CHAPTER FOUR

SUBLINGUAL GLAND

The sublingual gland of the rodents contains serous demilunes in addition to the principal mucous elements, but it is difficult to separate clearly the demilunes from the mucous cells; since they are in intimate relationship to one another. Myoepithelial cells are the feature of sublingual gland acini and are found between the acinar cells and enclosing basement membrane (Leeson, 1967). Intercalated ducts of the sublingual glands differ from those of the parotid and submaxillary as no secretory granules are present in the intercalated ducts. Striated ducts are composed of one cell type (Leeson, 1967).

Mucous acini in the sublingual glands of all the species considered so far except cat and dog contain large amount of acidic mucosubstances including guinea pig (Wislocki et al., 1947; Spicer and Warren, 1960a, 1960b); rat (Spicer and Warren, 1960b; Warren and Spicer, 1961), mouse (Spicer and Warren, 1960b, Warren and Spicer, 1961), Macaca irus (Jacobsen et al., 1971), Ceropithecus aethipos (Jacobsen and From, 1973), human (Quintarelli and Robinson, 1961). Sacramento and Miraclia (1972) reported two types of cells in the sublinguals of marmaset, some cells elaborating neutral polysaccharides and sialic acid and other elaborating a mixture of neutral polysaccharides, sulfomucins and little amount of sialic acid. Reifel and TraVill (1972) reported three types of cells in the sublingual glands of dog, first

cell type elaborating only neutral polysaccharides, second sulfomucins and third sialomucins. Jacobsen et al. (1971) showed that in Macaca irus monkey sublingual gland secretes fucose containing glycoproteins. Mucous glands of sublingual glands of the rodents are surrounded by demilunes. Demilunes of sublingual glands are seromucous in human (Leppi and Spicer, 1966; EverSole, 1972; Sirigu et al., 1974), cat (Tachi, 1972), tree shrew (Hara, 1976) and North American opossum (Pinkstaff, 1975). Serous demilunes have been reported in the sublingual gland of rat (Enomoto and Scott, 1971; Kim 1976) and cat (Tandler and Poulsen, 1977).

Though the extensive work is carried out on the sublingual gland there is scanty literature on its sexual dimorphism. It is worth noting that sexual dimorphism of the hamster sublingual gland reported by Shackleford and Klapper (1962b); Spicer and Duvenci (1964), Davi and Jacoby (1966) and Flon and Gerstner (1968). Spicer and Duvenci (1964) corroborated Shackleford and Klapper's observations and studied sexual dimorphism of the hamster sublingual gland. The sexual dimorphism of the hamster sublingual gland was revealed by staining for sulfomucins. Many more sulfomucin laden cells were seen in the sublingual glands of males than the females. Nakamura et al. (1974) have studied sublingual glands of mouse. Their studies did not reveal sex dimorphism of these glands, at least with respect to glucose-6-phosphate

dehydrogenase; neither there appears to be any sex difference with respect to the activities of hexokinase, phosphofructokinase or pyruvate kinase.

Material and Methods

Sublingual glands were removed from 5 male and 5 female adult squirrels and fixed within a fraction of time in various fixatives. Samples were fixed in 10% neutral buffered formalin for histology, neutral buffered formalin fixed paraffin wax embedded sections were stained haematoxylin and eosin. Additional samples were fixed in 2% calcium acetate in 10% formalin for PAS and Alcian blue pH 2.5 techniques. 1% calcium chloride in 5% formalin fixed frozen sections were used for the histochemistry of esterase and alkaline phosphatase.

For the histochemistry of esterase sections were incubated at 37°C in the incubating medium containing 5-bromoindoxyl acetate as substrate and for alkaline phosphatase histochemistry, sections were incubated at room temperature in the incubating medium containing naphthol-AS-MX phosphoric acid as substrate.

Observations

The squirrel sublingual gland was a compound tubuloacinar gland containing two secretory cell types, the

mucous (M) and Serous (S). (Plate No. 3, Figs. 17, 18). The serous cells were primarily present in the form of demilunes associated with the mucous tubules (↑). The duct system of the sublingual gland of squirrel was very simple. The only prominent ducts were located in the intralobular connective tissue and were apparently excretory ducts (ED) (Plate No. 3, Figs. 17, 18). No intercalated ducts were seen and rarely intralobular ducts were noted. There was distinct difference in between male and female sublingual glands. Mucous acini of male (M) were larger than female mucous acini (M) (Plate No. 3, Figs. 17, 18).

The mucous tubular cells were PAS-positive, but the staining was quite uneven, there being some mucous cells moderately positive (MF) (Plate No. 3, Figs. 19,20). Some were intensely stained (MI) (Plate No. 3, Figs. 19,20). Mucous tubules which showed PAS reaction were also stained with Alcian blue pH 2.5 (M) (Plate No. 3, Figs. 21, 22). In between the mucous cells forming mucous acini, there were some cells which did not stain with PAS and alcian blue, these might be the serous cells (S) in the mucous acini.

Number of these serous cells was more in male than that in female. Mucous tubules of the female sublingual glands were strongly PAS and less AB PH 2.5-positive whereas compared to female, in male they were not (Plate No. 3, Figs., 21,22).

The ducts of the sublingual did not exhibit any appreciable staining of mucosubstances.

The esterase activity was mainly localized in the serous elements of sublingual glands of male and female. The staining was intense and diffused nongranular and granular (↑) in nature (Plate No. 3, Figs. 23, 24). Staining intensity was more intense in male serous demilunes than that of female. Mucous acini of female did not show any esterase activity (Plate No. 3, Fig. 24). But slight granular esterase activity was observed in the mucous acinar cells of sublingual gland of male. The cells bordering the ducts gave strong esterase activity (ED) which was granular and diffused (Plate No. 3, Figs. 23, 24).

The alkaline phosphatase activity visualized by naphthol AS-MX phosphate technique was found to be localized in the serous demilunes surrounding the mucous acini and in the myoepithelial cells(↑). The mucous acini did not exhibit any alkaline phosphatase activity. No difference could be observed in the distribution and localization of alkaline phosphatase in the sublingual gland of male and female squirrel (Plate No. 4, Figs. 25, 26).

Discussion

Squirrel sublingual gland closely resembles the

sublingual glands of pig, cow, sheep (Shackleford and Wilborn, 1968) rat and mouse (Spicer and Warren, 1960b; Davies and Davies, 1962; Kurtz, 1964) where secretory acini were mucous and demilunes were serous in nature.

Sublingual gland has been studied by a number of investigators from different mammals (Wislocki et al., 1947; Spicer and Warren, 1960a,b; Dreizen et al., 1968; Jacobsen and From, 1973; Hara, 1976) where they described only histological feature while Nakamura et al. (1974) studied glucose-6-phosphate dehydrogenase, hexokinase, phosphofructokinase in the sublingual gland of mice but they could not record any sexual dimorphism in the sublingual gland. Pinkstaff (1975) who studied mucopolysaccharide distribution in the sublingual glands of Opossum, could not record sexual dimorphism in these glands. Pillai (1974) studied lysosomal enzymes of the sublingual glands of rat, but she also could not get difference in activities and distribution of lysosomal enzymes in the sublingual glands of male and female rat. But in the present investigation there is clear sexual dimorphism in the sublingual glands.

i) Mucous acini of male sublingual glands are larger than the mucous acini of female,

ii) Mucous acini of female are more PAS-positive and less alcianophilic than male,

iii) In the mucous acini of the male and female there are some serous cells, but the number of these serous cells is larger in male than that in the female,

iv) Esterase activity is more intense in the demilunes of male than the female and slight enzymatic reaction is present even in the mucous acini of male. These points indicate that though acini of sublingual glands of both male and female are mucous in nature, there are some serous elements in them and male sublingual gland contains more serous elements than the female sublingual gland.

Captions to Figures

Plate No. 3, Figs. 17 to 24

Fig. 17: Sublingual gland of male squirrel stained for H-E. Photomicrograph showing mucous acini (M), serous demilunes (arrow) and excretory duct (ED). x 150

Fig. 18: Sublingual gland of female squirrel stained for H-E. Photomicrograph showing mucous acini (M), serous demilunes (arrow) and excretory duct (ED). x 150

Fig. 19: Photomicrograph of sublingual gland of male squirrel. PAS staining reaction, intense staining mucous cells (MI), faint moderate staining mucous cells (MF). Unstained serous cells (S). x 150

Fig. 20: Photomicrograph of sublingual gland of female squirrel. PAS staining reaction. Intensely stained are mucous cells (MI) and moderately stained mucous cells (MF). x 150

Fig. 21: Sublingual gland of male squirrel stained for AB pH 2.5. Moderate staining mucous cells (M) and unstained cells serous cells (S). x 150

Fig. 22: Sublingual gland of female squirrel stained for AB pH 2.5 showing mucous cells (M) stained. x 150

Fig. 23: Sublingual gland of male squirrel stained for esterase. Photomicrograph showing staining reaction in the serous demilunes (↑) and in the excretory duct (ED). x 150

Fig. 24: Sublingual gland of female squirrel stained for esterase. Photomicrograph showing staining reaction at the site of serous demilunes (↑) and in the excretory duct (ED). x 150

PLATE NO.3