CHAPTER - IV

EFFECT OF TESTOSTERONE ON CYSTEAMINE INDUCED DUODENAL ULCERS AND BRUNNER'S GLANDS OF MALE MICE

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I) INTRODUCTION

The pathogenesis of duodenal ulcer is extensively studied and found to be comprised of several components. Gastric acid that enters the duodenal bulb from the stomach is continuously buffered and neutralized by duodenal bicarbonates. The resultant pH in the first part of the duodenum influences a number of physiological and pathological processes including gastric emptying (Lichtenberger et al., 1977; Poulsen et al., 1982; Wormsley, 1983), further secretion of acid (Fordtran and Walsh, 1973), release of the secretin (Grossman, 1978) and possible occurrence of duodenal ulcer (Howard and Jorden, 1960). Duodenal ulcers are formed due to inappropriate acidification or improper neutralization of gastric acid. Due to acid, duodenal mucosa gets irritated predisposing ulcer. Against this gastric acid there are defensive factors in the duodenum secreted by various cells of duodenum, one of them being glycoprotein.

Biological functions of glycoproteins are protection, lubrication and transport. In the gastroduodenal tract a thick mucus layer is formed by glycoprotein which is the physical barrier between cells of stomach and proteases and in the duodenum between HCl and gastric acid coming in the lumen. The role of mucus material in the protection of duodenal
tract has been described in detail by Richard (1980); Allen (1981a,b); Hotta (1981); Allen et al. (1982); Fosterner et al. (1982); Sellers and Allen (1984); Azzum et al. (1986) Touber and Gerok (1987) and Vatier et al. (1987). In the duodenum glycoprotein are mainly secreted by cells of crypts of Lieberkuhn, goblet cells and Brunner's glands. It is usually assumed that juice collected from the portion of the duodenum arises mainly from the Brunner's glands rather than from the glands of Lieberkuhn and goblet cells, because fistula of the Brunner's glands usually secrete spontaneously and show an increase in rate of secretion in response to feeding. Perfusion of dilute hydrochloric acid showed that mucin content of the Brunner's glands are greatly decreased whereas that of glands of Lieberkuhn is essentially remained unaffected (Grossman, 1958). This and other experimental studies have indicated impairment of Brunner's glands.

The Brunner's glands are present in all mammals secrete an alkaline fluid containing mucins (Leeson and Leeson, 1967). Impairment in the secretion of the Brunner's glands is responsible in the development of duodenal ulcer (Hartalia et al., 1950; Perkins and Green, 1975; Kirkegaard et al., 1981; Nadar and Pillai, 1991; Kirkegaard et al., 1981). In recent period it has
been proved that Epidermal Growth Factor (EGF) (Gregory, 1970; Grossman et al., 1974; Isenberg et al., 1975; Heitz et al., 1978; Schoon et al., 1978; Poulsen et al., 1981; Kirkegaard et al., 1981; Kirkegaard et al., 1983) and prostaglandins (Emas et al., 1981; Vantrappen et al., 1982) secreted from Brunner's glands is also responsible in protection and healing of duodenal ulcer.

Thus from the knowledge available it can be deduced that main function of Brunner's glands is to protect the surface epithelium of the first part of the duodenum from the damaging action of the acid chyme ejected from the stomach.

The glands of Brunner which are present in all mammals composed of remifying tubules into which acini open (Schwalbe, 1872). The secretion of Brunner's glands is highly viscous and it has stickiness. Analysis of the Brunner's glands juice indicate that the major organic constituent is mucoprotein (Bensley, 1903). Brunner's glands drain into the crypts of Lieberkühn (Cooke, 1967; Krause and Leeson, 1969a,b; Ham, 1974; Bloom and Fawcett, 1975; Leeson and Leeson, 1976; Treasure, 1978). Treasure (1978) showed clearly the presence of ducts traversing the mucosa drain into the lumen of the duodenum between villi. The cells of these
ducts are also PAS-positive in rat (Treasure, 1978) whereas in mice along with PAS-positive material in the Brunner’s gland the duct cells also synthesize and secrete acid mucopolysaccharides (Obuoforibo, 1975). Support for the hormonal possibility comes from the observation that intravenous injection of intestinal mucosal extracts containing secretin stimulates the flow of Brunner’s glands juice in cat (Fogelson and Bachrach, 1939; Blickenstaff et al., 1949). The question of whether the stimulating action of these extracts is due to secretin itself or due to another hormone extracted with it. It is settled by intravenous inclusions of vasoactive intestinal polypeptide (Kirkegaard et al., 1981) where they have shown powerful effect of vasoactive intestinal polypeptide.

Relationship between exocrine system and endocrine system has been under investigation for more than six to seven decades. Lacassagne (1940a) discovered sexual dimorphism in the submandibular gland of mice. Influence of testicular androgen on functional changes in various tissues are vivid (Ambadkaar and Gaugarani, 1980). Bulk of literature available deals with alterations after few weeks of archidectomy (Konopkova and Nedvidek, 1972; Moor et al., 1977; Guraya and Abrans, 1981; Takayasu and Saloshi, 1981).
The previous histochemical studies on glycoprotein of Brunner’s glands of rat showed the decrease in PAS-positive in archidectomised rats (Kurane, 1985). It has also been observed that fucose content is higher in the Brunner’s glands of male than female (Maldar, 1991) and higher ulcer index is observed in male than in female rat (Nadar and Pillai, 1992). Thus from the knowledge available we presumed that androgen may regulate glycoprotein synthesis of Brunner’s glands. To prove this following work is carried out:

Material and Methods

1) Material: Male mice weighing about 25 to 30 gm. and about 2 months of age were used to study effect of testosterone on cysteamine-induced duodenal ulcers and Brunner’s glands. Selected male mice were maintained in separate cages and were starved for overnight with water ad libitum. The skin on abdomen was painted with 95% alcohol and shaved. Castrations were carried out at 9.00 to 10.00 A.M. under ether anesthesia in bell jar. A midventral cut towards posterior side of abdomen was taken. The skin was retracted laterally and testes were pushed out through the incision. The blood vessels at the two end of the testis were tied by means of a thread and both the testes were removed and excision was sutured with the help of a thread and needle.
The castrated mice were maintained for 15 days in separate cages with optimum care of light, temperature, humidity, food and water. On the 16th day half the number of mice were injected (i.p.) consecutively for 3 days with 4 mg/100 g BW Testosterone propionate (Sigma, Batch No. T 1875) in olive oil (M3). The remaining castrated mice were injected with olive oil only (M2). These mice (M2 and M3) were then used for ulcer induction by cysteamine.

Out of different methods tried "Cysteamine Induced Gastroduodenal Ulceration" (Selye and Szabo, 1973) method was found quite suitable to induce ulceration. The mice initially were starved for 24 hours during which only water was supplied ad libitum. The mice were injected with cysteamine-HCl in water (40 mg/100 gm BW) subcutaneously twice at the interval of 4 hours (CM2, CM3). The remaining castrated (M2) and castrated + hormone injected (M3) were injected with water only and were used as control for cysteamine-treated mice (CM2 and CM3). Twenty-four hours after the second dose the animals were sacrificed, the pyloroduodenal junctions were dissected out opened along greater curvature of the stomach and mesentery of the duodenum and processed for different methods.
2) Methods

1) Gross Morphological Observations

   i) the mucosa of pyloroduodenal junction was stained for alkaline phosphatase to observe the mucosal nature under stereomicroscope and to critically evaluate changes in the mucosa due to castration and cysteamine injection.

   ii) Ulcer index was calculated by using Szabo’s method (1978). The mean severity was compared statistically to find out significance of the difference.

2) Histology: The pyloroduodenal junctions were fixed in 10% neutral buffered formalin, washed and routinely processed for histological technique. The sections were stained with H-E. Histology of pyloric glands, duodenal villi, crypts of Lieberkuhn and Brunner’s glands was studied.

3) Histochemistry: To study the nature of glycoprotein from Brunner’s glands of cysteamine treated (CM₂ and CM₃) and untreated (M₂ and M₃) mice following histochemical techniques were used.

   i) PAS technique was utilized to study glycoproteins in general (McManus, 1946; Hotchkiss, 1948).
ii) Acidic glycoproteins were studied by using AB pH 2.5 technique (Mowry, 1956).

iii) AB pH 1.0 was employed to study sulphated glycoproteins (Lev and Spicer, 1964).

iv) To distinguish between acidic and neutral glycoproteins AB pH 2.5 + PAS technique was used (Mowry and Winkler, 1956; Mowry, 1963).

v) To study the differences between sulfated and carboxymucins acid hydrolysis technique was used (Quintarelli et al., 1961).

4) Biochemistry: Brunner’s glands were isolated by using the method described by Smits et al. (1982) and glycoprotein from the Brunner’s glands was isolated by using the method described by Satakopen and Kurup (1977).

i) Estimation of fucose (Dische Shettle, 1948)

Fucose was estimated by using cold sulfuric acid, cystein reagent and fucose α-D(+) as a standard.

ii) Estimation of Hexose (Dubois et al., 1956)

Hexose was estimated by using phenol sulfuric acid reaction. D-glucuronic acid was used as a standard.

iii) Estimation of Sialic acid (Warren, 1959)

Sialic acid was determined after the hydrolysis of the sample by 0.1 N sulfuric acid at 80°C for 1 hour by thiobarbituric acid assay method.

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iv) Estimation of Protein (Lowry et al., 1951)

Protein from glycoprotein was estimated by using Folin ciocalteu phenol reagent and Bovine serum albumin as a standard.

III) OBSERVATIONS

1) Gross Morphology

A) Castrated Mice (M2)

Figure No.53 describes gross morphology of the duodenum of castrated mice. Castrated male mice did not show any morphological changes in mucosa of pyloroduodenal junction and distal duodenum. When mucosa stained for alkaline phosphatase the staining was almost uniform except certain patches of faintly stained area. Villi from these areas were short desquamated. Erosion or ulceration were not found. Rest of the area showed normal, tall, leaf formed villi.

B) Cysteamine-Treated Castrated Mice (CM2)

Cysteamine-HCl when administered in castrated mice subcutaneously in double dose led to the mucosal damage forming duodenal ulcers (U). Mucosa stained for alkaline phosphatase showed decrease in staining. the staining decreased with increase in severity of damage. Deep ulcerated areas did not stain at all. Some affected areas showed faint staining (Fig.54).
Duodenal ulcers were formed mainly in proximal part of duodenum. Usually ulcers were not formed at pyloroduodenal junctions but in severe cases ulcers were formed at the junction also. In less affected areas, the villi were short, desquamated.

2) Ulcer Index

The Table No.3 describes ulcer index in castrated cysteamine treated mice. The percentage incidence of duodenal ulcer was 100%. The deep ulcers involving submucosa and destroying Brunner's glands were 60%, 30% perforating ulcers, and only 10% superficial ulcers. The mean severity was 3.2 ± 0.054 and duodenal ulcer index was 5.2.

3) Histology

A) Castrated Mice (M2)

The figures 55, 56 and 59 describe histological structure of pyloroduodenal junction of castrated male mice.

1) Pyloric glands

The pyloric glands (PG) and pyloric pits (PP) stained intensely with H-E. The pyloric glands were simple, branched, tubular glands situated deep in the submucosa. The pyloric pits were comparatively lengthier occupying greater part of the submucosa. The gland cells showed the similar structure as in normal. Nuclei of pyloric glands were basally situated (fig.55).
ii) Duodenal Villi

The villi (V) were tall, leaf formed and uniformly arranged. The lamina appeared normal with few infiltrated lymphocytes and mast cells. The epithelial covering showed columnar cells and goblet cells arranged on delicate basement membrane. Both the cells stained brightly with H-E. Nuclei of the columnar cells were basely situated. The mucous membrane was continuous unbroken (Fig. 56).

iii) Crypts of Lieberkühn

The crypt cells (CL) stained more intensely with H-E. The crypts showed similar structure as in normal no change was observed in operated male mice when compared to normal (Fig. 59).

iv) Brunner's glands

The sections stained for H-E showed presence of parenchyma of gland, divided into lobules which were separated from one another by connective tissue septa. The acini (AC) and tubulo acini were made up of pyramid shaped cells. The nuclei were basaly situated. The lumen was very narrow. The duct cells were eosinophilic, their shape was low cubiodal. The connective tissue showed staining with eosin (Fig. 59).
B) Cysteamine-Treated Castrated Mice (CM₂)

i) Pyloric glands:

The castrated male mice when treated with double dose of cysteamine showed tremendous changes. The structure of pyloric pit (PP) was disturbed. In pyloric glands (PG) the eosinophilia was increased. Due to damage number of pyloric glands (PG) also seemed to be decreased (Fig.57). Their lumen was enlarged.

ii) Duodenal villi:

Most of the duodenal villi (V) showed signs of destruction. The villi were short and broadened with desquamation of apical surface having fissures and ramifications. The more affected villi were very low or nearly flat with at certain points the epithelial barrier was broken and an erosions were formed ( ). Pronounced inflammation was found in lamina propria, with increased number of lymphocytes and mast cells (Fig.58)

iii) Crypts of Lieberkühn:

The crypt cells (CL) showed increased eosinophilia. The normal arrangement of cells was disturbed. The nuclei appeared irregular and picnotic (Fig.60).

iv) Brunner's glands:

The Brunner's glands (BG) also showed increase in eosinophilia. The cells of acini (AC) and tubulo acini
showed decrease in height, lumen dilated. The interparenchymal gap was increased. The nuclei were enlarged appeared picnotic and centrally placed. The lumen of ducts was dilated (D) (Fig.60).

4) Histochemistry

A) Castrated Mice (M₂)

The figures 61, 62, 65, 66 describe the histochemical nature of castrated mice.

i) The pyloric glands:

The pyloric glands (PG) of castrated male mice gave positive reaction with PAS. The gastric pit (PP) cells were more strongly positive than the gland cells (Fig.61). With alcian blue at pH 1 the pyloric gland cells were slightly alcianophilic while pyloric pit cells did not show alcianophilia (Fig.65), at pH 2.5 the alcian blue staining was not stronger in pyloric gland cells (PG) as well as in pyloric pit cells (PP) (Fig.69). When sections were stained for AB pH 2.5 + PAS, combined technique the pyloric gland (PG) cells were stained blue and pyloric pit (PP) cells dark pink (Fig.73) with acid hydrolysis the alcianophilia was partially reduced.

ii) Duodenal villi:

PAS activity was seen in goblet cells (G) of duodenal villi, the other cells were PAS negative
(Fig. 62). With alcian blue at pH 1 and pH 2.5 the goblet cells showed alcianophilia (Fig. 66, 70). With AB pH 2.5 + PAS technique some of the goblet cells stained pink while others blue green (Fig. 74).

iii) Crypts of Lieberkühn:

The cells of crypts of Lieberkühn (CL) showed strong PAS reaction (Fig. 62), with alcian blue at pH-1 the cells stained blue (Fig. 66) but the alcianophilia was stronger at pH 2.5 (Fig. 70). With alcian blue pH 2.5 + PAS technique the cells stained purple (Fig. 74) with acid hydrolysis the alcianophilia was partially lost.

iv) Brunner’s glands (BG):

Sections stained for PAS showed positive reaction in cells of acini (AC), tubulo acini and duct cells. Though the reaction was intense it was observed only at 2/3 of the luminel cytoplasm, in basal region the PAS reaction was absent. Luminel side of duct cells also showed intense PAS positive reaction. Brunner’s glands from distal part of duodenum showed less PAS positive activity. The Brunner’s glands (BG) when stained with alcian blue at pH 1 the cells did not show alcianophilia (Fig. 65, 66). With alcian blue at pH 2.5 duct cells (D) and some acini from proximal most part of duodenum did show alcianophilia (Fig. 69, 70). But majority of the acini, did not stain. When sections
were stained with alcian blue at pH 2.5 + PAS technique the most of acini (AC) and stained pink magneta but some acini (AC) and duct cells (D) from proximal duodenum stained purple (Fig.73,74) with acid hydrolysis technique the alcianophilia was partially lost.

B) Cysteamine Treated Castrated Mice (CM₂)

i) Pyloric glands

Castrated mice when treated with cysteamine-HCl showed in general reduction in staining. The cells of pyloric gland (PG) and pyloric pit (PP) showed reduction in PAS activity (Fig.63). The alcianophilia at pH-1 and pH-2.5 found to be lost (Fig.67,71). But when AB pH 2.5 + PAS technique was employed no reduction in staining intensity was observed (Fig.75) with acid hydrolysis further loss of alcianophilia was observed.

ii) Duodenal villi

The goblet cells (G) from duodenal villi (V) were stained with PAS but the activity was reduced (Fig.64). The alcianophilia at pH 1 and pH 2.5 also was found to decreased. The number of alcianophilic goblet cells was reduced (Fig.68,72). With AB + PAS technique the staining intensity was reduced (Fig.76).
iii) Crypts of Lieberkühn

The crypts cells also showed reduction in staining with PAS (Fig. 64), AB pH 1 (Fig. 68), AB pH 2.5 (Fig. 72), AB pH 2.5 + PAS (Fig. 76).

iv) Brunner's glands (BG)

In cysteamine treated mice there was in general reduction of staining. The PAS activity from acini (AC) was reduced and was found to present at the luminal side only. The PAS activity was diffused rather than granular as in normal. The reduction of the activity was more at the Brunner's gland (BG) from the distal duodenum (Fig. 63, 64). The alcianophilia also reduced both at AB pH 1 (Fig. 67, 68) and AB pH 2.5 (Fig. 71, 72). With AB pH 2.5 + PAS the acini (AC) and duct cells did stain but the staining was not reduced (Fig. 75, 76) with acid hydrolysis the alcianophilia was further reduced.

5) Colorimetric Estimations of Sugars and Protein from Brunner's Gland Glycoprotein:

Values of colorimetric estimations of sugars and protein from glycoprotein isolated from Brunner's gland of castrated and castrated cysteamine treated mice are given in Table No. 4. In general the values are found to be decreased in castrated mice but pronounced decrease was observed in cysteamine treated castrated mice.
i) **Hexose**: Hexose content is expressed as ug/mg glycoprotein. In normal mice (M₁) hexose content was 80.125 ± 0.7212, whereas in castrated male (M₂) it was 67.475 ± 0.5058. In cysteamine treated normal mice (CM₁) the content was reduced to 31.358 ± 0.4142 and it further reduced to 15.295 ± 0.223 in cysteamine treated castrated male mice (CM₂). The reduction in cysteamine treated (CM₁ and CM₂) as compared to normal and castrated (M₁ and M₂) was statistically significant M₁ : CM₂ = P < 0.005, M₂ : CM₂ = P < 0.001.

ii) **Fucose**: Fucose content was measured in terms of ug/mg glycoprotein. The fucose content in normal male (M₁) was 3.8474 ± 0.0074 and 1.2538 ± 0.0111 in cysteamine treated male mice (CM₁). The reduction in value was highly significant M₁ : CM₁ = P < 0.001. In castrated mice (M₂) the fucose content was reduced 2.817 ± 0.0001. The castrated mice when treated with cysteamine (CM₂) there was further reduction in the fucose content 0.4915 ± 0.0057. The reduction in value was statistically significant M₂ : CM₂ = P < 0.0001.

iii) **Sialic Acid**: Sialic acid was measured in terms of ug/mg glycoprotein. The sialic acid content in normal and cysteamine treated normal male mice was found to be 0.2609 ± 0.0016 and 0.0409 ± 0.0013 respectively. The sialic acid content in castrated (M₂) and cysteamine
treated castrated mice (CM2) was 0.1116 ± 0.0033 and 0.0307 ± 0.0016. In castrated (M2) the sialic acid content was reduced. In cysteamine treated mice the sialic acid reduced more than normal and castrated. The reduction was stastically significant. M1 : CM1 = P < 0.001, M2 : CM2 = P < 0.005.

iv) Protein : The protein content eas estimated in terms of ug/mg glycoprotein. The protein content in normal (M1) and cysteamine treated normal (CM1) was 20.4845 ± 0.1437 and 14.899 ± 0.1835. The reduction was stastically significant M1 : CM1 = P < 0.001. In castrated (M2) and castrated cysteamine treated mice (CM2) the values were 16.47 ± 0.174 and 13.525 ± 0.1442. The reduction in value was significant M2 : CM2 = P < 0.005.

IV) OBSERVATIONS
Castrated mice were injected with testesterone (M3) and then they were used to induce duodenal ulcers (CM3). M3 and CM3 individuals were seperately studied.

1) Gross Morphological Observations

A) Castrated + Hormone Injected Mice (M3)

The figure 77 describes gross morphological changes of duodenum of castrated + Hormone injected mice. Pyloroduodenal junctions when stained for alkaline phosphatase, the staining was almost uniform except certain patches in the duodenum were less
stained. In general the villi were tall, leaf like and uniformly arranged like in normal. The patches showed short, brodened, desquamated villi.

B) Castrated + Hormone Injected + Cysteamine Treated Mice (CM₃)

The figure 78 describes gross morphological changes of duodenum of cysteamine treated castrated + Hormone injected mice. The double dose of cysteamine gave 100% incidence of duodenal ulcers. The target area of ulcer formation was proximal part of duodenum. The affected part showed variation in staining for alkaline phosphatase. The reduction in staining increased with increase in severity of ulcer. Areas at the deep ulcers did not stain at all.

2) Ulcer Index:

The table III describes ulcer index in cysteamine treated + castrated + hormone injected male mice. When cysteamine was given in double dose the percentage of duodenal ulcer was 100%. The deep ulcers were 60%, perforating ulcers were 10% and 30% were superficial ulcers. The mean severity when calculated it was found to be 2.4 ± 0.13 which was reduced as compared to control (M₃) (3.2 ± 0.054) stastically the difference was significant CM₂ : CM₃ = P < 0.001. The ulcer index was 4.4 which was also found to be slightly less than the control (UI.5.2).
3) Histology

A) Castrated + Hormone Injected Mice (M3)

i) Pyloric gland

Figures 79, 80, 83 describe the histological structure of pyloroduodenal junction. These were found to be simple tubular gland, which open into deep pyloric pits. Most of the pyloric gland cells (PG) were mucus secreting cells. The pyloric pit (PP) were lined by surface epithelium cells. The pyloric gland and pyloric pit cells stained intensely with H-E. Comparatively pyloric gland cells stained more brightly than the pit cells (Fig.79).

ii) Duodenal villi

These were tall, leaf formed at most of the places (Fig.80). The surface epithelial cells were tall with large nucleus at the base. Among these many goblet cells (G) were seen. The mucosal border was continuous and lamina propria was thin containing loose connective tissues with few mast cells.

iii) Crypts of Lieberkühn

These are simple tubular glands. They open on the mucosal surface between the villi and extend through the lamina propria to the muscularis mucosae (Fig.83). The epithelium of the crypt is continuous with the surface epithelium of the villi. The crypt cells were strongly eosinophilic.
iv) Brunner's glands

The cells of acini (AC), and duct (D) were stained intensely with H-E. Brunner's gland cells were pyramidal and nuclei were basal in position.

B) Cysteamine Treated Castrated + Hormone Injected Mice (CM3)

i) Pyloric gland

In cysteamine treated mice the eosinophilia was decreased PG and PP were destroyed and mucosa showed erosion (Fig. 81).

ii) Duodenal villi

In cysteamine treated mice the duodenal villi were short, broadened with desquamation of upper surface. The villi were fissured and lamina propria with inflammation or oedema (Fig. 82). In more affected areas showed avillous condition (Fig. 81). The number of goblet cells was decreased considerably (Fig. 82).

iii) Crypts of Lieberkühn:

The cells of crypts of Lieberkühn the eosinophilia was decreased considerably (Fig. 84).

iv) Brunner's glands

The cysteamine treated castrated + hormone injected mice the shape and size of acinar cells were changed, the height of the cells was reduced. The lumen of the acini was dilated. The cells were devoid of
alcinophilia, the nuclei were enlarged and picnotic. The lumen of ducts (D) also showed dilation (Fig.84).

4) **Histochemistry**

A) **Castrated + Hormone Injected Mice (M₃)**

i) **Pyloric glands**

The pyloric pit cells (PP) were strongly PAS positive but pyloric gland cells (PG) were mildly reactive to PAS (Fig.85). The alcianophilia was mild at pH 1 (Fig.89) but increased considerably at pH 2.5 (Fig.93.97). With AB pH 2.5 + PAS technique the pyloric pit (PP) cells stained purple and gland cells (PG) stained blue green (Fig.97).

ii) **The duodenal villi**

The goblet cells of the villi were strongly PAS positive (Fig.86). With AB pH 1 some goblet cells were stained intensely (Fig.90) other goblet cells stained with AB pH 2.5 (Fig.94). The alcianophilia was partially lost in some of the goblet cells with acid hydrolysis technique. With AB pH 2.5 + PAS technique some of the goblet cells stained violet while others blue green (Fig.98).

iii) **Crypts of Lieberkühn**

The crypt cells (CL) showed strong positive reaction towards luminal side with PAS (Fig.86). The cells also stained intensely with AB pH 1 and AB pH 2.5 (Fig.90,94). With AB pH 2.5 + PAS technique the cells
appeared dark purple (Fig. 98) and with acid hydrolysis technique the alcianophilia was lost partially.

iv) Brunner's glands (BG)

The cells of acini and duct showed positive reaction with PAS (Fig. 85, 86). The reaction was mainly towards luminal side, the basal side was free of PAS material. The cells did not stain with AB at pH 1 (Fig. 89) but with AB pH 2.5 the duct cells and some acinar cells from duodenal bulb region stained dark green (Fig. 93). With AB pH 2.5 + PAS technique majority of the acini and tubulo acini stained pink magenta (Fig. 98) some acini and duct cells from duodenal bulb region which stained purple (Fig. 97).

B) Castrated + Hormone Injected + Cysteamine Treated (CM₃) Mice

1) Pyloric gland

In cysteamine treated, castrated but hormone injected mice, the PAS reactivity was reduced completely, the reaction was observed in pyloric pit cells (PP), but it was not properly localised indicating distruction of the structure (Fig. 87), at AB pH 1.0, there was no alcinophilia in gland and pit cells (Fig. 89) but at AB pH 2.5, it was observed, but again it was not properly localised (Fig. 93). At AB + PAS pyloric pit cells showed intense, but not properly
localised PAS reactivity, and slight reaction was observed for AB in pyloric gland cells (Fig. 99).

ii) Goblet cells

When the castrated + hormone injected mice treated with double dose of cysteamine the reactivity either was reduced or lost completely with PAS (Fig. 88). Alcianophilia which was observed at AB pH 1.0 (Fig. 92) AB pH 2.5 (Fig. 96) in goblet cells of castrated hormone injected mice was lost in castrated hormone injected and cysteamine treated mice. Intense purple reaction which was observed with at AB + PAS was also lost and most of the cells stained blue (Fig. 100).

iii) Crypt of Lieberkühn

In cysteamine treated, castrated hormone injected mice showed reduction in staining intensity. The PAS activity was diffused (Fig. 88). The alcianophilia at AB pH 1.0 and AB pH 2.5 was very much reduced (Fig. 92, 96). With AB pH 2.5 + PAS technique the purple staining intensity was reduced (Fig. 100) with acid hydrolysis the alcianophilia was partially lost.

iv) Brunner's Glands (BG)

The Brunner's glands in cysteamine treated mice the PAS staining intensity was reduced and not localised. It was seen only on luminal side. Alcianophilia at pH 1 was absent (Fig. 87, 88) and at pH 2.5 the duct cells and acinar cells showed reduction in
alcinophilia (Fig. 95, 96). With AB pH 2.5 + PAS the reactivity was very much reduced (Fig. 99, 100). There was reduction in the purple colour, but PAS reactivity was still intense, but not properly localised (Fig. 99, 100).

5) Colorimetric Estimations

Values of colorimetric estimations of sugars and protein are given in Table No. 4. The values were found to be less in castrated mice as compared to normal. There was maximum loss in castrated cysteamine injected mice but it was prevented in castrated hormone injected mice i.e., with testosterone injections the loss was regained.

i) Hexose: The hexose content is expressed as ug/mg glycoproteins. The hexose content of castrated + testosterone injected mice (M₃) was 80.2082 ± 0.3507 which was approximately equal as normal (M₁). But in castrated + testosterone injected + cysteamine treated mice (CM₃) it was reduced to 28.958 ± 0.1492. The reduction was statistically significant (M₃ : CM₃ = P < 0.001).

ii) Fucose: The fucose content is expressed as ug/mg glycoprotein. The fucose content of castrated + testosterone injected male mice (M₃) was 3.5396 ± 0.0092 which was almost same as normal (M₁). But in castrated + testosterone injected + cysteamine treated
Table 3

Effect of Testosterone on Cysteamine Induced Duodenal Ulcers in Male Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Change in Ulcers (%)</th>
<th>Mean Ulcer Severity</th>
<th>Ulcer Index</th>
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<td></td>
<td>Incidence</td>
<td>Superficial (cm²)</td>
<td>Deep (cm²)</td>
</tr>
<tr>
<td>CM₁ Normal ♂ + Cysteamine</td>
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<td>15</td>
<td>75</td>
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<td>100</td>
<td>10</td>
<td>60</td>
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<tr>
<td>CM₃ Operated ♂ + Hormone treated + Cysteamine</td>
<td>100</td>
<td>30</td>
<td>60</td>
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Values are Mean ± Standard Error.

P < 0.05 is significant.

CM₁ : CM₂ = P < 0.001
CM₂ : CM₃ = P < 0.001
Table 4

Carbohydrates and Protein Contents of Soluble Glycoprotein Isolated from Brunner's Glands of Male Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Hexose</th>
<th>Fucose</th>
<th>Sialic Acid</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Normal ♂</td>
<td>80.125</td>
<td>3.8474</td>
<td>0.2609</td>
<td>20.4845</td>
</tr>
<tr>
<td>± 0.7212</td>
<td>± 0.0074</td>
<td>± 0.0016</td>
<td>± 0.1437</td>
<td></td>
</tr>
<tr>
<td>CM1 Normal ♂ + Cysteamine</td>
<td>31.3583</td>
<td>1.2538</td>
<td>0.0409</td>
<td>14.899</td>
</tr>
<tr>
<td>± 0.4142</td>
<td>± 0.0111</td>
<td>± 0.0013</td>
<td>± 0.1835</td>
<td></td>
</tr>
<tr>
<td>M2 Operated ♂</td>
<td>67.475</td>
<td>2.817</td>
<td>0.1116</td>
<td>16.47</td>
</tr>
<tr>
<td>± 0.5058</td>
<td>± 0.0001</td>
<td>± 0.0033</td>
<td>± 0.174</td>
<td></td>
</tr>
<tr>
<td>CM2 Operated ♂ + Cysteamine</td>
<td>15.2958</td>
<td>0.4915</td>
<td>0.0307</td>
<td>13.525</td>
</tr>
<tr>
<td>± 0.0233</td>
<td>± 0.0057</td>
<td>± 0.0016</td>
<td>± 0.1442</td>
<td></td>
</tr>
<tr>
<td>M3 Operated ♂ + Hormone</td>
<td>84.2083</td>
<td>3.5396</td>
<td>0.2698</td>
<td>20.484</td>
</tr>
<tr>
<td>± 0.3507</td>
<td>± 0.0092</td>
<td>± 0.0052</td>
<td>± 0.684</td>
<td></td>
</tr>
<tr>
<td>CM3 Operated ♂ + Hormone +</td>
<td>28.958</td>
<td>1.5424</td>
<td>0.03411</td>
<td>15.9144</td>
</tr>
<tr>
<td>± 0.1492</td>
<td>± 0.0134</td>
<td>± 0.0012</td>
<td>± 0.4683</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Error.
P > 0.05 is non-significant.

**Hexose**

- \( M_1 : CM_1 = P < 0.005 \) HS
- \( M_2 : CM_2 = P < 0.001 \) HS
- \( M_3 : CM_3 = P < 0.001 \) HS

**Fucose**

- \( M_1 : CM_1 = P < 0.0001 \) HS
- \( M_2 : CM_2 = P < 0.0001 \) HS
- \( M_3 : CM_3 = P < 0.001 \) HS

**Sialic acid**

- \( M_1 : CM_1 = P < 0.001 \) HS
- \( M_2 : CM_2 = P < 0.001 \) HS
- \( M_3 : CM_3 = P < 0.005 \) HS

**Protein**

- \( M_1 : CM_1 = P < 0.001 \) HS
- \( M_2 : CM_2 = P < 0.005 \) HS
- \( M_3 : CM_3 = P < 0.005 \) HS
male mice (CM3) it was reduced to $1.542 \pm 0.0134$. The reduction was statistically significant ($M_3 : CM_3 = P < 0.001$).

**iii) Sialic Acid** : The sialic acid content is expressed as ug/mg glycoprotein. In castrated + testosterone injected male mice (M3) the sialic acid content was found to be $0.2698 \pm 0.0052$ which was almost same as normal (M1). In castrated + testosterone injected + cysteamine treated mice (CM3) the value was reduced to $0.03411 \pm 0.0012$. The difference was statistically significant ($M_3 : CM_3 = P < 0.005$).

**iv) Protein** : The protein content is expressed as ug/mg glycoprotein. In castrated + testosterone injected male mice (M3) the protein content was $20.484 \pm 0.684$ which was almost same as normal (M1). In castrated + testosterone injected + cysteamine treated male mice (CM3) the protein content was reduced to $15.9144 \pm 0.4683$. The difference between $M_3 : CM_3$ was stastically significant $P < 0.005$.

**DISCUSSION**

In normal male mice alkaline phosphatase activity was intense and staining was uniform, the duodenal villi were tall and leaf-formed, pyloric gland cells and pyloric pit cells were normal, and the nuclei in these cells were situated at the basal region. These
cells showed reactivity towards PAS, AB pH 2.5 and an intense purple colour was formed with AB pH 2.5 + PAS. Goblet cells were normal, nuclei were situated at the basal region. The cells were rich in glycoprotein material. Similarly, the cells of crypts of Lieberkühn and Brunner's gland cells were rich in glycoprotein. Majority of the Brunner's cells of distal duodenal did not show positive reaction for AB pH 2.5 but duct cells of Brunner's gland and some of the acini of the proximal side did show positive reactivity with AB pH 2.5 and AB pH 2.5 + PAS. In castrated males, duodenal mucosa did not show uniform alkaline phosphatase activity at various sites there were unstained or less stained patches. The narrow band present at the pyloroduodenal junction formed of short villi was normal but there was slight reduction in the activity. Pyloric gland cells and cells of pyloric pit showed staining reduction for PAS and AB pH 2.5. Similar changes were observed in cells of crypts of Lieberkühn and Brunner's gland cells; cells which were strongly AB pH 2.5 positive and showed deep purple color with AB pH 2.5 + PAS, showed reduction in staining reactivity. Changes which took place in castrated mice were recovered after the injection of testosterone. When castrated mice were treated with double dose of cysteamine, there were remarkable changes at
pyloroduodenal junctions as well as Brunner's glands, there was maximum destruction at duodenal mucosa, maximum reduction in alkaline phosphatase activity and complete loss of short villi located at the extreme proximal site of the duodenum. Superficial, deep and perforating ulcers were formed. Significant difference was observed in ulcer severity between normal mice and castrated mice who received cysteamine. Number of goblet cells was reduced mostly from the proximal site. Substantial loss was observed in staining reactivity for glycoprotein from secretory cells like pyloric gland cells, goblet cells and the cells of crypts of Lieberkühn. There was complete loss of PAS positivity and alcianophilia of Brunner's gland acini and duct cells. But castrated mice treated with testosterone did not show severe damage of duodenal mucosa. When they were replenished with testosterone and then treated with cysteamine, lesions were less in number and severity was reduced, there was increase in alkaline phosphatase activity which also was uniform. Glycoprotein content of pyloric gland cells, goblet cells, cells of crypts of Lieberkühn and Brunner's gland cells was increased.

In biochemical studies on Brunner's gland glycoprotein, it was found that sugar and protein levels were decreased in castrated mice. The levels
were further decreased in castrated but cysteamine-treated mice. Upon administration of testosterone values were increased in castrated mice but not significant in castrated and cysteamine-injected male mice.

Similar morphological and histological changes were observed in cysteamine-treated normal rats (Groves et al., 1974; Robert et al., 1974; Dzau et al., 1975; Haith et al., 1975; Poulsen and Szabo, 1977; Kirkegaard et al., 1981; Poulsen et al., 1986). Inhibition of alkaline phosphatase activity of duodenal mucosa in cysteamine treated rats has been observed by Japuridzia et al. (1988, 1991).

Typical mucosal lesions of coeliac disease develops with 8 to 12 hours after local application of gluten in asymptomatic patients (Trier, 1973). Intestinal changes in the jejunum seems to start with shortening and fusion of microvilli (Rubin et al., 1966; Shiner, 1974) and result in flat mucosa (loss of normal villous structure) (Trier, 1973). A nonspecific gastroenteritis has been described in children where the duodenal biopsy shows mucosal damage similar to that occuring during coeliac disease, flattening of villi, replacement of columnar epithelium with cuboidal cells and infiltration of lamina propria with
inflammatory cells (Barnes and Townley, 1973). Similar morphological changes have been described in the duodenum after the administration of norwalk agent (i.e., broadening and shortening of microvilli) (Agus et al., 1973; Schreiber et al., 1973). The similarity of these intestinal changes to the changes found after administration of cysteamine suggests that response of duodenal cells to cysteamine is nonspecific. Though nonspecific, there is fine correlation between ulcer formation and loss of glycoprotein content in the duodenum. The recent studies also indicate that in cysteamine-treated animals there is decrease in the secretion of glycoprotein in the duodenum (Spicer and Meyer, 1960; James, 1964; Schrater, 1964; Lev and Spicer, 1965; Nadar and Pillai, 1989; Kurebayashi et al., 1985).

Properties of these mucins have been observed in different secretory cells of duodenum. The pyloric gland cells, goblet cells, crypts of Lieberkuhn consist of both neutral and acidic mucins (Gregory et al., 1982). In some animals a few acini and Brunner’s gland duct cells also possess acidic mucins. But in most of the animals Brunner’s gland cells consisted of neutral mucins. Glycoproteins secreted by these cells at the duodenal mucosa have viscosity, adhesiveness and
cohensiveness properties. Glycoprotein and bicarbonate contribute to form an unstirred layer over gastric and duodenal mucosa. Thickness of this layer is 73 to 82 μm in rat and mice. This thickness reflects a dynamic balance between mucus secretion and erosion by gastric acid and mechanical balance. Animal and human data were presented showing that this mucus bicarbonate gel serves as a mixing barrier to acid. Therefore, the secretion of bicarbonate and mucus may play an important role in protecting the duodenum from damage. It has been established that secretions of Brunner's glands contribute the resistance of the duodenum to acid (Hartalia et al., 1950; Griffith and Harkins, 1956; Grossman, 1958). Inhibition of the Brunner's gland secretion may be playing a role in the formation of cysteamine-induced duodenal ulcer (Poulsen et al., 1981; Kirkegaard et al., 1981). Cysteamine depletes Brunner's gland secretion (Kirkegaard et al., 1981; Poulsen et al., 1981; Adler et al., 1982). Histochemical demonstration of glycoproteins during ulceration in pyloric gland cells, goblet cells and cells of crypts of Lieberkuhn and reduction in the number of goblet cells in cysteamine-treated mice also suggest their contribution of glycoprotein at duodenal mucosa and protection against the formation of duodenal ulcers. Apart from glycoprotein and bicarbonates.
Prostaglandins and Epidermal Growth Factors are also involved in the protection of duodenal mucosa. About sixty years ago it was shown that extract of pregnant woman's urine have beneficial effect on peptic ulcers. Later on it was reported that extract from pregnant woman's urine causes an inhibition of gastric secretion (Culmer et al., 1939; Gray et al., 1939), which contains urogastrin (urine EGF). Importance of Brunner's glands in the protection of duodenal mucosa against acid hypersecretion released by gastrin is supported by the findings of epidermal growth factor in the secretion of Brunner's glands (Heitz et al., 1978; Murphy et al., 1979; Kirkegaard et al., 1983; SkovOlsen et al., 1983; Kirkegaard et al., 1984; SkovOlsen et al., 1984; SkovOlsen et al., 1985). The epidermal growth factor is a mitogenic peptide, it is also produced in salivary glands and is present in large amount in urine (Hollenberg, 1979). Intragastric instillation of EGF increases the synthesis and content of DNA and RNA in the gastroduodenal mucosa (Dembinski et al., 1982) and intraduodenal instillation of EGF prevents the development of experimental duodenal ulcers in the rat (Kirkegaard et al., 1983; SkovOlsen et al., 1984). Though prostaglandins have been implicated by exerting a novel type of gastric protection (Robert, 1979; Hillier et al., 1985) and its presence has been detected in
duodenal mucosa (Gregory, 1977) but exactly, where it has been synthesized is not yet been discovered.

From above, discussion it is clearly established that Brunner's gland secretions like bicarbonates, glycoproteins and epidermal growth factors protect duodenal mucosa. The mucus bicarbonate barrier contributes to save duodenal mucosa from the damaging action of acid chyme ejected from the stomach and EGF has been shown to inhibit gastric acid secretion (Elder et al., 1975).

Florey et al. (1935) and Kirkegaard et al. (1984) have shown that a humoral mechanism is important in regulation of secretion from Brunner's glands. A nervous effect on secretion has also been reported (Wright et al., 1940; Kirkegaard et al., 1981). Inhibitory effect of sympathetic nervous system on the Brunner's gland secretion was confirmed by Skov-Olsen and his coworkers (1985), whereas alpha adrenergic agonist, noradrenaline, inhibited the secretion. This result is consistent with the findings of adrenergic nerve surrounding the secretory acini of Brunner's glands (Stach et al., 1978). Substantial evidence also has been accumulated showing that cholinergic, andrenergic and vasoactive intestinal polypeptide (VIP) containing nerves innervate and thereby influence the
secretion of Brunner's glands (Stach et al., 1978; Wright et al., 1940; Kirkegaard et al., 1981). Adrenergic nerves and VIP containing nerves stimulate exocrine secretion from submandibular gland. Many workers have investigated the effects of various hormones on the submandibular gland (Luckman, 1961; Spicer and Duvenci, 1964; Caramia, 1966a,b; Kaiho et al., 1975). Biochemical studies on the glands revealed that the activities of esteropeptidases (Junqueira et al., 1949), amylase (Angeletti et al., 1967), amount of glycoprotein (Hosoi and Ueha, 1977) synthesis and secretion of nerve growth factor - c are stimulated by testosterone.

The above short reviews on regulation of secretion of Brunner's glands and submandibular glands suggest similarity in the regulation of secretion of Brunner's and submandibular glands. Therefore, there may be possibility that the exocrine secretion of Brunner's gland can also be regulated by testosterone. The development of experimental duodenal ulcer, maximum damage and increase in ulcer index was observed in the duodenum of castrated + cysteamine-treated male mice than normal mice injected with only cysteamine. The decrease in the exocrine secretion of glycoprotein was observed from Brunner's gland in castrated mice. Decrease in acid glycoprotein from the Brunner's gland
duct cells is also not worthy. In addition, we have also observed more depletion of glycoprotein content of other exocrine cells, i.e., pyloric gland cells, cells of crypts of Lieberkuhn and goblet cells from the duodenum of castrated + cysteamine-injected mice than only cysteamine-injected male mice.

In conclusion, this study suggests that secretion of glycoprotein is very much essential for the protection of duodenal mucosa. This is contributed by various exocrine cells present in the duodenum. This secretion is influenced by testosterone. Perhaps duodenum of male might be more protected than female as Robert and his colleagues in 1987 showed that the female rats were more sensitive than males to cysteamine-induced duodenal ulcers. In recovery study (Robert et al., 1974), it was reported that in males there was 68% recovery within 25 days after the formation of duodenum ulcer which was only 25% in females. But in the present study we could not find any sex difference in staining intensity of glycoproteins and sugar content of Brunner's glands, but though not significant ulcer index was found to be higher in males than females. This shows better duodenal protection in female. To find this it is essential to study effect of estrogen on induced duodenal ulcers and secretion of Brunner's glands in female mice.