CHAPTER-2

INFLUENCE OF ESTROUS CYCLICITY ON CERTAIN METABOLIC ALTERATIONS IN SUBMANDIBULAR GLAND OF FEMALE RAT.

It is well known that there exists sexual dimorphism in case of rodent submandibular gland and that administration of sex hormones leads to structural changes in both sexes (Laccasagne, 1940a; Janqueria *et al.*, 1949; Shafer and Muhler, 1953; Atkinson *et al.*, 1959; Mudd and White, 1975; Hatakeyama *et al.*, 1987; Rumio and Pardini, 1989; Jayasinghe *et al.*, 1990; Yang *et al.*,1993). Liu *et al.* (1969) observed that long term estradiol benzoate treatment causes a significant decrease in tubular diameter of submandibular gland of female rats. In adult rats, the distribution and size of granules in the granular ducts of the submandibular gland were shown to increase due to estrogen (Mudd and White, 1975). Rumio and Pardini (1989), in their review, have also referred to the presence of several biologically active polypeptides in granular duct region exhibiting sexual dimorphism. Laine and Tenovuo (1983) have demonstrated definite estrogen responsive nature of rat salivary glands with respect to peroxidase enzyme activity.

Specific androgen and estrogen binding capacities have been observed in the mouse and rat submandibular glands (Verhoeven and Wilson, 1976; Verhoeven, 1979; Laine and Tenovuo, 1983; Sakabe *et al.*, 1987). Furthermore, it has also been reported that 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 gland may serve to promote gender-based differences with respect to submandibular-EGF content; possibly to mediate changes in saliva composition during the female reproductive cycle and also for regulating the release of EGF for cyclic uterine growth.

Changes in salivary contents are known to occur during menstrual cycle (Puskulian, 1972) 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 (Oster and Yang, 1971) in saliva have been proposed as clinical indicators of ovulation. Significantly elevated phosphate concentration was observed at mid-cycle in normally ovulating women by Ben-Ayreh *et al.* (1976).

Some literature is available on the possibility of correlation of enzymic activities of saliva with the onset of ovulation. Boyer and France (1976) reported an increase in salivary alkaline phosphatase activity at the time of ovulation in women. On the contrary, Cockle and Harkness (1978) could not find any change in this enzyme activity at the time of ovulation. Such contradictory observations were probably due to variation in methods of collection as well as processing saliva samples. Treves *et al.* (1986) reported on daily changes of lactate dehydrogenase (LDH) activity in both saliva and cervical mucus. These authors found optimum activity at mid-cycle in case of both the secretions. Peroxidase enzyme activity of both stimulated (Tenovuo *et al.*,1981) as well as unstimulated (Cockle and Harkness, 1978) saliva showed peak activity at the midcycle of normally ovulating women. Salivary lactate dehydrogenase and leucine aminopeptidase activities have been reported to show peaks at the mid cycle (Pal and Bhattacharya, 1989). On this basis, these authors suggested that assaying salivary enzyme activities may prove to be a convenient way for detecting the day of ovulation.

In the light of above-cited information it is logical to expect metabolic alterations of the salivary glands under the influence of cyclically varying patterns of sex hormones. It was, therefore, thought desirable to investigate possible changes in certain metabolites as well as concerned enzyme activities different phases of the estrous cycle of laboratory rats. This would throw some light on functional role of salivary glands as affected by estrous cyclicity.

MATERIAL AND METHODS

Adult female albino rats weighing 140 ± 20 gms served as experimental animals. Rats were maintained on balanced diet and water *ad libitum*. Only those animals which had normal 4-day estrous cycle were utilized in this study. The stages of estrous cycle were confirmed by observing vaginal lavages daily at 09:00 am. Animals were sacrificed at each stage of estrous cycle under mild ether anaesthasia. Submandibular glands were excised and freed of connective tissue. Following parameters were assayed as per the methods described in chapter-1 :-

Glycogen
Total lipid
Cholesterol
Total protein
Glycogen phosphorylase
Cyclic AMP-specific phosphodiesterase
Total ATPase
Na^+-K^+ ATPase
Succinate dehydrogenase
Plasma glucose

RESULTS

The results showed a sustained but graded reduction of glycogen concentration through proestrous to diestrous stages (Table 2.1). It was well marked during transition from estrous to metestrous Total phosphorylase and succinate dehydrogenase (SDH) activities were found to increase from proestrous to metestrous, however, by diestrous stage a marked reduction was noticed. The c.AMP-specific phosphodiesterase, total ATPase and Na^+-K^+ ATPase activities were found to be enhanced from proestrous to estrous, however, reduction was observable at metestrous with further reduction at diestrous stage. The highest

TABLE-2.1

Showing influence of cyclic variations of ovarian hormones on different parameters of submandibular gland of rat.

Parameters	Diestrous	Proestrous	Estrous	Metestrous
Glycogen	0.066	0.136[a]	0.121[a]	0.078[c]
mg/100 mg tissue	± 0.006	± 0.008	± 0.007	± 0.005
Phosphorylase	69.040	98.848[a]	108.750[a]	127.650[a]
µmoles PO_4 released/ mg protein/ H	± 03 505	± 04.246	± 003.812	± 002.512
c AMP-PDE	4.844	6.226[c]	8.689[a]	5 736
µmoles PO_4 released/ mg protein/ H	± 0.515	± 0 276	± 0.519	± 0.426
Total ATPase	200.328	278 392[a]	307.794[a]	274.731[a]
µmoles PO_4 released/ mg protein/ H	± 012.000	± 005.646	± 006 640	± 013.020
Na^+-K^+ ATPase	41.824	79 509[a]	83.283[a]	72.591[a]
µmoles PO_4 released/ mg protein/ H	± 03 174	± 05.596	± 02.200	± 04 807
SDH	30.304	41.914[a]	42.095[a]	50 957[a]
mg formazan formed/ mg protein/ H	± 01.726	± 02.306	± 02.238	± 02 152
Protein	24.467	11.871	18.227	15.079
mg/ 100 mg tissue	± 01.446	± 00.409	± 00.662	± 00.283
Plasma Glucose	112.500	84.600[a]	139.500[a]	101.250[a]
mg/ 100 ml plasma	± 002.012	± 001.374	± 002.010	±001.006

Values are mean ± SE (n = 8)

a - P<0.0005; b - P<0.005; c - P<0.05

Levels of significance have been calculated with reference to diestrous values.

TABLE - 2.2

Showing influence of cyclic variations of ovarian hormones on different parameters of submandibular gland of rats.

Parameters	Diestrous	Proestrous	Estrous	Metestrous
Lipid mg/ 100 mg tissue	6.846 ± 0 561	9.466[c] ± 0.712	11.567[a] ± 00.98	7.234 ± 00.510
Cholesterol mg/ 100 mg tissue	0.775 ± 0.045	0.775 ± 0.034	0 882 ± 0.067	0.908 ± 0 083

Values are mean ± SE (n = 8)

a - P<0.0005; b - P<0.005; c - P,0.05.

Levels of significance have been calculated with references to diestrous values

level of total protein concentration in submandibular gland was recorded during diestrous stage. It was found to be reduced drastically ($P<0.0005$) in the proestrous stage, whereas a significant increase ($P<0.0005$) was noted while estrous lasted. Again, during the metestrous stage the same was found to be lowered. Thus, the fluctuations in protein concentrations were very obvious.

Total lipid concentration was found to increase from diestrous to estrous. It was reduced at metestrous stage. Cholesterol concentration did not show any significant alterations; except that its level was highest at metestrous stage.

Plasma glucose level was maximum at estrous and lowest during proestrous.

DISCUSSION

The data presented here showed variations worth noticing in respect of most of the parameters under study. During transition from diestrous to proestrous stage (the follicular phase) rising level of estrogen (Horn *et al.*, 1968; Brown-Grant *et al.*, 1970; Naftolin *et al., 1972;* Butcher *et al.*, 1974) might have been responsible for increase in glycogen concentration even though there was an apparent increase in phosphorylase activity. On the basis of data on hand, however, it is not possible to explain what exact role could the increasing level of c.AMP-specific phosphodiesterase be playing in this context. Taking into consideration the variations in the ATPase activities and those of plasma glucose alongwith previously published report (Ambadkar *et al.*, 1994) on increased glycogen synthetase activity, it can be suggested that there is an enhanced uptake of glucose and incorporation of the latter into glycogen. Under these transitional circumstances; it can be seen that the metabolic condition were in favour of increase in total lipids, but with a reduction in cholesterol percentage. It can also be said that there appears to be a general hightening of oxidative metabolism of the glandular tissue as is evident from the rising level of SDH activity.

Considering the alterations from proestrous to estrous, it could be seen that, the estrogen primed tissue would come under gradually increasing influence of progesterone as well as the pre-ovulatory surge of LH (Goldman *et al.*, 1969; Barraclough *et al.*, 1971). These circumstances could have produced a condition that was probably responsible for observed maximum level of c.AMP-PDE activity. Logically, this should lower intracellular c.AMP concentration, which situation may be expected to lead to acceleration of glycogen synthesis, eventhough there was some increase in phosphorylase activity. Glycogenolytic effect of the latter could not, as yet get manifested so significantly, as was evident from more or less sustained glycogen concentration of the salivary gland during this period. Additionally, the SDH activity was also noted to have increased. Therefore, it could be surmized that during this transition of stages of estrous cycle, glandular metabolic pattern is hightened, in general. However, if one takes into consideration the sustained Na^{+}-K^{+} ATPase activity observed here and the previously reported increased activity of glycogen synthetase (Ambadkar *et al.*, 1994) it explains continued uptake of glucose by acinar cells and its incorporation into glycogen to a comparatively similar level to that of previous period. Here it may be cited for the sake of comparison that, Sladek (1977) could demonstrate influence of estrous cyclicity on hepatic glycogenesis and gluconeogenesis, both being affected differentially. He has opined that fluctuations in plasma levels of ovarian steroids are sufficient enough to cause alterations in gluconeogenesis and hepatic glycogen metabolism. The present findings on salivary gland, though at some variance in stage-wise details, corroborate this opinion regarding the influence of variations in ovarian steroid hormonal levels on metabolic processess of sensitive organs. The situation in respect of total lipid concentration and its ratio with cholesterol too, remains similar to that of the earlier period. In a different context, Biswas and Mukherjea (1973) reported that in uterus, liver and kidney of female rats the total lipid and cholesterol concentrations are affected significantly during estrous and diestrous stages of the cycle. Present findings were at variance in that the highest level of lipids were observable during estrous stage as compared to minimal level in uterine tissue studied by Biswas and Mukherjea

(1973). This is probably a good proof of organ-specific differences in response to ovarian steroids. However, it is obvious in the present circumstances that the hormonal *milieu* was more in favour of protein synthesis.

The transition from estrous to metestrous, led to significant reduction in c.AMP-PDE permitting enhanced availability of intracellular c.AMP that would result in stimulation of the activity of glycogen phosphorylase (Hers *et al.*, 1970; Jain, 1978) . At the same time Na^{+}-K^{+} ATPase also exhibited reduced activity. This became obvious from the abrupt but significant drop in the glycogen concentration of the gland. Additionally, the glandular protein and lipid concentrations were also seen to be reduced. Reduction in glandular lipid was accompanied by rise in cholesterol percentage. It is, therefore, obvious that during metestrous stage catabolic trend appears to gain predominance.

For proper comprehension of the foregoing account it is necessary to take into consideration the alterations during transition from metestrous to diesterous. In this context, it can be easily seen that when the circulating gonadal hormones are at minimum levels (Hori *et al.*, 1968; Butcher *et al.*, 1974) the enzyme activities registered minimal levels. Total lipid and glycogen concentrations were seen at their lowest levels, the only obvious exception is that of glandular protein. Therefore, one can say that glandular metabolism gets geared up exclusively for protein synthesis. It may be added here that further work is essential for substantiating the tentative conclusions mentioned here.