also show cyclic variations. There observations and those presented here clearly indicate influence of ovarian hormones on sialic acid concentration of submandibular glands in female rats. The minimal sialic acid concentration in submandibular gland was noted during estrous stage. The same increases during the metestrous and diestrous stage reaching peak level, once again starts dropping down during proestrous. From these observations it can be said that rising titers of estrogens apparently suppress synthesis of sialic acid, but when the gland comes under increasing influence of progesterone glandular sialic acid build up is facilitated. However, ovariectomy was found to reduce glandular sialic acid concentration to a certain extent. This may be so due to comparative lack of both estrogen and progesterone. This contention finds support in the results obtained after 5 μg of E_2 admnistration by 2 H, when the level was more or less similar to estrous condition(lowest recorded level). Further 5 μg of E_2 plus 2 mg of P administration brought the sialic acid concentration in the gland close to metestrous level (higest recorded level) within 2 H. This also supports the view that both hormones favour build up of sialic acid in the gland as is normally seen at metestrous stage. From a different prospective angle, it could be surmized that, in all probablity, the saliva may become watery in consistancy during estrous stage, when the animals are known to resort to licking of the genital region. On the other hand, during metestrous and diestrous stages increased amounts of sialomucins may find their way in the secreted saliva making it more viscous.

It seems logical to suggest that about 5 μg of E_2 and 5 μg plus 2 mg progesterone are better replacement than the other doses tried out. The 15 μg of E_2 and 2 mg of progesterone dose is certainly non-physiologicle in its influence as far as this parameter is concerned. One more inference that could be drawn from the results of 48 H of ovariectomy is the possiblity of an adverse influence on the sensitivity of the submandibular gland to subsequent administration of hormones; probably due to the effect on the hormone receptors of the submandibular gland either quantitatively or qualitatively during that brief period of 48 H.

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). 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, most of the 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, and the possible effects on metabolic patterns thereof, seen in relation to the parameters dealt within earlier chapters. Histochemical localization of three hydroxysteroid dehydrohgenases did exhibit noteworthy variations in localizations as well as intensities in phase with the stages of the estrous cycle. In general, wherever the positive enzymic reactions were obtained the localization was usually

more intense in the ductal system than in the acinar regions. Earlier studies mostly under *in vitro* and histochemical conditions did report on presence of above enzyme activities commenting that more intensities were met with in the ductal system of the gland (Sirigu *et al.*, 1982; Bloom *et al.*, 1993), but none of these say anything about variations due to ovarian cyclicity. Due to lack of such reference to stages Sirigu *et al.* (1982) were led to state that 3α-HSDH was not detectable histochemically in human salivary gland. From the present report it is clear that though 3α-HSDH is absent during estrous stage it is easily demonstrable in diestrous and proestrous stages. Moreover, Furuyama *et al.* (1990) succeded in isolating 3α-HSDH from cytosol of rat submandibular gland, having a 30 KD m.w. The initiation of this enzyme activity during metestrous and progressive increase upto proestrous stage is a pointer to the variable sensitivity of submandibular gland that is dependent on the stages of the cycle. It may be surmized that the role of 3α-HSDH through these stages facilitates formation of DHT, a potent androgen, substantiating androgen dependence of the gland even in female rats. It is known that during the proestrous stage estrogen titre goes high causing catabolic influence on glandular sialic acid and protein. A noteworthy point was absence of 3α-HSDH activity during the estrous stage. These two facts may favour full expression of metabolic influence of estrous stage on these two parameters Hence, atleast during this stage of estrous cycle, the submandibular gland of female rat is estrogen dependent. This contention is supported by lack of preference for testosterone and androsterone as substrates by 17β- and 3α-HSDH enzyme activities during estrous and metestrous stages. Probably the rising level of progesterone during metestrous stage sets in a shift towards greater enzymic affinities for estradiol and DHEA which may reinitiate androgen dependence. One of the metabolic effects of this state was reflected in increasing sialic acid concentration in submandibular gland; probably leading to more viscous saliva being secreted in consonance with licking behaviour of the animal during metestrous stage. It appears that minimal ovarian hormone levels influence submandibular gland metabolism in directing it more towards synthetic activities. Kyakumoto *et al.* (1986) and Furuyama (1989) have clearly demonstrated sex-

dependent differences in the distribution of cytosolic and nuclear androgen receptors of mouse and rat submandibular glands. Colaterally, it is also known that the sex hormone-receptor populations are influenced by female sex hormones (Jenne *et al.*, 1978; Robel *et al.*, 1981; West *et al.*, 1983) and this may be the basis of cyclic variations in the manifestation of hydroxysteroid dehydrogenases and their subsequent metabolic influences. The recent literature on sex hormone-receptors, their influences on hormone metabolising enzymes, regulation of receptor populations and subcellular distributions may be said to corroborate the above statement.