CHAPTER - 6

INFLUENCE OF OVARIAN HORMONES ON THE SIALIC ACID CONCENTRATION IN THE SUBMANDIBULAR GLAND OF FEMALE ALBINO RATS.

It is a well documented fact that sialic acid (SA) exhibits wide occurence in animal tissues as an important constituent of sialomucins, mucoproteins and lipoprotein-carbohydrate complexes. Sialic acid as an ubiquitous component of complex carbohydrate moities seemingly imparts varied significant biological properties. Sialic acid constitutes predominent portion of the carboxylated mucosubstances (Smith and Frommer, 1975). Such wide distribution of sialo-mucoproteins in animal secretions and excretions indicates a protective rather than a structural function. As a rule cells of the animal body do not possess rigid cell walls. The significance of sialo-mucoproteins as regular components of the viscous mucins covering the epithelial surfaces of respiratory, digestive and uriogenital tracts is rather obscure.

Reports are available on the influence of normal cyclic variations of female sex hormones, gonadectomy, replacement with female sex hormones and synthetic estrogenic compounds on SA content of various mammalian tissue and secretions. The levels of SA in the ovaries, oviduct, uterus, vagina, pitutary gland, thyroid gland, blood serum and urine varies during the estrous cycle of the rat (Jensen, 1967). Carborg (1966) observed high level of vaginal SA content in mouse during pregnancy, who opined that this could be due to the simultaneous action of progesterone and estrogen. In laboratory rats; the plasma SA content was shown to decrease after spaying and increase with administration of estradiol benzoate (Houssay and Blumenkrantz, 1964 and Curbelo *et al.*, 1974a). Coppola and Ball (1966) showed that uterine SA concentration in rat was directly proportional to estrogen levels and inversely proportional to that of progesterone. Galletti and Gardi (1972) reported increase in SA content of rat vagina after ovariectomy and decrease after subsequent treatment with estradiol. On the other hand,

ovariectomy in gerbils has been reported to bring about depletion of uterine and vaginal SA contentsand administration of cyproterone acetate depleted it further (Dixit and Arya, 1975).

Estrogen have been shown to lead to an increase of protein-bound sialic acid in human serum, either during pregnancy (Rajan *et al.*, 1980) or during prolonged application of contraceptives (Klinger *et al.*, 1981a & b), followed by a decline at parturition or discontinuation of the drug. In case of women, the concentration of SA in cervical mucus decreases significantly at mid-cycle and increases after ovulation (Moghissi *et al.*, 1975). The urinary SA level was found to vary during the menstrual cycle. The pattern of variation being characterized by a near-mid cycle SA excretion peak. It was suggested that the mid cycle SA excretion peak is associated with the time or approximate time of ovulation. Saliva from normally mensturating women has been reported to exhibit a ferning pattern in the proliferative phase of the cycle which disappears in the secretory phase (Androli *et al.*, 1957; Kullander and Sonessan, 1965). The ferning phenomenon of saliva is associated with the physicochemical properties of mucopolysaccarides which, in turn have been observed to be governed by the sialic acid content of the negatively charged macromolecules. Later on, Oster and Yang (1971) and Tenovuo *et al.* (1983) have shown cyclic variation of SA content of saliva and that the SA content of whole saliva is at its minimum close to mid-cycle.

Ravetto *et al.* (1965) reported on the occurence of SA in the submandibular glands of rats. Poddar and Jacob (1978) demonstrated histochemically the presence of carboxylated sialomucins in mongoos salivary glands. Denny and Denny (1984) observed diurnal variation of sialomucin concentration in female mouse submandibular glands. Further, Denny *et al.* (1991) have also reported about effect of aging in mice on mucine content of submandibular gland. Kuyatt and Baum (1981) reported age and sex dependent difference in SA content of submandibular glands of rat. Various researchers have shown that the submandibular glands of rodents are androgen and estrogen sensitive (Kronman

and Spinale, 1965; Laine and Tenovuo, 1983 and Desai, 1989). In male rats, castration was shown to lead to reduction in SA concentration in the submandibular gland, while replacement with testosterone propionate increased the same (Zebrowski, 1973 ; Curbelo *et al.*, 1974b; Desai, 1989). On the other hand, with respect to mice, Devalle (1968) reported decrease in submandibular gland SA content after replacement with testosterone propionate. Curbelo *et al.* (1974a) observed non-significant alteration due to ovariectomy in rats. Further, these authors have shown that long term (1 month) administration of estradiol benzoate to ovariectomized rats resulted in increase in SA content of submandibular gland.

Careful perusal of the literature cited in fore-going account reveals the fact that most of the studies on SA content of salivary glands/salivary constituents were studied over comparatively longer periods of time after either ovariectomy or subsequent administration of gonadal hormones. However, in the light of recent progress dealing with influence of hormones on metabolism it has been well recognized that effects of hormonal manipulations get manifested within a matter of few hours. Hence, the present study was carried out after much shorter intervals of experimental manipulations of sex hormones to understand the influence of normal cyclic variations of female sex hormones to compare with rapid effects of ovariectomy and replacement with ovarian hormones on SA concentration of submandibular glands of female rats.

MATERIAL AND METHODS

Adult female albino rats weighing between 140 $\pm$ 20 gms served as experimental animals. Only those animals which had normal 4-day estrous cycles were utilized for this study. For the study of normal cyclic variations, animals were sacrificed at 09:00 am at each stage.

OVARIECTOMY (OvX) :- Only the diestrous females were bilaterally OvX or sham-operated. Following three post OvX intervals were selected to study the effects of OvX on SA concentration of submandibular glands i.e. 24, 48 and 72 H.

REPLACEMENT WITH 17β-ESTRADIOL (E_2) :- 48 H ovariectomized females were injected intramuscularly with 0.1 ml of vehicle (propylene glycol) alone, serving as control or with 0.1 ml of E_2 solution containing three differeent doses of 17β-estradiol *viz.*- 5 (D-1), 10 (D-2) and 15 (D-3) ug as a single injection at 09:00 am. The animals were sacrificed at the end of 1, 2 and 4 H post-injection intervals.

SIMULTANEOUS REPLACEMENT WITH 17β-ESTRADIOL(E_2) AND PROGESTERONE (P) :- 48 h OvX animals were injected with 0.5 ml of vehicle alone to serve as controls. Individuals of different experimental groups, each comprising of ten 48 H OvX females, were injected with three different doses of E_2 i.e. 5 (CD-1), 10 (CD-2) and 15 (CD-3) μg simultaneously, in each case, with 2 mg of P dissolved in 0.5 ml of propylene glycol. All the hormone replaced as well as control females were sacrificed after two H of injection.

The submandibular glands were excised immediately after sacrificing, freed of connective tissues and weighed accurately (upto 0.1 mg). The glandular tissue was hydrolysed serially in weak (0.5 N and 0.1 N) sulphuric acid solution for one hour each. The hydrolysate was eluted successively over Dowex-2 and -50 columns and the total SA concentration was assayed colorimetrically as per the method of Svennerholm (1958), and was expressed as μg SA/100 gms fresh tissue weight. Statistical analysis was carried out employing student's *t* test. As it was noted that after sham-operation the females showed normal estrous cyclicity, the results obtained with sham-operated females were not taken into consideration. The obtained results were therefore compared with the values obtained in case of intact diestrous females. Similarly, the vehicle injected 48 H

OvX rats, did not show any variation when compared with 48 H OvX results, hence, the readings were considered as redundant.

RESULTS

NORMAL ESTROUS CYCLE :- Sialic acid concentration in the submandibular gland showed cyclic variation. The SA concentration exhibited a statistically significant graded reduction from diestrous to estrous, the latter being the lowest concentration recorded. The highest SA concentration was recorded during metestrous stage.

EFFECT OF OVARIECTOMY :- The SA concentration was observed to get reduced significantly, with reference to diestrous level, after OvX within 24 H. Thereafter, as the time elapsed, it showed further gradual decrease upto 72 H.

EFFECTS OF REPLACEMENT WITH 17β-ESTRADIOL :- The results obtained with all the three intervals after administration of D-1 revealed reduction, with reference to the level of 48 H spayed female, in SA concentration of the submandibular gland. Within an hour after D-2 administration SA concentration of the submandibular gland showed increase upto the level of 48 H spayed value. Thereafter, it increased gradually by 2 and 4 H intervals going above the 48 H spayed level. Thus, this dose of 17β-estradiol could bring about fairly good recovery of glandular SA concentration. Results obtained after administration of D-3 were not at much variance with those of D-2, neverthless, by 2 H interval maximum level of glandular SA concentration was registered; though it was noticeably below the normal diestrous level.

EFFECTS OF REPLACEMENT WITH 17β-ESTRADIOL PLUS PROGESTERONE:- The results revealed increase in SA concentration of the submandibular gland at 2 H with three different combinations of E_2 plus P administered. However, the best possible response leading to restoration of SA

TABLE - 6.1

Variations of sialic acid concentration in submandibular gland as influenced by normal 4-day estrous cycle expressed as µg/100 g of fresh tissue weight.

Stages of estrous cycle			
Diestrous	Proestrous	Estrous	Metestrous
198.714 ± 002.228	167.714[a] ± 002.447	126.857[a] ± 003.454	218.166[a] ± 001.816

Vales 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 - 6.2

Effects ovariectomy after 24, 48 and 72 H on sialic acid (µg/ 100 mg of fresh tissue weight) concentration in submandibular gland.

Diestrous female normal	Post-operative intervals		
	24 H	48H	72H
198.714 ± 002.228	159.600[a] ± 002.054	152.400[a] ± 005.635	143.333[a] ± 003.158

Vales 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 - 6.3

Influence of 5 (D-1), 10 (D-2) and 15 (D-3) μg of 17β - estradiol administration on glandular sialic acid (μg/ 100 g of fresh tissue weight) levels.

Dosage	Post-injection interval		
	1 H	2 H	4H
5 μg	92.344[a]	120.879[a]	138.057[a]
	05.031	004.722	003.567
10 μg	157.978	167.444[c]	173.743[c]
	002.461	006.466	005.506
15 μg	167.149[c]	169.557[b]	166.098[c]
	002.032	001.333	001 617

The value of 48 H OvX female was 152.400 μg/ 100 g of fresh tissue weight
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 - 6.4

Effects of simultaneous administration of a fixed 2 mg dose of progesterone along with 5 μg E_2 (CD-1), 10 μg E_2 (CD-2) and 15 μg E_2 on glandular sialic acid (μg /100 g of fresh tissue weight) concentration.

Dosage	Post injection interval
	2 H
5 μg E_2 + 2 mg P	217.428[a]
	002.546
10 μg E_2 + 2 mg P	201.833[a]
	001.801
15 μg E_2 + 2 mg P	278.500[a]
	002.195

The value of 48 H OvX female was 152.400 μg/ 100 g of fresh tissue weight.
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.

concentration close to that of diestrous stage, during which stage of the cycle the female were spayed, was observable with 10 ug E_2 plus 2 mg P (CD-2) combination dose, whereas that with CD-1 and CD-3 could be considered as leading to abnormally high levels.

DISCUSSION

The results reported here do exhibit some conformity with previous report of Rybakova (1978), who studied the mucopolysaccharides of rat submandibular gland. Oster and Yang (1971), working on salivary SA content in normally cycling women, have also observed cyclic variations. There observations clearly indicate influence of ovarian hormones on salivary glands as well as their secretion. From table-6.1 it can be seen that during estrous stage, when the estrogen primed glandular tissue comes under the influence of rising titre of progesterone, the SA concentration of submandibular gland showed minimal level, when ovulation is known to occur in laboratory rats. This may be construed to indicate loss of this important component of salivary mucoprotein by way of metabolic breakdown and ultimate release of glucose, as the latter was shown to rise to a maximum level in secreted saliva around the time of ovulation in women (Davis and Balin, 1973). However, it should be mentioned here that no direct experimental evidence regarding enzymes involved in such a process is available. More work on this line may clarify this suggestion. From a different perspective angle it could be surmized that in all probability the saliva becomes more watery in consistency during estrous stage, when the rats commonly resort to licking of genital regions.

Further, the conditions during metestrous, when minimal estrogenic as well as gestagenic levels are known to occur (Hori *et al*, 1968) may facilitate far greater build up of SA concentration in the submandibular gland. When the diestrous stage sets in; the sex hormone titres start to rise, which obviously reduce glandular SA concentration. During the succeeding proestrous stage, when an LH surge is known to occur (Goldman *et al*., 1968; Barraclough *et al*, 1971), the SA reducing

trend already initiated in the previous stage becomes more obvious. This may be said to ultimately lead to the lowest SA level observed during estrous stage of the cycle. Hence, it may be suggested that estrogenic hormones probabably favour lowering of glandular SA level and that progesterone acts in a synergistic manner as far as submandibular gland is concerned. This suggestion finds support in the observations presented in Table-6.2 where in it is clearly seen that the lack of ovarian hormones (spaying) favours rise in SA concentration of submandibular gland, but the effect gradually wanes off upto 72 H. From the results depicted in Table-6.3 it can be said that only the D-1 at 2 H interval mimicks fairly well the glandular SA concentration of metestrous stage, and that the other two higher doses are apparently non-physiological in this context. Further, it could be seen from Table 6.4 that CD-1 and CD-2 combinations of P and E_2 act synergistically but in a totally opposite manner by increasing the glandular SA concentration close to metestrous level. Under these circumstances it could be said that increased amounts of sialomucins may find their way into secreted saliva making it more viscous. The present author confess that this enigmatic influence of higher E_2 doses and the E_2 plus P combination cannot be explained properly on the basis of data at hand. The only tentative explanation that could be put forward is that, experimentally induced lack of ovarian hormones exerts its influence within 48 H, and an adverse influence on the sensitivity of submandibular gland to subsequent administration of hormones; probably by affecting glandular receptors qualitatively or quantitavely.