CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUDING REMARKS

i) Studies on Glycoproteins of Brunner's Glands

The present investigation was taken up with a view to augmenting the understanding of the secretion of Brunner's glands from male and female white rats. At gross anatomical level it is observed that Brunner's glands are spread from pyloroduodenal junction to posterior upto the opening of main pancreatic duct. In female the distance between pyloroduodenal junction to pancreatic duct is more than male, and hence at least apparently it seems that the area occupied by Brunner's gland is more in female than male. Population of Brunner's glands at pyloroduodenal junction is thicker in female than that of male. Kuczynski (1890) classified mammalian species into three categories according to distance, they extend from pylorus to distal side; in the carnivores they are short, in omnivores they are intermediate and long in herbivores. Villemin (1922) showed that they extend upto pancreatic duct. Landboe - Christensen (1944a,b) who used the method for staining gross specimen with hematoxylin after clearing with acetic acid. According to him continuous gland area did not extend below the interior papilla (minor pancreatic duct) in a few instances, but in no instance did it reach upto the superior papilla (main pancreatic duct). All these reports about the extension of Brunner's gland area are related with diet. The secretion of Brunner's glands may protect the duodenum from ulceration by acid pepsin secreted from stomach (Hartiala et al., 1950) and one of the conspicuous features of duodenal ulcer is

its common occurrence in adult male. According to Doll and Avery Jones (1951) the chance of a man developing a duodenal ulcer remains remarkably constant between ages of 20 and 65. By contrast chances of woman developing duodenal ulcer remain relatively low throughout the whole of her reproductive life but increase sharply at the menopause. Brunner's glands from female may be producing more secretory material which may be helpful to her to protect the duodenal mucosa from acid pepsin; this may be accomplished by larger number of Brunner's glands in female than in male. Histologically there is no difference in the secretory end pieces, ducts and connective tissue of Brunner's glands from male and female white rats. In both the sexes glands are globular as well as tubular in nature named as acini and tubulo acini. Cells of ducts are low cuboidal in nature and connective tissue surrounds the parenchyma of the glands. These histological units of the glands do not change after castration and after androgen and estrogen injections in male and female respectively. But in both the sexes area between parenchyma of the gland which is occupied by connective tissue is increased. It leads to conclude that dimensions of acinar cells might have been reduced in castrated animals.

The cells of acini, ducts and connective tissue showed PAS activity, which is not abolished after malt diastase digestion and cells do not stain with AB pH 2.5 even after pepsin digestion. These observations indicate the presence of

neutral glycoproteins in the secretory cells, duct's cells and connective tissue. PAS activity is granular and more towards the luminar side of the cells demonstrating the presence of glycoprotein material in the secretory granules. There is no sex difference in staining intensity and localisation of glycoprotein material in acinar cells, duct cells and connective tissue of the glands. Various studies dealing with mucosubstance histochemistry of Brunner's glands in large numbers of mammals show marked species variations and variation within species. Poddar and Jacob (1979) reviewed mucosubstances of Brunner's glands of about twentyseven mammals. They all have iterated differences between species in the expression of mucosubstances. Brunner's glands of mouse (Spicer, 1960; Sheahan and Jervis, 1976; Jenning and Florey, 1956), rat (Spicer, 1960; Belanger, 1963; Sheahan and Jervis, 1976; Jenning and Florey, 1956), dog (Sheahan and Jervis, 1976), hamster (Sheahan and Jervis, 1976), marmoset (Miraglia et al., 1967), duck billed platypus (Krause, 1971), opossum (Krause and Leeson, 1969), Kangaroo, marsupial and Bandicoot (Krause, 1973), armadillo (Carvalho et al., 1970), Rhesus monkey and Baboon (Sheahan et al., 1970; Sheahan and Jervis, 1976), man (Belanger, 1963; Ganter and Marche, 1970; Berger and Pizzotato, 1915; Sheahan and Jervis, 1976), ferret (Poddar and Jacob, 1979) contain neutral mucopolysaccharides but they did not notice any sex difference in Brunner's glands of above species as far as their glycoproteins are concerned. But Shackleford and Wilborn (1978) showed sexual difference in the Brunner's glands of

hamster. Difference was seen in AB staining in males and females' glands by atomic absorption method. Isolated sections of duodenal glands stained with AB in 0.1 M $MgCl_2$ showed 2.3 micro gm Cu per mg tissue for females as compared with 4.4 µg for males. Thus acidic mucins in male's duodenal glands, as demonstrated by AB staining, were present at almost twice the level found in female glands. Neutral mucosubstances, by contrast, were more concentrated in duodenal glands of female.

Staining reaction for glycoprotein which was observed in normal male and female Brunner's glands was reduced considerably 1 month after castration. PAS positive material which was observed more towards the luminar side of the cells of acini, tubulo acini and duct cells and in connective tissues of normal animal was reduced and slight PAS reaction for glycoprotein was observed only in luminar borders of acini. But the intensity of PAS reaction was increased after hormone injections of estrogen and androgen in ovariectomised and testectomised animals respectively. There are several reports showing the effect of steroid hormones on the secretory material of exocrine glands of mammals reviewed by Leeson and Leeson, 1967; Pinkstaff, 1980; Barka, 1980; Young and Van Lennep, 1978. But there is paucity of literature describing the effects of steroids on Brunner's glands. Even though Baylis and Starling (1902) suggested and now it is firmly established that substances localised in endocrine glands and autonomic nerves are principal controlling agents of body functions.

Receptors for sex steroids on the membranes of different exocrine cells have been shown by Grossman et al. (1969), Verhoven and Wilson, 1976; Takuma et al., 1977; Grossman et al. (1983) indicate control of steroids on secretory cells. Decrease in glycoprotein contents of Brunner's glands of male and female rat after castration and increase in glycoprotein contents of glands after hormone injections in castrated animals provoke the effect of steroids on synthesis and secretion of glycoproteins in Brunner's glands.

ii) Studies on Lysosomal Enzymes of Brunner's Glands

The classic studies of De-Duve et al. (1960) established that cells contain a degradative organelle, the lysosome, in which esterases, proteases, glycosidases and acid hydrolyses are concentrated. This organelle thus has capacity of hydrolysing lipids, proteins, glycoproteins and phosphate esters. Material which is not wanted by the cell is engulfed by way of autophagy or by crinophagy and this material undergoes rapid digestion. β-glucuronidase is one of the glycosidases present in all cell types of Brunner's glands of male and female rats. It is present in the form of granules all over the cytoplasm of acini and tubulo acini of normal male and female rats. It is present at the luminar border of duct cells in the form of granules and non-granular and granular activity is observed in connective tissue. All over the sites the enzyme activity was intense. In

ovariectomised and testectomised animals considerable reduction in the enzyme activity was observed at the luminar side of the cells of acini and tubulo acini. The enzyme activity from duct cells and connective tissue also decreased considerably. But β-glucuronidase positive granules were observed at the basal side of the cells but the number of these granules also reduced considerably. Upon respective hormone injections the activity for β-glucuronidase was increased considerably. The increase in enzyme activity was more towards the basal side of the cells of Brunner's glands along with presence of β-glucuronidase positive granules towards luminar side of the acinar cells. The number of glycoprotein containing secretory granules was decreased in castration and increased after hormone injections to castrated animals. Concomitantly there was decrease and increase in β-glucuronidase-positive granules. Tappel (1967) showed that lysosomes are the intracellular site of catabolism of glycoprotein. Enzymes for various glycoprotein hydrolysis are found in lysosomes. An example of one of the best known pathways is the degradation of hyaluronate. Hyaluronate is hydrolysed by hyluronidase of lysosomes mainly to tetrasaccharides (Aronsen and Davidson, 1967) and these in turn are hydrolysed by β-glucuronidase and B-N acetylglucosaminidase to the final product glucuronic acid and N-acetyl glucosamine.

There are a number of esterases present in the lysosomes known as lipase, cholinesterase, phospholipase acetyl

cholinesterase, spingolipase (Mahadevan and Tappel, 1968a,b, Mellors and Tappel, 1961; Brady, 1966). All these esterases are involved in the degradation of various fats and lipids; they require different substrates for their action. But all of them can act on substrate 5-Bromoindoxyl acetate. An enzyme esterase is present in all cells of Brunner's glands of male and female rats. Enzyme activity was demonstrated using 5-bromoindoxyl acetate as substrate and hence esterase which is demonstrated in glands of Brunner in the present investigation is non-specific. The intense enzyme activity was present in the forms of granules all over the cytoplasm of acinar cells of acini and tubulo acini and in duct cells. In connective tissue it is non-granular. In ovariectomised and testectomised rats activity was reduced considerably. All esterase-positive granules disappeared from the cytoplasm of acinar cells but non-granular activity was still evident at the basal border of the cells. Injections of androgen and estrogen to castrated male and female rats respectively showed intense enzyme activity in the form of granules more towards the basal region of the cells of acini and duct. Secretory granules containing glycoprotein were decreased in glands of castrated animals which were increased in glands of hormone-injected castrated males and females. Decrease and increase in enzyme activity in the form of granules coincides with decrease and increase in the number of secretory granules in cells of Brunner's glands. Secretory granules which remained to be secreted out their material in the lumen of the acini

and membranes of secretory granules which were secreted, were the subject of lysosomes for degradation. Membranes are rich in protein, lipids and glycoproteins. Lipids from membranes are phospholipids, phosphoglycerides and spingomylein. These lipids may be degraded in lysosomes as lysosomes have complete complement of hydrolytic enzymes for degradation of lipids. These subcellular organelles are with highest activities of lipase for acid triglycerides, acid esterase for higher fatty acid esters, and acid phospholipase and spingolipid hydrolases (Tappel, 1969).

iii) Concluding Remarks

While concluding the present investigation on endocrine control over Brunner's glands of white rats, it should be mentioned that the aim with which the present work was undertaken has been satisfactorily accomplished. The aim has been documented at the end of the introductory chapter of the present dissertation. Thus, in summary, it has been shown that -

i) Brunner's glands of male and female rats are present in duodenal submucosa and area occupied by Brunner's glands of female is wider than male.

ii) Secretory end pieces, ducts and connective tissue of

Brunner's gland contain glycoproteins and glycoprotein containing granules were decreased in castration and increased after hormone injections like estrogen and androgen in female and male respectively.

iii) β-glucuronidase responsible for degradation of glycoprotein is decreased and increased in gland cells as glycoproteins.

iv) An enzyme esterase responsible for digestion of fatty acid esters also showed concomitant changes as do glycoprotein and β-glucuronidase.

v) The results are discussed from the point of view that Brunner's gland cells respond to androgen and estrogen, their secretory activity at least partially controlled by these steroids. The main secretory product of Brunner's glands in rat is glycoprotein which is enclosed in secretory granules. The granules which remained to be secreted and membranes of secreted granules may be digested in lysosomes by the action of β-glucuronidase and esterase.

Though maximum efforts were made to complete this investigation, some aspects are yet to be elucidated such as: i) it was very necessary to measure actual area occupied by

Brunner's glands from male and female duodenum . ii) While searching out effects of androgen and estrogen on Brunner's glands adrenalectomy remained to be carried out which was necessary as adrenal glands do secrete certain amount of estrogen and androgen. iii) Biochemical estimations of glycoprotein, β-glucuronidase and esterase were necessary to discuss these chemical agents and their role in more details. iv) The study of glycoproteins from duodenal contents would have been more picturesque while discussing the role of steroids in secretion of Brunner's glands.

Investigation was carried out for M.Phil. degree and hence it was time bound. It was, therefore, impossible to tackle the above problems, which will be done in due course of time in this laboratory.