CHAPTER THREE

SUBMANDIBULAR GLAND

Sexual dimorphism of salivary glands of mammal dates back to 1923 when Hammett (1923) and Tupa (1926) described sexual dimorphism in the submaxillary glands of rat. But real credit for the discovery of sexual dimorphism in the submaxillary gland is generally given to Lacassagne (1940a). Since then a number of investigators have worked and have been working in this field and they have confirmed sexual dimorphism at least in the submaxillary glands. This sexual dimorphism has been noted by many investigators including Lacassagne (1940a,b,c); Lacassagne and Chamarro (1940); Causse and Lacassagne (1942); Raynaud (1943); Feyel Cabenes (1947); Arvy and Gabe (1950); Harvey (1952); Abouharb (1955); Borghi (1963); Hetem (1967); Flon et al. (1970); Floridi et al. (1976). They have described greater gross weight in relation to body weight of the submaxillary gland of adult males than the females. They have reported that gland of adult male showed preponderance of the granular ducts over acini. The cells of these ducts in male are tall and columnar and the number of cytoplasmic granules was larger in the male than the female. A number of quantitative studies were also made which confirmed the larger diameter and larger relative area occupied by the granular ducts in the males. Dominance of acinar cells in the female submaxillary is reported by Lacassagne (1940a) in rat. Raynaud (1960) in mice, Shackleford and Klapper (1962a) in Syrian hamster, Girod (1964)

in crab eating monkey, Mandel et al. (1964) in man and Spicer and Duvenci (1964) in rabbit.

In many investigations periodic acid schiff and Alcian Blue pH 2.5 techniques have been used to demonstrate sexual dimorphism in the submandibular glands of mammals, Shackleford and Klapper (1962a) in Syrian hamster submandibular gland and Girod (1964) in crab eating monkey submandibular gland reported stronger staining of acini by Alcian Blue in female hamster than the male. But Pinkstaff (1972) gave different results than the above, according to whom, there were seromucous demilunes in the male and female submandibular glands and they were intensely stained with AB pH 2.5 whereas the mucous acini of female pig submandibular gland were more Alcian Blue-positive than the mucous acini from male submandibular gland. Booth et al. (1973) reported somewhat different structure of the submandibular gland of White Essex pigs than the miniature pigs. They did not describe seromucous demilunes in both male and female submandibular gland. And the percentage of serous acini was more in the male submandibular gland than the female submandibular gland. On the contrary the percentage of mucous acini was more in the female glands where the mucous acini were strongly alcian blue-positive.

There are certain reports which are exactly opposite

to above one. Smith and Frommer (1972a,b) studied submandibular glands of chilian rodent (Octodondegas). They have reported that acinar cells from male submandibular gland were strongly PAS-positive, whereas acinar cells from female submandibular glands were only moderately stained with PAS. Sato et al. (1977) made quantitative histochemistry of submandibular glands of mouse, where submandibular glands were stained for PAS technique; in this study they have reported higher periodic positive groups in male gland than the female gland.

The above literature shows marked diversity in the nature of sexual dimorphism in the submandibular glands of mammals; so it makes it extremely difficult to compare sexual dimorphism among different species; hence the purpose of this study was to determine whether sexual dimorphism exists in submandibular glands of squirrel and the nature of sexual dimorphism.

Material and Methods

For the present investigation about 20 mature (10 male and 10 female) squirrels were used. Immediately after trapping, they were killed by cervical dislocation and submandibular glands were dissected out and fixed in (i) 10% Neutral buffered formaline for haematoxylin-eosin technique, (ii) 2% calcium acetate formalin for PAS and ABPH2.5 technique,

and (iii) 1% calcium chloride in 5% formalin for esterase and alkaline phosphatase.

10% Neutral buffered formalin fixed paraffin sections were used for haematoxylin-eosin technique and iron haematoxylin technique. Calcium acetate formalin fixed paraffin embeded sections were used for PAS and Alcian blue pH 2.5 technique. And 1% calcium chloride in 5% formalin fixed frozen sections were used for the demonstration of alkaline phosphatase and esterase. For the demonstration of esterase 5-bromoindoxyl acetate is used as substrate (Pearson and Gross, 1959; Holt and Withers, 1958; Pearson and Defendi, 1957) and for alkaline phosphatase Naphthol AS-MX Phosphate is used as substrate (Burstone 1958, modified by Barka, 1962).

Observations

Haematoxylin and eosin stained sections of submandibular glands of both male and female gave the appearance of mixed gland containing serous, seromucous and mucous acini as secretory unit and ductal elements containing intralobular ducts, granular ducts, striated ducts and excretory ducts (Plate No. 1, Figs. 1 and 2). In both male and female glands there were three types of acini. One type was intensely stained with eosin which could be called as serous acini (S), and the other one was moderately stained with eosin called

as seromucous cells (SM). And the third type remained unstained with eosin might be called as mucous cells (M) (Plate No. 1, Figs. 3 and 4).

In the staining with periodic schiff mucous acini were intensely stained; number of these PAS stained acini (M) was larger in the male (Plate No. 2, Fig. 9) whereas seromucous acini (SM) were moderately stained. Such a type of acini were observed in the female submandibular gland (Plate No. 2, Fig. 10). But in both the glands (Figs. 9 and 10) there were a number of acini remained unstained and these were the serous acini (S). These acini were large in size in male (Fig. 9) but in the female they were small in size but their number was larger (Plate No. 2, Fig. 10).

Mucous or seromucous cells were also demonstrated by staining with Alcian blue pH 2.5. Cells which were intensely stained with Alcian blue were mucous cells. Such type of mucous acini (M) were observed in the male gland (Plate No. 2, Fig. 11). Cells from female submandibular gland were moderately stained with Alcian blue and acini were called as seromucous acini (SM) (Plate No. 2, Fig. 12). Serous cells which remained unstained in PAS technique did not stain with Alcian blue pH 2.5 also.

The structure of granular tubules in submandibular

gland could not be revealed by haematoxylin-eosin staining technique. But the number of granular tubules was larger in the male than that in the female (GT) (Plate No. 1, Figs. 1 and 2). Tubules were more convoluted in the male than in the female glands. Staining with iron haematoxylin revealed difference between male and female granular ducts where the number of granules was larger in the male gland than that in the female gland (Plate No. 1, Figs. 7 and 8).

Enzymorphology of esterase of submandibular glands of male and female was carried out with 5-bromoindoxyl acetate which showed intense granular activity for esterase in the granular tubules (GT) whereas in the acini (AC) activity was granular and moderate. There was no difference in the localization, intensity and nature of esterase activity in the granular tubules (GT) and acini (AC) of submandibular glands from male and female (Plate No. 2, Figs. 13 and 14). But in the male some cells did not show esterase activity and these might be the mucous cells.

Myoepithelial cells could not be revealed either with haematoxylin-eosin or with PAS and AB pH 2.5. These cells were not stained for esterase. But these cells could be stained for alkaline phosphatase. Myoepithelial cells were observed surrounding the acini of male and female glands, but these cells were absent surrounding the striated

Captions to Figures

Plate No. 1, Figs. 1 to 8

Fig. 1: Submandibular gland of male squirrel stained for Haematoxylin-Eosin (H-E) showing acini (↑) and granular tubules (GT). x 150

Fig. 2: Submandibular gland of female squirrel stained for H-E. Light photomicrograph showing acini (↑) and granular tubules (GT). x 150

Fig. 3: Light photomicrograph of submandibular gland of male squirrel stained for H-E showing mucous (M) and serous acini (S). x 225

Fig. 4: Light photomicrograph of submandibular gland of female squirrel stained for H-E showing serous (S) and seromucous acini (SM). x 225

Fig. 5: Submandibular gland of male squirrel stained for H-E. Photomicrograph showing granular tubule (GT) and mucous acini (M). x 675

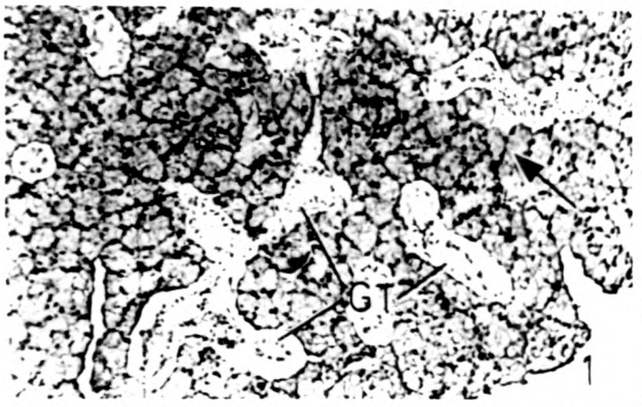
Fig. 6: Submandibular gland of female squirrel stained for H-E. Photomicrograph showing granular tubule (GT) and seromucous acini (SM). x 675

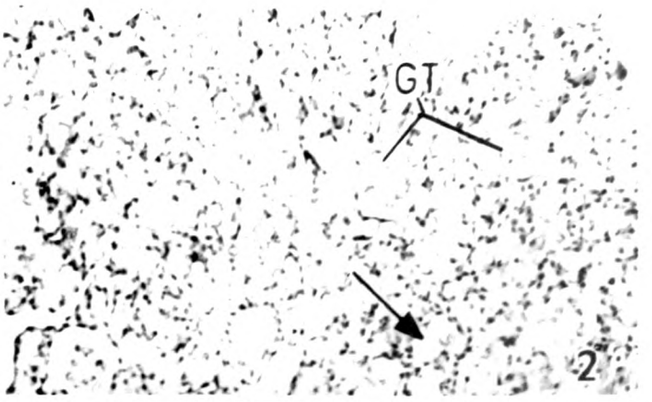
Fig. 7: Light photomicrograph of submandibular gland of male squirrel stained for Iron-haematoxylin showing granules (G) in the granular tubule (GT). x 675

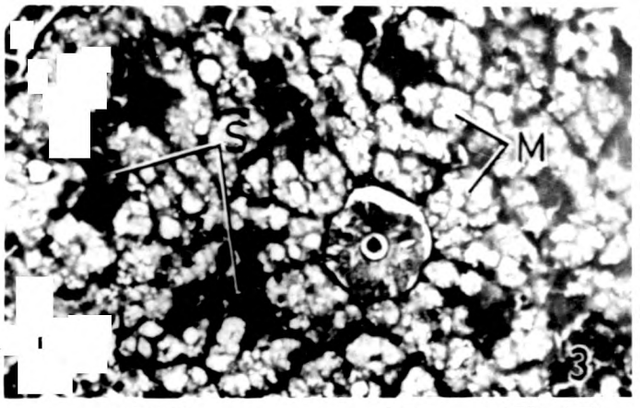
Fig. 8: Light photomicrograph of submandibular gland of female squirrel stained for Iron-haematoxylin showing granular tubule (GT) x 675

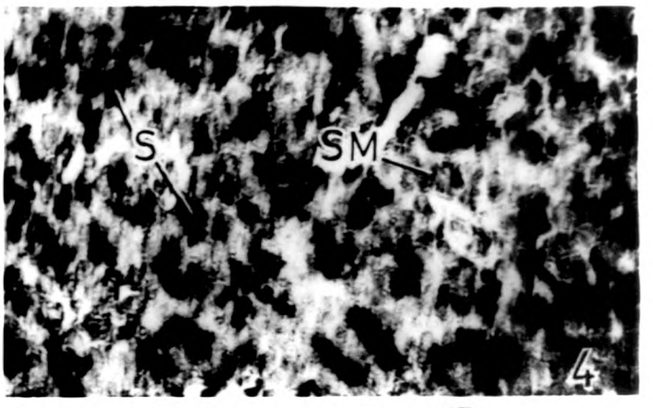
..

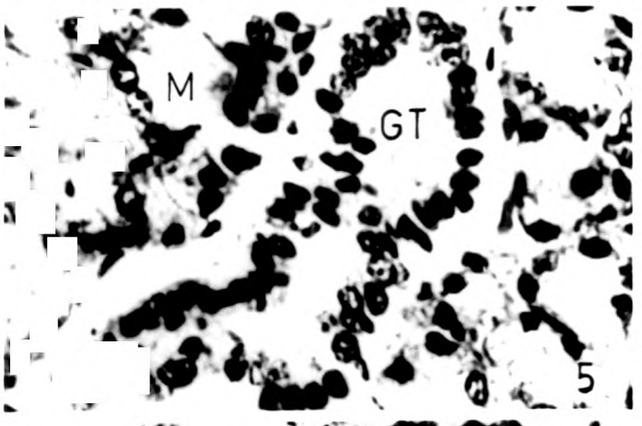
PLATE NO.1

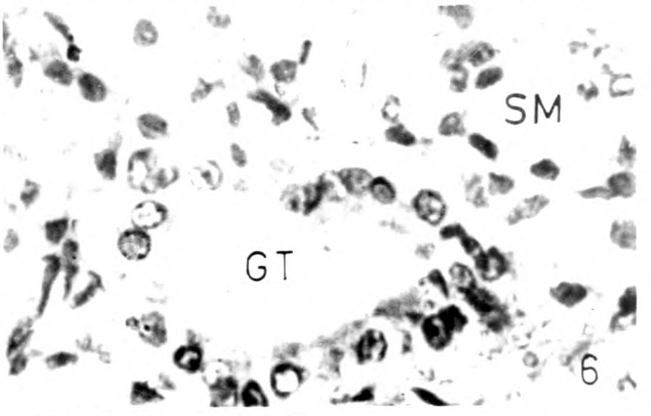


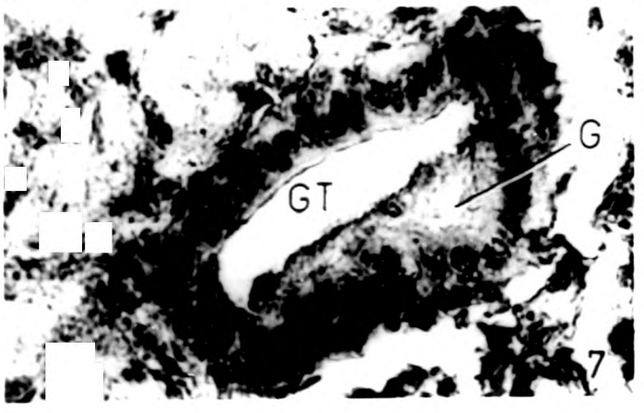


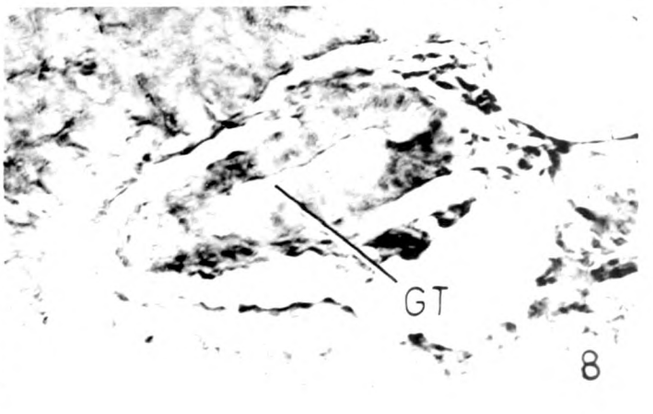












ducts and granular tubules. There was no difference in the number and nature of the myoepithelial cells (↑) from submandibular glands of male and female squirrel (Plate No. 2, Figs. 15, 16).

Discussion

Morphological structure of male and female submandibular gland is apparently similar, as glands from both sexes consist of acinar cells and granular ducts and striated ducts etc. But the number of acini is larger in the female than in the male. Dominance of acinar elements in the female submandibular gland is also shown by Lacassagne (1940a); Shackleford and Klapper (1962a); Girod (1964); Mandel et al. (1964); Spicer and Duvenci (1964); and Pillai (1974). In the male submandibular glands mucous cells are more in number. They do not get stained with eosin because of pale cytoplasm. Cytoplasm becomes pale due to destruction of mucigen droplets during fixation (Leeson, 1967). Mucous nature of these cells is confirmed with the help of PAS technique and with AB pH 2.5 staining reaction. These cells are intensely stained with PAS and Alcian blue technique and hence these cells from submandibular gland of male are mucous acini. In the submandibular gland of male, mucous acini are also reported by (Grad and Leblond, 1949; Smith and Frommer, 1972a,b; Sato et al., 1977); but Shackleford and Klapper (1962a); Girod (1964); Pinkstaff (1972);

Booth et al. (1973) reported presence of mucous acini in the female. In the female submandibular gland there are seromucous acini. Seromucous term should only be applied to those cells that contain appreciable amount of acidic mucopolysaccharides (Shackleford and Klapper, 1962a). In the submandibular gland of the female squirrel the acini are moderately stained with eosin (SM) (Plate No. 1, Fig. 4). These acini are stained for PAS and Alcian blue (Plate No. 2, Figs. 10 and 12), but complete cytoplasm of cells does not stain for Alcian blue pH 2.5 technique. The moderately stained acini with Alcian blue stain in female submandibular gland indicates the presence of seromucous acini.

There are a number of serous acini in the submandibular glands of both male and female. These serous acini possessed staining with eosin-haematoxylin technique, but they do not stain with PAS and Alcian blue technique. However, intense granular esterase activity can be revealed in these cells of both sexes. In the serous acini there are a large number of secretory granules containing watery secretory material. These secretory granules do not get destroyed due to fixation; they stain acidophilic (Leeson, 1967). Though serous acini are present both in male and female submandibular glands their percentage is larger in the female. However, intense granular esterase activity can be revealed in these cells of both the sexes (Plate No. 2, Figs. 13 and 14). Hence

in the male submandibular gland there are serous and mucous acini whereas in the female submandibular gland there are serous and seromucous acini; number of serous acini is more in the female than the male, so the acinar elements in male submandibular gland can be considered as mucous in nature while acinar elements in the female submandibular gland can be considered as serous in nature.

The granular ducts are more in male submandibular gland than in the female submandibular glands. The dominance of granular ducts in the male submandibular glands is also shown by Lacassagne (1940a,b,c); Gresik (1966); Caramia (1966). These granular ducts contain granules and their number is larger in the granular duct of the submandibular gland of male than the granular duct of the submandibular gland of the female (Plate No. 1, Figs. 7, 8). These cytoplasmic granules reported with iron haematoxylin technique in the granular duct of submandibular gland by Abouharb (1955). He noted that males of two species of Gerbils Meriones shawi and Meriones libyeus contained numerous secretory granules in the granular duct of submandibular gland but very few granules were seen in the granular duct of female. These granular ducts are purely serous in nature as they remain unstained for PAS and alcian blue technique. But intense esterase activity is observed in these ducts (Plate No. 2, Figs. 13, 14). At histochemical level apparently there is no difference in the

esterase activity in the granular ducts from male and female. But more number of granular tubules from male submandibular gland may contain more amount of esterase. At biochemical level more esterase activity in the male submandibular gland than the female submandibular gland is reported by Angeletti and Angeletti (1967); Calissano and Angeletti (1968); and Pillai (1974).

The myoepithelial cells are non-secretory cells; they are associated with secretory end-pieces. They cannot be revealed with haematoxylin-eosin technique, but they can be stained for alkaline phosphatase. In the squirrel submandibular glands also, these cells are stained for alkaline phosphatase. But there is no difference in the distribution pattern of these cells in the gland of male and female. There is no difference in the staining activity for alkaline phosphatase also.

Differences described above in submandibular glands of male and female squirrels may result from the action of sex hormones on the salivary glands as indicated by many investigators in their earlier studies (Lacassagne 1940a,b,c; Chretien, 1966, 1977; Calissano and Angeletti, 1968; Davalle et al., 1968) eventhough exact mechanism of interaction between the endocrine gland and the salivary gland is not known.

Captions to Figures

Plate No. 2, Figs. 9 to 16

Fig. 9: Light micrograph of submandibular gland of male squirrel, stained for PAS showing mucous acini (M) and serous acini (S). x 225

Fig. 10: Light micrograph of submandibular gland of female squirrel, stained for PAS showing seromucous acini (SM) and serous acini (S). x 225

Fig. 11: Submandibular gland of male squirrel stained for AB pH 2.5. Photograph showing mucous acini (M) and serous acini (S). x 225

Fig. 12: Submandibular gland of female squirrel stained for AB pH 2.5. Photograph showing seromucous acini (SM) and serous acini (S). x 225

Fig. 13: Submandibular gland of male squirrel stained for esterase. Photomicrograph showing intense granular activity for esterase in granular tubules (GT) and granular and moderate esterase activity in acini (AC). x 225

Fig. 14: Submandibular gland of female squirrel stained for esterase. Photomicrograph showing intense granular activity for esterase in granular tubules (GT) and granular diffused moderate activity in acini (AC). x 225

Fig. 15: Microphotograph of submandibular gland of male squirrel stained for alkaline phosphatase. The alkaline phosphatase reaction could be seen at the border of acinar cells which is the site of myoepithelial cells (arrow). x 225

Fig. 16: Submandibular gland of female squirrel stained for alkaline phosphatase. The alkaline phosphatase reaction could be seen at the border of acinar cells which is the site of myoepithelial cells (arrow). x 225

PLATE NO.2