CHAPTER ONE

INTRODUCTION

The first mention of the glands of Brunner was made by Wepfer (1679) and then by Brunner (1688), Kuczynski (1890), Bensley (1903), Carleton (1935), Elias (1947) and Florey (1955). Brunner (1688) called the glands "glandulae duodeni" or "Pancreas Secundarium". Middeldorf (1846) was the first to record that these glands are found only in mammals, they are abundant in herbivores than in carnivores. He also pointed out that secretion of these glands differs from pancreatic juice, and who therefore, suggested that they be designated as the glands of Brunner instead of Pancreas secundarium. The term duodenal glands, first introduced by Brunner, is some times used to designate these glands, but it has the disadvantage that it may be misinterpreted to mean all the glands present in duodenum. The Brunner's glands are of interest because they are contained only in mammals and their function is uncertain. The early literature on these glands has been reviewed by Grossman (1958), Cooke and Grossman (1966) and Cooke (1967).

A) Gross Anatomy

The glands of Brunner begin at the transition from gastric to intestinal mucosa, but the distance they extend from pylorus is highly variable between species and moderately variable within species. Kuczynski (1890) classified mammalian species into three categories - short in carnivores, intermediate in

omnivores, and long in herbivores. Oppel (1897) stated that in the higher mammals the glands of Brunner extend limited portion of the duodenum above the entrance of bile duct. Villemin (1922) stated that the entrance of pancreatic duct is more correct than the entrance of bile duct as a guide for the distal extension of Brunner's glands.

Landboe-Christensen (1944a,b) who used method for staining gross specimens with hematoxylin after clearing with acetic acid, and stated the glands are located in the submucosa beneath the muscularis mucosa, except near the pylorus where a few glands are found in the lamina propria of the mucosa. The lower limit of the gland is quite variable. In Landboe-Christensen's material the continuous gland area did not extend below the inferior papilla (minor pancreatic duct) in a few instances, but in no instance did it reach the superior papilla. In one third of the cases, the glands of the Brunner were seen distal to the beginning of mesentry, that is, in jejunum. With aging there is a clear tendency for the Brunner's glands to extend short distance to get densely packed.

B) Microscopic Anatomy

i) Secretory End Pieces

The glands of Brunner are composed of branched tubules

with lumen into which acini open (Maziarski, 1901; Peiser, 1902; Schwalbe, 1872). Their ducts open into the base or sides of the crypts of Liberkühn (Ham, 1974; Bloom and Fawcett, 1975; Leeson and Leeson, 1976), but in opossum, the ducts open on the surface of the intestinal mucosa (Krause and Leeson, 1969a). The cells of acini and tubules are composed of cells of one type. Some workers divided the mucin secretory cells of gastrointestinal tract into two categories - true mucus secreting cells and mucoid cells (Clara, 1940; Gomori, 1952). According to this classification Brunner's glands of man fall into mucoid category in contrast to goblet cells which are classified as secreting true mucin. Brunner's glands of some species fall into mucoid category in contrast to pig and rabbit which are true mucin category.

The glands of Brunner of rabbit and horse are unique in that they contain two kinds of cells (Schwalbe, 1872; Bensley, 1903; Florey and Harding, 1933; Hosoda, 1956). The main type is mucoid which is similar in form to that of other species. In addition many terminal tubules and acini are composed of second cell type "dark cells" because they stain deeply with dyes such as haematoxylin. Histologically these cells have all the characteristics of cells which secrete digestive enzymes. The most important features are basal perinuclear basophilia and presence of dense secretory zymogen granules in the apical

portion.

In rat the main bulk of the glands lies at the pyloroduodenal junction as a comma shaped mass, with a narrow tappering tail extending into duodenum. The glands are tubuloalveolar and their structure and nuclear polarity vary with the functional activity (Bensley, 1903). In general the glands are lined by typical mucous cells which are conical in shape and had a dark granular cytoplasm. There are occasional Argentaffin cells and Paneth cells found in the glands of Brunner, the latter being found principally near the opening of the glands into the base of the crypts of Liebekühn.

In the resting state the cells of Brunner's glands are full of stainable secretory material, and nuclei are flattened against the base of the cells. After the strong stimulus to secretion has been applied, such as perfusion with hydrochloric acid, the cells become almost free of stainable mucin and nuclei become rounded and appear at lower third of cell. In such exhausted cells there are some stainable mucins just above the nucleus. On feeding both mucous and serous cells become free of stainable mucin and basophilia of serous cells decrease (Wolff, 1961). Pilocarpine causes depletion of stainable mucin from mucous cells but it is ineffective on the serous cells.

ii) The Ducts of Brunner's Glands

Wepfer (1679) and Brunner (1688) gave the earliest description of Brunner's glands in the duodenal mucosa. Later on Kuczynski (1890) described the presence of ducts ascending through the mucosa as far as the duodenal lumen in the cat, and Bensley (1903) also described ducts in the mucosa of cat and human duodenum.

Ham (1974), Bloom and Fawcett (1975), and Leeson and Leeson (1976) have stated that Brunner's glands drain into the crypts of Lieberkühn. Electron microscopic studies of Brunner's glands by different scientists have repeated this statement without illustration or further comment (Moe, 1960; Friend, 1965; Leeson and Leeson, 1966, 1967, 1968; Grossman, 1958). Treasure (1978) studied the ducts of Brunner's glands in rat, cat and man by using PAS technique. PAS gives a positive (granular) reaction for mucus in apical cytoplasm of cells of ducts of the Brunner's glands. Treasure (1978) further stated that ducts of Brunner's glands drain into base of the crypt in the rat, at a variable level in man, while in cat long discrete ducts drain independently into the duodenal lumen.

C) Ultrastructure

Electrone microscopic studies have detailed differences

between Brunner's glands of different species with regard to their fine structure. The ultrastructure of these glands has thus been described for cat (Moe, 1960), guinea pig (Cochrane et al., 1964), mouse (Friend, 1965), rat (Leeson and Leeson, 1966; Anand and Han, 1975), rabbit, (Martin, 1954; Leeson and Leeson, 1967), man (Leeson and Leeson, 1968), opossum (Krause and Leeson, 1969 a,b), echidna and platypus (Krause, 1970, 1971). All the species studied show morphological evidence of high secretory potential with an elaborate endoplasmic reticulum in acini, microvilli were seen on the luminal surface of the Brunner's gland cells. The cells in the guinea pig (Cochrane et al., 1964), and rat (Leeson and Leeson, 1966) are reported to be typical mucous cells, but Anand and Han (1975) indicated that, despite the electron lucent and mucous nature of secretory granules, the cells in their general feature belong to serous type. Whereas in cat (Moe, 1960) and mouse (Friend, 1965) they include both serous and mucous cells. In rabbit they are serous. The close association of mitochondria with RER in the secretory cells of Brunner's glands has been observed by Friend (1965) in mouse and Krause (1971) in platypus. Similar observations have been made in rabbit and in man by Leeson and Leeson (1967, 1968). They suggested that RER was involved in the synthesis of secretory material. The same authors however, did not find a close association of the two organelles in rat (Leeson and Leeson, 1966), but Anand and Han (1975) suggested intimate

association of mitochondria with desmosomes along the lateral borders of the secretory cells. It is apparent from these studies that there are numerous species differences with regard to fine structural features of cells of Brunner's glands which leads to further cytochemical and biochemical studies on Brunner's glands.

D) Cytochemistry

Various studies dealing with mucosubstance histochemistry of Brunner's glands in a large number of mammals show marked inter-species and intra-species variations. In all species the periodic acid Schiff (PAS) and related reactions which stain all gastrointestinal mucins to some degree are positive in the cells of Brunner's glands (Jenning and Florey, 1956). Florey and Harding (1934) using mucicarmine, found that Brunner's glands stained very well in rabbit, well in guinea pig and goat, faintly in pig and not at all in rat, cat and dog. Bignardi (1939, 1940), Boerner-Patzelt (1942) and Lillie (1949a) found that all the cells of the Brunner's glands in guinea pig and rabbit were metachromatic while those in hedgehog, rat, mouse, and pig did not show any metachromasia. Jenning and Florey (1956) obtained metachromasia with toluidine blue in Brunner's glands of the rabbit but not in cat and rat. Belanger (1954) and Jenning and Florey (1956) correlated metachromasia with the uptake of S^{35} labelled sulfate by autoradiographic methods,

this indicates the presence of sulfated mucins in Brunner's glands. Alcian blue is generally considered to be specific for acid mucopolysaccharides (Mowry, 1956). Alcian blue at pH 1 (Mowry, 1963a), the high iron diamine reagent (Spicer, 1965), and aldehyde fuschin (Gomori, 1950; Abul Haj and Rhinert, 1952) are all judged to be specific histochemical test for sulfated acid mucosubstances. The combined HID-AB (Spicer, 1965) and AF-AB (Spicer and Meyer, 1960) methods are used for histochemical differentiation between carboxylated and sulfated acid mucosubstances. Sialomucins alone in Brunner's glands were present only in man (Ganter and Marche, 1970; Sheahan and Jervis, 1976), otherwise they were present with neutral mucin and with foci of sulphomucin (Sheahan and Jervis, 1976). Krause (1973) studied Brunner's glands in a series of marsupials, Odour-Okelo (1976) studied them in the cat and horse. Sheahan and Jervis (1976) investigated the gastrointestinal mucosubstances in eleven mammals, and Poddar and Jacob (1979) reviewed mucosubstances of Brunner's glands of about twentyseven mammals. They all have iterated the 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; and Belanger, 1954), gerbils (Sheahan and Jervis, 1976), marmoset (Miraglia et al., 1967),

duck billed platypus (Krause, 1971), opossum (Krause and Leeson, 1969a,b), Kangaroo, native cat, 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 Pizzolato, 1975; Sheahan and Jervis, 1976), ferret (Poddar and Jacob, 1979) contain neutral mucopolysaccharides, whereas in guineapig (Lillie, 1949b; Cochrane et al., 1964; Spicer, 1963; Jervis et al., 1973; Sheahan and Jervis, 1976; Jenning and Florey, 1956), rabbit (Lillie, 1949b; Sheahan and Jervis, 1976; Jenning and Florey, 1956) cells of Brunner's gland are metachromatic with some neutral mucin and sialomucin either in deep glands or superficial glands. Presence of carboxylated mucin in cells of Brunner's glands has been shown in horse (Odour-Okelo, 1976), echidna and koala (Krause, 1970). It is clear from the above review that the variability between species in the staining reactions of Brunner's glands and within species in staining of the various types of mucoid cells is related to chemical differences in mucins.

E) Enzyme Histochemistry

Brunner's glands in Duckbilled Platypus have been studied by Krause (1971). He observed the positive reaction achieved with acid phosphatase appeared specific for areas in which a high concentration of dense granules is found in both secretory cells

and duct lining cells. The lining epithelium of the ducts usually gives an intense staining reaction for esterase whereas the secretory parenchyma is relatively unstained. However, a considerable number of ducts were also observed that gave only a light staining reaction. The reason for this variation in staining was not determined.

The acid phosphatase activity found in both the secretory parenchyma and the duct system of the platypus may indicate the presence of hydrolytic enzymes in the apical granules, as the staining reaction is generally confined to areas occupied by granules. Lysosomes were not identified ultrastructurally. Acid phosphatase activity also has been observed in Brunner's glands of guineapig (Cochrane et al., 1964) and marmoset (Miraglia et al., 1967). With the exception of the cat (Moe, 1952), the Brunner's glands of the other species give a negative reaction to alkaline phosphatase activity. Non-specific esterase is intensely active in the lining epithelium of the ducts. This finding indicates that the duct system may function not only in conduction but also in elaboration of secretory material. Richterich (1952) found esterase activity in the albino mouse in Brunner's glands of the rabbit give positive reaction for true lipase. Brunner's glands of the platypus failed to give positive reaction for lipase.

F) Hormonal Control

Florey and Harding (1935), Wright et al. (1940), Sonnenschein et al. (1947) established that the duodenal glands of Brunner have a hormonal mechanism of control. Florey and Harding (1935), Fogelson and Bachrach (1939) demonstrated that impure intestinal extracts containing secretin stimulate flow of juice from Brunner's glands. It has been confirmed by Cooke and Grossman (1966). But Sonnenschein and co-workers (1947) with highly purified secretin extract showed that it was without significant effect on Brunner's glands. Cooke and Grossman (1966) gave evidences that secretin is not the hormone regulating Brunner's gland from the experiments with secretin vitrum and histamine. The pure polypeptide gastrin-I was also an ineffective stimulant of Brunner's gland (Cooke and Grossman, 1966). The various crude mucosal extracts were found to stimulate Brunner's gland secretion, but still it is not known which mucosal region or regions release stimulants of Brunner's glands secretion in response to feeding.

Blickenstaff and co-workers (1949) studied secretion and motility of Brunner's glands and postulated that there may be two factors - one stimulating secretion directly and the other stimulating motility. However, Cooke and Grossman (1966) found that in all the stimulants they studied, except Bethanecholchloride, secretion was associated with motility.

Bethanecholchloride, however, increased motility alone. They also gave evidences to prove that Brunner's glands behave differently from other parts of the small intestine.

G) Sexual Differences

Shackleford and Wilborn (1978) have studied the sexual difference in the Brunner's glands of hamster. The glands at the deep zone are more eosinophilic as compared to those at the surface zone which are mostly unstained with eosin. In females the deep zone was twice the width of the deep zone of males. Surface zone was wider in males than in females. The deep zone was intensely stained with PAS while the surface zone reacted moderately. Alcian blue staining brought out the sexual differences in zonation even more dramatically, the deep zone was totally unstained while the surface zone reacted strongly with AB at pH 2.5.

Atomic absorption data confirmed the difference in AB staining in males and females. Isolated sections of Brunner's 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 micro gm for males. Thus, acidic mucosubstances in male duodenal glands, as demonstrated by AB staining, were present at almost twice the levels found in female glands. Neutral mucosubstances, by

contrast, were more concentrated in the Brunner's glands of females.

H) The Secretory Process in Exocrine Cells

i) Synthesis and Discharge of Secretory Material

Secretory cells, by their very virtue, have been endowed with the double duty of synthesizing proteins or glycoproteins not only for their internal need but for some functions are to be carried out extracellularly. The first step in the secretory process is the incorporation of amino acids into polypeptide chains by polyribosomes (polysomes) located on the granular endoplasmic reticulum, this occurs in cytosol. In the second step of the process, the newly synthesized polypeptides are transferred to the cisternal spaces of granular endoplasmic reticulum, a process commonly referred to as segregation. The third step is transport of protein to Golgi complex but according to Novikoff (1976) they are transported to GERL - a region of granular endoplasmic reticulum located in the inner aspect of the Golgi complex and is thought to be producing lysosomes. In GERL concentration of protein in condensing vacuoles takes place, these vacuoles later on unite to form secretory granules. These granules are filled with proteins or glycoproteins. These granules are maintained in a "ready to release state", and upon appropriate

nervous or hormonal stimulation, are discharged into lumina of the secretory endpieces by a process called exocytosis, the final step in the secretory process (Palade, 1975; Case, 1978; Grossman, 1984). In this secretory process a few points are worth making with respect to some aspects of the secretory process, particularly with regard to Golgi complex, GERL and exocytosis. The Golgi complex, or possibly GERL, appears to be important in packing and storage of secretory material and it has been shown that the Golgi complex is also involved in addition to the material to the secretory granules. Leblond and Bennett (1977) have discussed the role of Golgi complex in terminal glucosylation of glycoproteins. Berg and Austin (1976) have also shown that the Golgi complex adds material to the secretory granules. Hand and Oliver (1977) has suggested that the GERL has a major function in the formation of secretory granules in exocrine secretory cells. The GERL is known to possess an acid hydrolases, like acid phosphatase, and function of these particular enzymes in the secretory process is still uncertain. It has been proposed that secretory granule membrane are returned to the cell by endocytotic vesicles. The phenomenon of exocytosis is well demonstrated in salivary gland cells of the serous type (Nagasawa, 1977), where fusion of granular membrane takes place with the luminal plasmalemma as has been shown to occur in rat parotid acinar cells (Amsterdam et al., 1969; Selinger et al., 1974). The

biosynthetic mechanism involved in the production of the glycoproteins of mucous cells is in some ways very similar to the mechanism operating in serous cell secretion. The protein core of the glycoprotein is probably synthesized in the same manner as the proteins of the serous cells (Bogart, 1975, 1977). The first sugars are added to the protein core while polypeptides are being formed on polyribosomes of the granular endoplasmic reticulum; the enzymes involved in this stage of glycoprotein synthesis are called glycosyl transferase (Schachter, 1974, 1977; Phelps and Young, 1977; Phelps, 1978; Carlson, 1977). The protein core as it passes along the membranes of endoplasmic reticulum apparently has other sugars added, and the final glycosylation of the compound apparently occurring in Golgi complex (Carlson, 1977; Phelps and Young, 1977; Leblond and Bennett, 1977; Buscher et al., 1977). The action of glycosyl-transferases, each of which appears to be specific for one sugar (Schachter, 1977) are responsible for the final form of glycoprotein being secreted. The manner of discharge of mucous droplets from gland cells varies to a considerable extent. The human labial gland cells appear to secrete their mucin via an apocrine secretory method (Tandler et al., 1969 a) as do the rat sublingual mucous cells (Kim et al., 1972) and human submandibular gland mucous cells (Testa-Riva, 1977). In all these glands the secretory material reaches the end piece lumen via breaks in the apical plasmalemma. But Bogart (1975),

Tandler and Poulsen (1976), Ruby and Canning (1978) suggested merocrine secretion by mucous cells, where secretory droplet membrane fuse with apical plasmalemma of the cell and fused membranes undergo modification that eventually results in rupture of the fused membrane, thereby allowing secretory material's release into the endpiece lumen. This process is well described by Palade (1975) and Tandler (1978).

ii) Membrane Turnover in the Secretory Cycle

During exocytosis granule membrane fuses with the plasma membrane, thus increasing the apparent surface area of the plasma membrane. It would be wrong to conclude that the granule membrane, thus inserted, would be identical to the adjacent plasma membrane. The freeze-fracture studies by de Camilli et al. (1976) on the rabbit parotid showed that, after extrusion of the secretion granules, the apical plasma membrane forms a mosaic of areas with high particle density interspersed with batches of low particle density i.e., granule membranes transiently incorporated into the apical membrane. Even so, the excess must eventually be removed by reabsorption. Geuze and Poort (1973) and Kramer and Geuze (1974) investigated the reuptake of membrane, they considered that three mechanisms account for the re-uptake - (1) re-absorption of the deep caveolae left by the discharge of the granules by a process

of pinching off smaller vesicles, (2) micro pinocytosis from the apical plasma membrane, and (3) re-uptake of lateral plasma membrane by infolding and pinching off. They find that ferritin injected into the pancreatic duct becomes entrapped into pinched off vesicles and finally ends off in lysosomes. Thus, redundant granule membrane would be reabsorbed and degraded in the lysosomes.

iii) Secretion Granule Degradation

If there is no stimulation there is very less discharge through the secretory granules. Production of secretory granules probably continues albeit at reduced rate. This would eventually lead to an excessive number of secretion granules at the periphery of the cells. Hand (1972a,b) studied the process of zymogen granule degradation in the rat parotid. After 16 hrs. of fasting, lipid droplets appear in the basal cytoplasm and lysosomes with mouth eaten appearance are found. After 48 hrs, lysosomes and lipid droplets are quite abundant. A similar type of secretion granule degradation has been described earlier in lactotrophe cells of the rat pituitary gland following removal of sucking young from their mother (Smith and Farquhr, 1966). Degradation of secretion granules by lysosomal enzymes is probably a normal phenomenon in the secretory tubule cells of the sheep parotid gland (Van Lennep et al., 1977).

iv) Catabolism of Secretory Granule Membrane and Material in Lysosomes

It is now well known that membranes consist of integral proteins, peripheral proteins and fatty acids and cholesterol etc. Nature of peripheral protein and integral protein changes from membrane to membrane. Protein hydrolysis is a branching and very complex pathway with somewhat random sequences of reactions of enzymes with their protein and polypeptide substrates. Lysosomes contain the requisite enzymes in sufficient amounts to account for catabolic hydrolysis of different proteins. Generally, hydrolysis of proteins to amino acids is catalysed by cathepsin D; Cathepsin E and collagenase; Cathepsin A and B, Cathepsin C, Arylamidase, dipeptidase etc. (Tappel, 1969). The second constituent of membrane is lipid. Lysosomes may have a complete complement of hydrolytic enzymes for the degradation of lipids. Lysosomes are the subcellular organelles with the highest activities acid triglyceride lipase (Mahadevan and Tappel, 1968a), acid esterase for higher fatty acid esters (Mahadevan and Tappel, 1968b), acid phospholipase (Mellors and Tappel, 1967) and Spingolipid hydrolases (Brady, 1966; Weinreb, et al., 1968). All these lipase, esterase and phospholipase and spingolipid hydrolases are grouped into non-specific esterase. A substrate 5-bromo-indoxyl acetate is non-specific for esterase. It is hydrolysed by practically all esterases, the optimum pH

for the reaction was between 4.08 and 5.8. But reaction could be performed at all pH levels, the intensity of the staining reaction was observed to be lessened at pH above 7.3 (Pearson and Defendi, 1957).

Since nearly all secretions contain some glycoprotein, lysosomes involved in degradation of secretory granules have to carry out hydrolysis of glycoproteins. These are complex of proteins with relatively short often branched, carbohydrate chains, usually with no indication of a specific repeating unit. These are also called as glycurono-glycosaminoglycans. The carbohydrate moieties of these glycoproteins that have been defined biochemically have shown to include at least two of the following compounds, hexoses, fucose, hexosamines, sialic acid. In addition, the carbohydrate may be sulfated to a variable degree. If at neutral pH, the number of negative charges from carboxyl (including sialic acid) and sulphate groups does not greatly exceed the number of positive charges from free amino groups, the glycoprotein classed as neutral glycoprotein. Enzymes degrading glycosaminoglycans present in lysosomes are lysozyme (Jolles, 1960; Gibian, 1966); hyluronidase (Meyer et al., 1960; Aronson and Davidson, 1967; Gibian, 1966; Vaes, 1967); β-glucuronidase (Levvy and Marsh, 1959, 1960; Fishman, 1961; Levvy and Conchie, 1966), arylsulfatase (Roy, 1960; Dodgson, 1966; Mehl and Jatzkewitz,

1963), β-N acetyl hexosaminidase (Sellinger et al., 1960) n-N-acetyl hexozaminidase (Walker, 1966), β-galactosidase (Wallentels and Malhotra, 1961; Furth and Robinson, 1965; Sellinger et al., 1960), β-glucosidase (Beck and Tappel, 1968; Fisher et al., 1967; Robinson et al., 1967), α-fucosidase (Levvy and McAllan, 1961; Woessner, 1965).

I) Role of Steroids

In case of pancreas there is no continuous secretion but usually it is in response to peptide hormones elicited by the gut after feeding, signal from parasympathetic nervous system or by steroid hormones. Maintenance of zymogen granulation in acinar cells is dependent on glycocorticoid and/or estrogenic steroids. Since these organelles disappear after adrenalectomy of male rats or adrenalectomy and ovariectomy of females (Grossman et al., 1969; Grossman et al., 1983). Although binding site for estrogens was initially found associated with female reproductive tissue such as the uterus (Jensen and Jecobson, 1962; Gorski et al., 1968; Chan and O'Malley, 1978; Baxter and Funder, 1979), estrogen binding proteins and/or physiological responses to estrogen have been observed in pancreas (Grossman et al., 1983), pituitary gland (Davies et al., 1975); hypothalamus (Davies et al., 1975; Beers and Rosner, 1977; Smirnov et al., 1977; Weinberger et al., 1978); kidney

(DeVries et al., 1972), adregenic nerves (Hamlet et al., 1980), thymus (Imanishi et al., 1980), adipose tissue (Gray and Wade, 1980), skin (Punnonen et al., 1980), adrenal medulla (Weichman and Borowitz, 1979). A major source of estrogen in male is probably the adrenal glands or testosterone may be converted into estrogen, as in adipose tissue an aromatase has been described that converts testosterone to estrogen or estrogen-like steroids (Nimrod and Ryan, 1975; Gray and Wade, 1980). In the rodents granular convoluted (GCT) cells of salivary glands are under multihormonal control and the synergistic action of androgen, thyroxine and adrenal cortical steroids are necessary for their maintenance. Verhoeven and Wilson (1976) showed that protein in salivary gland binds dihydrotestosterone (DHT). Takuma et al. (1977) have detected specific binding of DHT in the cytosol of the submandibular gland of mice as young as five days of age, and have confirmed the attaindence of adulthood of this protein by 2 to 3 weeks of age.

J) Reasons for the Present Investigation

Glands of Brunner are of interest because they are confined to mammals and their function is uncertain. The early literature has been reviewed by Grossman (1958) and Cooke and Grossman (1966). Their general opinion is that the glands of Brunner secrete an alkaline fluid containing mucin to protect the

proximal duodenal mucosa against acid insult from gastric content. The Brunner's gland area is more resistant than lower region of duodenum to the damaging effect of dilute HCl (Florey and Harding, 1934) and chronic drainage of gastric juice over isolated Brunner's gland area do not cause ulceration (Florey et al., 1939).

One of the conspicuous features of duodenal ulcer is its common occurrence in adult male. In childhood ulcer is uncommon and two sexes are equally affected (Sandweiss et al., 1939). After puberty its frequency increases, and a heavy male preponderance becomes apparent. From the age of 20 years onwards, duodenal ulcer is common in men. It has been shown by Doll and Avery Jones (1951) that the chance of a man developing a duodenal ulcer remains remarkably constant between the ages of 20 and 65. By contrast, the chance of a woman developing a duodenal ulcer remains relatively low throughout the whole of her active reproductive life but increases sharply at the menopause. Clark (1953) has confirmed this, and further found that when a woman with chronic peptic ulcer becomes pregnant it is usually found that she is symptom-free during pregnancy, although symptoms are likely to recur during the early months after delivery. The male-female ratio for uncomplicated duodenal ulcer varies from 3:1 to 10:1 and ratio might be expected to be similar for perforations. Korbsch (1937), Schulz (1940),

Winkelstein (1936) have related these peculiarities of ulcer in relation to sex with the anti-ulcer effect of the circulating oestrogens. The oestrogen possibly diminishes the secretory activity of the stomach (Ojha and Wood, 1950). Another possibility is that oestrogens have a beneficial effect upon healing of lesions. (Arey, 1936; Sjovall, 1953, 1954; Sjostedt, 1953). The reason seems that oestrogens are general stimulators of mitosis (Bullough, 1946, 1955). The metabolic actions of the oestrogens in increasing protein anabolism and depressing blood cholesterol might be highly relevant to healing. It has been shown that steroids (naturally occurring human oestrogens and synthetic stilbestrol which mimic the biological actions of oestrogen) act as coenzymes and facilitate hydrogen exchange in nuclear reactions in variety of tissues (Hurlock and Talalay, 1958). H.M. Sinclair (1959, unpublished, cited by Truelove, 1960) has interpreted duodenal ulcer as manifestation of lack of essential fatty acids. This deficiency causes a structural defect in cell membranes and connective tissue, and the body in consequence becomes increasingly susceptible to injurious agents like ultraviolet light, X-rays, chemical carcinogens and hydrochloric acid in the duodenum. Therefore, it is quite possible that the origin of duodenal ulcer might be the deficiency of essential fatty acid, the requirement of which in lower animals is about 7 times greater in males than in females during the reproductive periods (Sinclair, 1958).

Sex hormones play an important role in regulating mucous secretions in hamster salivary glands (Shackleford and Klapper, 1962). Shackleford and Wilborn (1978) studied the Brunner's glands in male and female hamster and concluded that sexual differences were evident in duodenal glands of hamster. They, with the help of Alcian blue staining and atomic absorption spectrophotometry showed the duodenal glands of males to contain about double the acid mucosubstance of the female glands and periodic acid Schiff (PAS) staining was stronger in the females than in males.

In the light of the above observations made by different researchers, it appears that sex hormones might be playing an important role in mucus secretion in body. And, therefore, it was decided to study the effect of sex hormones on Brunner's glands in rat.

K) Plan of Proposed Work

The present dissertation concerns with the gross anatomy, histology, secretion and hormonal regulation of Brunner's glands of white rats. Efforts are directed to study -

i) the gross anatomy of Brunner's glands of male rat.

ii) the histology of Brunner's glands of normal, castrated and hormone-injected male rats.

iii) the secretory nature of Brunner's gland whether it is mucous or serous in normal, castrated and hormone-injected male rat. The work is carried out using certain techniques used in mucopolysaccharide histochemistry.

iv) the fate of secretory material which is not secreted out in normal, castrated and hormone-injected male. This work is carried out by studying histochemistry of β-glucuronidase and esterase both are lysosomal enzymes and are responsible for degradation of glycoproteins and certain components of the membrane.

v) the gross anatomy of Brunner's glands of female rat.

vi) histology of Brunner's glands of normal, overiectomised and hormone-injected female rats.

vii) the secretory nature of Brunner's glands of normal, overiectomised and hormone-injected female rats by using some methods established for demonstration of mucosubstances and glycoproteins.

viii) the catabolism of secretory granules which remained to be secreted out, and membranes of secretory granules which are secreted out by using histochemistry of β-glucuronidase and esterase.

Aim of the above studies was to find out the difference in distribution of Brunner's glands in male and female. What is the nature of secretion of Brunner's glands ? Whether the secretion of the Brunner's glands is regulated by steroid hormones, and what happens to the secretory material from secretory granules which are left over after secretion ? Are they digested or stored ?