CHAPTER FOUR

# OBSERVATION AND DISCUSSION ON FEMALE RAT

Wepfer (1679) first mentioned what is now known as the glands of Brunner. Extensive work has been done on anatomy, histology and physiology of these glands, which is described in review articles from time to time (Grossman, 1958; Cooke and Grossman, 1966 and Cooke, 1967). However, we are still ignorant as to the nature of the hormone regulating them. Florey and Harding (1935) showed in cat that transplanted and denervated pouch of duodenum secreted in response to feeding. This is confirmed in the pig (Wright et al., 1940) and dog (Sonnenschein et al., 1947). They believed that the hormone was secretin, since in cat secretion was shown to stimulate both the pancreas as well as Brunner's gland pouches (Mellanby, 1932) with a similar latent period intervals (Florey and Harding, 1935), but Cooke and Grossman (1965) in dogs Brunner's gland pouches showed that impure preparation of secretin given as rapid intravenous injection was an effective stimulus for secretion, but highly purified secretin was ineffective. Similarly, crude gastrin stimulated the secretion, whereas pure polypeptide did not (Gillespie and Grossman, 1963). There are number of reports describing regulation of estrogen over mucous secreting salivary gland acinar cells, which are also exocrine glands secreting glycoproteins like Brunner's glands.

It is interesting to observe that in these glands which

have an elaborate hormone control, such as pancreas and salivary glands, nervous system apparently does not have a prominent role in controlling their secretory activity. One is tempted to speculate on the view set forth by Prosser and Brown (1961), who stated that, for the digestive system a progressive series from nervous to hormonal control is evident. Thus, apparently, hormonal mechanism evolved during vertebrate phylogeny as an improvement, substituting to some extent for nervous control. The effect of sex hormones on exocrine glands of mammals probably may be comprising a more general phenomenon that one might suppose. There are a number of reports describing control of estrogen over glycoprotein secreting salivary gland cells; which are present in submaxillary glands (Pinheiro et al., 1970; Taga and Pinheiro, 1972; Foster and Raymond, 1967, 1968; Mandruzzato and Cecco, 1961; Schackleford and Klapper, 1962; Weber and Kittle, 1965; Barka, 1967; Herzberg and Barka, 1969; Puskalian, 1972). Acini of the submaxillary glands can be compared with Brunner's gland acini which are also rich in glycoprotein secretion. And hence it may be possible that the glycoprotein secretion from Brunner's glands may be controlled by estrogen. Taking this into consideration it was decided to study the effect of estrogen on Brunner's glands.

## Material and Method

For the study of distribution of Brunner's glands in

female rat, gross anatomical preparations were carried out as described in Chapter Two using 15 female rats.

For histological and histochemical study of Brunner's glands about 15 mature females within age group of 160 to 180 days, weighing about 225 to 250 gm were selected. They were isolated from males and kept separately in a room for about two weeks. They were kept in various cages avoiding crowding and the cages were kept completely in dark from 6 p.m. to 6 a.m. 200 watt bulbs were turned on at 6 a.m. and allowed to remain there till 6 p.m. This was repeated for four days. It was found that rats displayed more regular estrus cycle by this method. After 3-4 hours after 6 a.m. of the fourth day, it was found that about 10 rats were in estrous phase, some were in early estrus and some were in late estrous. Five female rats were utilized to study histology and histochemistry of Brunner's glands. In the remaining ten females overiectomy was carried out. The rats were anesthesized by employing ether by routine bell jar technique. A single longitudinal cut was taken in the ventral midline at the level of lower pole of the kidneys. The skin was then retracted laterally towards either side and ovary was exposed through skin and muscle wall just below the dorsal muscle mass. Care was taken to cut the skin of minimum length which could conveniently allow the extrusion of ovary together with fat, oviduct and small portion of the uterus. Both the

ovaries were removed. The wounds were closed with linen thread and then skin was sutured. The wounds were painted either with 95% alcohol or iodine. The day on which overiectomy was performed was taken as zero day of overiectomy. Thirty days after, 5 females were sacrificed to study the effect of overiectomy and five were injected 2 mg estradiol 17B per 100 gm of body weight, in corn oil, to observe effects of hormone on the Brunner's glands.

## Observation

### Gross Anatomy

The segment of gut containing pyloroduodenal depressions in mucosa are observed on the duodenal side of the junction. Pyloric mucosa broke up into a small villus like structure covered by gastric epithelium. These structures were replaced after a short interval by intestinal villi of duodenum. There was abrupt change from gastric to intestinal epithelium at the junction. Most of the villi and secretory product of Brunner's gland were washed out due to continuous washing in cold water and then cleared in 3% acetic acid. The staining of this cleaned material with hematoxylin showed presence of a number of glands in submucosal region. This number is larger towards the junction and reduced posteriorly. The amount of glandular tissue present was subject to considerable variation but did not extend less

than 6 cm and more than 7 cm beyond the junction (Fig. 14) upto the opening of pancreatic duct (Fig. 14D).

Histology

Brunner's glands of female rats are tubular gland consisting mainly acini and tubulo acini (Fig. 15 AC and TC). Nuclei which are oval or slightly flattened are basally located. Apical cytoplasm did not stain in eosin hematoxylin technique. Duct cells are cuboidal in nature, nuclei of these cells are situated at centre (Fig. 15 inset). The secretory end pieces are bounded basally by connective tissue. The connective tissue (Fig. 15↑) consisting mainly of fibres. In the overiectomised females, tubulo acini became shorter nuclei of which as situated towards the centre but not exactly in the centre. Cells were stained with eosin. There is no difference in the duct cells. Connective tissue between secretory unit increased considerably (Fig. 16↑). In overiectomised hormone injected rats, cells of acini again seemed to be hollow as they did not stain with eosin (Fig. 17).

Histochemical Observations

i) Mucosubstances

Sections of duodenum stained for PAS reaction showed PAS reaction mainly in tubulo acini and acini. Reaction is mainly

## Captions to Figures
Figs. 18 to 26

Fig.18: The Brunner's glands of normal female rat, stained for PAS, showing intense activity at the luminar side of the acinar (AC) and tubulo acinar (TC) cells. Connective tissue (↑) slightly positive x 240.

Fig.19: The Brunner's glands of overiectomised female rat, stained for PAS, showing acini (AC), tubulo acini (TC) and connective tissue (↑) with reduced PAS activity x 120.

Fig.20: The Brunner's glands of overiectomised hormone-injected female, stained for PAS, showing increased PAS activity, all over the cells of acini (AC), tubulo acini (TC) and connective tissue (↑) x 240.

Fig.21: The Brunner's glands of normal female, stained for β-glucuronidase, showing granular activity all over the cells of acini (AC) and tubulo acini (TC). Connective tissue (↑) is also β-glucuronidase positive x 540.

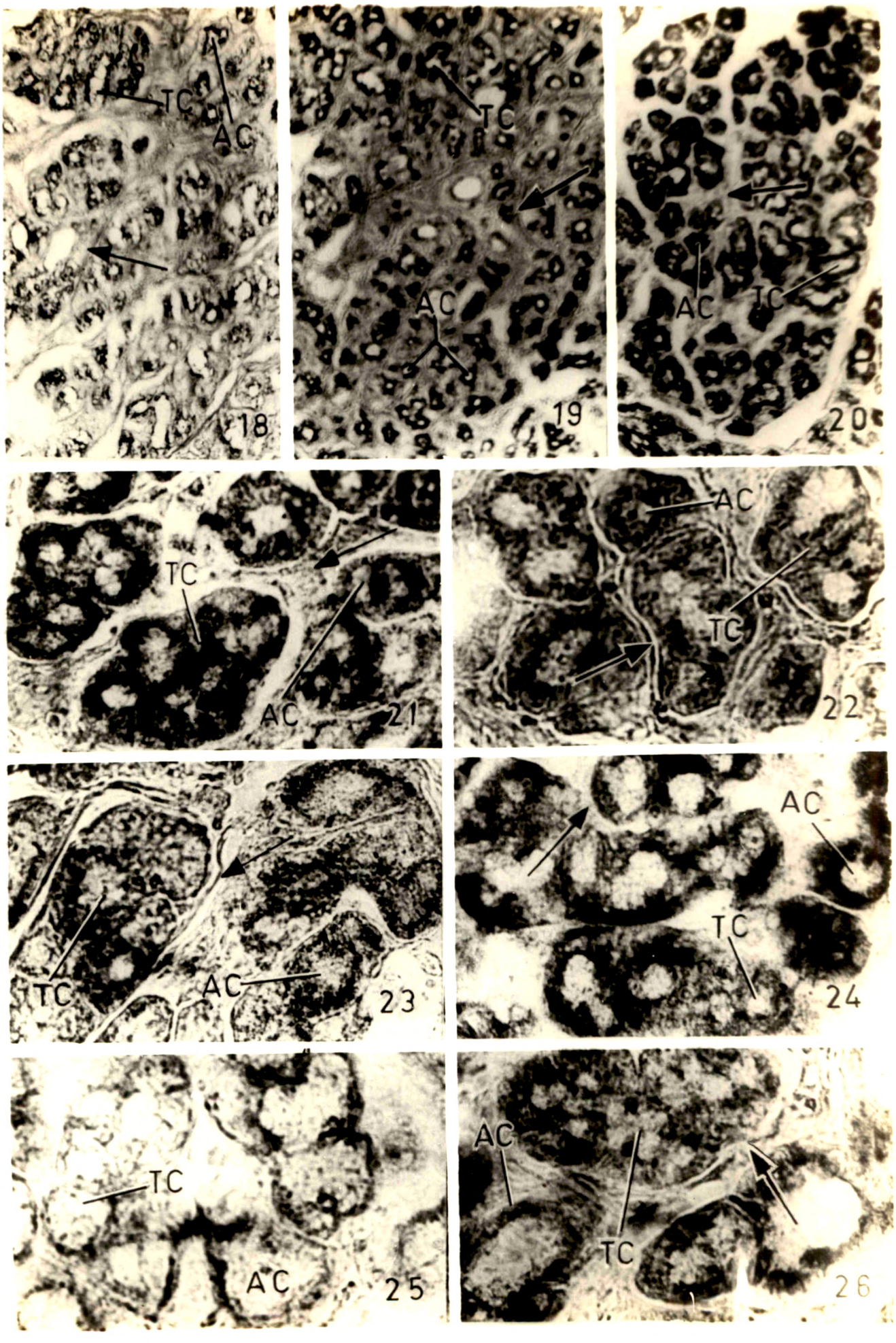
Fig.22: The Brunner's glands of overiectomised female rat, stained for β-glucuronidase, showing acini (AC), tubulo acini (TC) and connective tissue (↑) with considerably reduced activity x 540.

Fig.23: The Brunner's glands of overiectomised and hormone-injected female rat, stained for β-glucuronidase showing acini (AC) and tubulo acini (TC). Connective tissue (↑) with increased granular activity. Granules spreading all over the cells. x 540.

Fig.24: The Brunner's glands of normal female rat stained for Esterase, showing intense granular activity all over the cells of acini (AC) and tubulo acini (TC). Cells of connective tissue (↑) are weakly stained. x 540.

Fig.25: The Brunner's glands of overiectomised female rat, stained for Esterase, showing remarkable reduction in the activity in acini (AC) and tubulo acini (TC) cells. x 540.

Fig.26: The Brunner's glands of overiectomised and hormone-injected female rat, stained for Esterase, showing increased activity mainly in acini (AC). Connective tissue faintly stained x 540.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towards luminal border, that too, in the form of granules (Fig. 18 TC). Reaction was intense which was totally absent in the basal cytoplasm. Luminal side of the duct cells was also stained for PAS (Fig. 18 ). Connective tissue septa was also stained slightly with PAS. PAS activity did not alter in acini, tubulo acini, duct cells and connective tissue from malt diastase sections. The cells of these components did not stain with Alcian blue pH 2.5. Staining could not appear even after treating sections with pepsin and then staining with Alcian blue pH 2.5. In overiectomised females PAS activity which was localised at the luminal side of the acini (AC) and tubulo acini (TC) reduced remarkably (Fig. 19). There was reduction in the PAS activity from connective tissue and duct cells (Fig. 19 ↑). But overiectomised female rats receiving hormone depicted a different nature. Cells of both acini (AC) and tubulo acini (TC) were stained intensely with PAS. PAS activity was observed throughout the cytoplasm of acinar cells. Connective tissue cells are also stained for PAS (Fig. 20 AC, TC and ↑).

ii) β-glucuronidase

β-glucuronidase activity was found to be localised all over the cytoplasm of cells of acini (AC), tubulo acini (TC) and connective tissue (↑) (Fig. 21). Reaction was in the form of granules. The darkly stained sharply delimited granules were

present all over the cytoplasm. Thirtieth day after overiectomy β-glucuronidase activity was altered remarkably from acini (AC), tubulo acini (TC) and connective tissue (↑) (Fig. 22). Significant reduction in the enzyme activity is observed. β-glucuronidase-positive granules which were localised all over the cytoplasm were displaced towards the basal region of acinar cells. Intense β-glucuronidase positive reaction from the granules was reduced significantly. Enzyme activity from the connective tissue became non-granular which was remarkably granular in normal cells. In overiectomised but hormone receiving rats there was increase in staining intensity for β-glucuronidase from all cells of acini, tubulo acini, ducts and connective tissue. Activity was completely granular in form. β-glucuronidase positive granules appear to spread all over the cytoplasm of acini and tubulo acini. These granules were smaller in size compared to normal and they were in the form of clusters (Fig. 23).

iii) Esterase

Esterase activity was revealed with 5-bromoindoxyl acetate technique in all cells of Brunner's glands of normal rat. There was no difference in morphology of esterase in acinar cells of both acini (AC) and tubulo acini (TC). Esterase-positive granules were spread throughout the cytoplasm but they are more towards the basal region of the cytoplasm. They were densely stained and

hence could not be resolved one from the other. Connective septa were weakly stained for esterase. In overiectomised females cells of acini and tubulo acini of Brunner's glands lost their esterase activity remarkably. Very few esterase-positive granules could be observed at the extreme basal region of the cytoplasm. Activity was mostly nongranular in nature. Connective tissue also lost its esterase-positive activity. Brunner's glands of overiectomised female receiving estrogen showed esterase activity which was located throughout the cytoplasm of acinar cells but more towards basal region (Fig. 26 AC, TC). In the connective tissue faint nongranular esterase-positive activity was noticed (Fig. 26↑).

## Discussion

Gross anatomical picture of the female Brunner's gland indicates that these glands are more towards anterior side. Their population is decreased towards posterior region. They are spread up to the pancreatic duct (Fig. 14 D). Histologically these glands show two types of secretory end pieces spheroidal secretory end pieces are called as acini whereas of long axis are called as tubulo acini. In female Brunner's glands all secretory cells are eosin negative very few being stained with eosin but basal region of these cells was slightly stained with eosin. And hence in female Brunner's glands both acini and tubulo acini may be seromucous in nature. Nature of these cells is not much altered in overiectomised, hormone-injected females

but in overiectomised females acini and tubuloacini may reduce in size indicating increase in the connective tissue septa. The area occupied by these connective tissue was decreased in overiectomised hormone-injected females. Cells of acini and tubulo acini contain PAS positive granules at their luminar border indicating presence of mucous secreting material in the cells. This mucous material is not glycogen because PAS activity did not appear after diastase digestion. Cells of acini and tubulo acini did not stain with Alcian blue pH 2.5 even after pepsin digestion. This indicates that material present in the PAS positive granules from acini and tubulo acini is not acid mucopolysaccharides. Possibility of protein masked acid mucopolysaccharide is also ruled out with pepsin digestion technique. These observations lead to conclude that the cells of the Brunner's glands may be in the form of acini or tubulo acini contain neutral glycoproteins.

The secretion of neutral glycoprotein from acini and tubulo acini may be altered due to female hormones. Less PAS positive material is observed at the luminar border of the acinar cells of Brunner's glands of castrated females, which is again increased in castrated but hormone injected female Brunner's glands acinar cells.

β-glucuronidase activity is mainly in granular form

throughout the cytoplasm of cells of acini and tubulo acini connective tissue and duct cells but there is reduction in enzyme activity in overiectomised female. Reduction in enzyme activity is more towards luminal site of acinar cells at basal region still β-glucuronidase-positive material is observed. The population of β-glucuronidase-positive granules is increased after estrogen injection. Initially itself in normal rat β-glucuronidase-positive granules are less in number towards the luminal border of acinar cells and more towards the basal region. Their number and intensity are decreased; when there is less estrogen in the body and after estrogen injection this number is increased. High intensity of esterase activity which is present in granules in cell of acini and tubulo acini is more towards the basal region which decreases in overiectomised females but it increased there after estrogen injection.

These studies reveal that there is effect of estrogen on Brunner's gland and its secretory activity. Secretion of neutral glycoprotein is affected due to estrogen. Enzymes, β-glucuronidase and esterase which are involved in synthesis of mucus material and protein respectively, are also changed after overiectomy from cell of acini and tubulo acini of Brunner's gland of female rat. Changes occurred due to overiectomy in Brunner's gland cells can be reverted after injections of estrogen.