CHAPTER - III

SEX DIFFERENCE IN CYSTEAMINE-INDUCED DUODONAL ULCERS AND BRUNNER’S GLANDS OF MICE

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A) MATERIAL

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I) INTRODUCTION

It has been shown that there is sex difference in the formation of duodenal ulcers. Ulcer formation is predominant in men compared to women (Truelove, 1960; Kurata et al., 1985). Female sex hormones have been known to reduce the ulcer incidence in experimental models (Manekar and Namaji, 1977). Sex difference in experimentally induced duodenal ulcers has been described by Nadar and Pillai (1992), where they showed high incidence of duodenal ulcers in female rats. But Wingren et al. (1989) showed that healing of gastric ulcer is sex-dependent, female rats showed slow rate of healing. To resolve this controversy, it was decided to study the sex difference in cysteamine-induced duodenal ulcers and Brunner’s glands in Mice.

Extensive study of pathogenesis of duodenal ulcers comprises gastric acid hyper-secretion (Ishii et al., 1973; Kirkegaard et al., 1980), release of gastrin (Lichtenberger et al., 1977a); delayed gastric emptying (Lichtenberger, 1997b; Poulsen, et al., 1982) and inhibition of duodenal and Brunner’s gland secretions of bicarbonates and mucus and epidermal growth factor (EGF) (Poulsen et al., 1981; Kirkegaard et al., 1981), secretion from Brunner’s gland.
The glands of Brunner are localized in the submucosa of the proximal duodenum and experimental studies have indicated that the main function of the Brunner's gland is to protect the duodenal mucosa against the chyme ejected from the stomach (Florey et al., 1939; Griffith and Harkins, 1956). Duodenal ulcers are situated in the proximal duodenum in the Brunner's glands area and recent studies have demonstrated that an impaired Brunner's gland secretion might be involved in the development of experimentally induced duodenal ulcers (Hartial et al., 1950; Perkins and Green, 1975; Kirkegaard et al., 1981). To achieve favourable conditions for the formation of duodenal ulcers and to ensure reliability, well elucidated pathogens were used and various operations such as duodenal ligation (Shay et al., 1945) introduction of dil. HCl in duodenum (Nadar and Pillai, 1986 b) were carried out. The pathogenesis of these induced ulcers have been extensively studied and it seems that cysteamine is a potent ulcerogen in rats and induces duodenal ulcers in most of the cases.

To evaluate the reliability of sex difference in the formation of duodenal ulcers, it has been decided to study anterior part of the duodenum of mice in whom duodenal ulcers are induced with cysteamine. Duodenal
mucosa is protected by alkaline mucus layer of glycoprotein secreted by Brunner's glands and other cells of duodenum, which may also be contributing secretion of glycoprotein. There may be possibility of having sex difference in the formation and secretion of glycoprotein from all these cells. Therefore, it was decided to find out if there was any sex difference in the histology and histochemistry of glycoproteins of these cells. It was also been decided to study biochemically glycoprotein content of Brunner's glands.

II) MATERIAL AND METHODS
A) Material

Male and female mice with 25 to 30 gm body weight and about 2 months of age were used for the present investigation. They were maintained in proper conditions and were supplied with Gold Mohur mouse feed (Lipton, India) and drinking water ad libitum.

Different methods of duodenal ulcer induction were tried and cysteamine-induced gastroduodenal ulceration was selected. The mice initially were starved for 24 hours during which only water was supplied ad libitum. These mice were injected with cysteamine-HCl in water (40mg/100 gm BW) subcutaneously twice at the interval of 4 hours. The controls received water only. Twenty-four hours after the second dose the animals were
sacrificed by cervical dislocation. The pyloroduodenal junctions were dissected out as a single unit, opened along the greater curvature of the stomach and mesentry of the duodenum and processed for different methods.

B) Methods

1) Gross Morphology:
   i) The mucosa of pyloroduodenal junction and anterior of the duodenum was observed under stereomicroscope and was stained for alkaline phosphatase to highlight the mucosal nature and changes in mucosa. Method is described in detail in chapter.

   ii) The ulcers were critically evaluated under stereomicroscope and ulcer index was calculated using Szabo’s method (1978). The ulcer index of male and female were compared statistically to find out whether the difference was significant or not.

2) Histology:

   The pyloroduodenal junctions and anterior of duodenum were fixed in 10% neutral buffered formalin and routinely processed for histological technique. The sections were stained with Haematoxylene Eosin (HE). Histology of pyloric glands, duodenal villi, crypts of Lieberkunh and Brunner's glands was studied.
3) Histochemistry:

To study the nature of glycoproteins from various parts of pyloroduodenal junction, following histochemical techniques were used:

i) To study the glycoproteins in general, PAS technique was employed (McManus, 1946; Hotchkiss, 1948).

ii) To study the acidic glycoproteins AB pH 2.5 technique was used (Mowry, 1956).

iii) To study the sulphated glycoproteins AB pH 1.0 technique was used (Lev and Spicer, 1964).

iv) To study the difference between acidic mucins and neutral mucins AB + PAS technique was used (Mowry and Winkler, 1956; Mowry, 1963).

v) To study the difference between sulphated and carboxymucins acid hydrolysis technique was used (Quiterelli et al., 1961).

4) Biochemistry:

To study the nature of glycoproteins biochemical estimations of various sugars of glycoprotein and protein were carried out. With the help of present techniques available, it was not possible to separate pyloric glands, crypts of Lieberkühn and goblet cells but Brunner's glands were isolated by using the method...
described by Smits et al. (1982) and glycoproteins were isolated by using method described by Satakopan and Kurup (1977). The details of isolation of Brunner's gland and glycoproteins are described in the II chapter.

i) **Estimation of Fucose** (Dische Shettle, 1948)

Fucose was estimated using cold sulfuric acid and cysteamine 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 sample with 0.1 N sulfuric acid at 80°C for 1 hour by thiobarbituric acid method. Crystalline N-acetyl neuraminic acid was used as a standard.

iv) **Estimation of Protein** (Lowery et al., 1951)

The protein content of the glycoprotein was estimated using folin ciocalteu phenol reagent and Bovine serum albumin as a standard.
III) OBSERVATIONS

The different methods used for duodenal ulcer induction such as starvation, duodenal ligation, luminal acid and histamine were not suitable. In case of ligation technique ulcers were formed in the stomach region and histamine could induce ulcers in duodenum but incidences were not 100%. Cysteamine-induced ulcers were found mainly in duodenum and subsequent two doses of cysteamine gave 100% incidence of duodenal ulcer.

1) Gross Morphology:

A) Male (M₁) and Female (F₁) Mice

No sex difference could be noticed in the alkaline phosphatase reactivity in male and female. Gross morphological observations of pyloroduodenal junctions and duodenum of male and female mice are described in Fig.1 and 2 respectively. Villi from pyloroduodenal junctional region (PD) were short and arranged perpendicular to the duodenal villi (V). They showed very strong alkaline phosphatase activity. The duodenal villi appeared tall, leaf-formed. They also showed strong alkaline phosphatase activity.

B) Cysteamine-treated Male (CM₁) and Female (CF₁) Mice

Cysteamine-HCl administrated subcutaneously in double dose led to the formation of duodenal ulcers (U) in all mice of both the sexes. The ulcers were formed
in the proximal part of the duodenum. The acid-affected part of the mucosa showed reduced alkaline phosphatase staining or no staining according to severity. Alkaline phosphatase staining decreased with increase in severity of the ulcer. The deep ulcerated area did not stain at all (Fig. 3 and 4). In most of the cases the duodenal villi were low, in more affected areas the villi were eroded to form avillus conditions and in severely affected areas submucosa was involved in the erosion and sometimes muscularis mucosae was damaged to form perforating ulcers.

2) Ulcer index

Cysteamine-induced duodenal ulcer index is described in Table No.1. The percentage incidence of duodenal ulcer was found to be 100% in both male and female mice. Superficial ulcers were 15% in male and 21% female mice. Deep ulcers were 75% in male while 70% in female mice. Perforating ulcers were 10% in male and 9% in female. The mean severity was 2.05 + 0.14 in male and 1.09 + 0.2 in female. Though the ulcer severity was slightly higher in male than in female, the difference was not significant statistically (CM₁ : CF₁ = P < 0.3). The ulcer index was higher in male (4.05) than in female (3.9).
Table 1

Effect of Sex Hormones On Cysteamine-Induced Duodenal Ulcers in Normal Male and Female Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Incidence</th>
<th>Ulcers (%)</th>
<th>Mean Severity</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM₁ Normal O²⁺ Cysteamine (12)</td>
<td>100</td>
<td>15</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>CF₁ Normal O⁺ Cysteamine (9)</td>
<td>100</td>
<td>21</td>
<td>70</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Error.

P > 0.05 is non-significant.

CM₁ : CF₁ = P > 0.3
3) Histology

Pyloroduodenal junction was dissected out as a single unit and processed for histological studies, sagital sections of 7 µ were cut and processed for routine eosine hematoxyline technique.

a) Male (M₁) and Female (F₁) mice

Histology of pyloroduodenal junction of male and female mice is described in Fig.5 and 13 respectively.

i) Pyloric Glands

Pyloric part of the junction showed presence of pyloric glands (PG) (Fig.5 & 13) situated deep into submucosa. They were numerous and compactly arranged. The gland cell showed presence of large, ovoid nucleus at the base. Pyloric pits (PP) were occupying greater part of submucosa, opened on the mucosal surface. The PP were lined by epithelial cells. No differences could be noticed in the histological structures of pyloric glands of male and female mice.

ii) Duodenal villi

Duodenal villi (V) were tall, leaf-formed and uniformly arranged in both male and female mice (Fig.6 & 14). Each villus consisted of a core of delicate loose connective tissue (CT) and an epithelial covering (E) made up of single layer of columnar epithelium cells arranged on delicate basement membrane. The
epithelium showed mainly two types of cells, columnar epithelial cells and mucus-secreting goblet cells (G). Epithelial cells stained brightly with haematoxyline-eosin. The nuclei were situated at the base of the cells. Similar structure was observed for both male and female.

iii) Crypts of Lieberkühn

Figures 7 and 15 describe histological structure of crypts of Lieberkühn in male and female mice respectively. These are tubular glands located in the mucous membrane and extending into submucosa and open between the villi, on mucosal surface. The cells of crypts of Lieberkühn (CL) were continuous with epithelium of the villi. The crypt showed different types of cells among which were the mucus-secreting cells which did not stain with eosin-haematoxylin technique.

iv) Brunner's glands

Brunner's glands of male and female mice stained for haematoxylin and eosin are described in figures 8 and 16. The parenchyma of the gland was divided into lobules which were separated from one another by narrow mass of connective tissue septa ( ). The parenchyma consisted of secretory end pieces and their ducts. The spheriodal and elongated expansions are termed acini (AC). The cells of acini were pyramidal (in shape) with
nuclei situated towards the base. The acini showed very narrow lumen. The acinar cells and duct cells both showed mild staining with eosin, but the connective tissue ( ) showed bright staining. The difference in the structure in the Brunner's glands of male and female mice was not observed.

B) Cysteamine Treated Male (CM\textsubscript{1}) and Female (CF\textsubscript{1}) Mice

i) Pyloric glands

The cysteamine-treated male and female mice showed some alterations in the pyloric region (Fig. 9 and 17). The mucosa was eroded. The pyloric glands (PG) and pyloric pits (PP) showed increased staining with H-E. The lumen and pit gaps were dilated.

ii) Duodenal villi

The villi (Fig. 9, 10, 17 and 18) in both male and female mice showed changes. The less affected villi were short and broad with desquamation of apical surface (Fig. 10). Some of them showed fissures and ramifications, but the lamina propria was unaffected and without inflammation. The villi from more affected areas were nearly flat showing avillus condition. The surface epithelial cells were also low or nearly flat (Fig. 11) barely covering the lamina propria and other times bulging in small segments and showing necrosis. In heavily affected regions the epithelium barrier was broken and erosions ( ) were formed (Fig. 12). In

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this case pronounced inflammation was observed in the lamina propria. The centre of the affected area was most severely affected and showed deep erosion involving mucosa. The changes were same in male and female mice.

iii) Crypts of Lieberkühn

The cells of crypts of Lieberkühn (CL) of cysteamine-treated male and female mice (Fig.12 and 19) showed increase in eosin staining. The lumen was dilated and the cellular arrangement was disturbed the nuclei were picnotic.

iv) Brunner's glands

The size and shape of Brunner's glands cells of cysteamine-treated male and female mice were changed. The height of acinar cells was reduced considerably, a large lumen was formed and there was increase in interparenchymal gaps. The cells showed increased staining intensity with eosin, nuclei were enlarged and picnotic. The lumen of the ducts (D) also showed dilation (Fig.9, 12, 17 and 20).

4) Histochemistry

To study histochemistry of pyloroduodenal junction sections were stained for PAS, AB pH 2.5, AB pH 1.0, AB + PAS. Results of normal and cysteamine-treated male and female mice are described in figures from 21 to 52.
A) Male (M) and Female (F) Mice

i) Pyloric glands

The pyloric pit (PP) cells were strongly PAS-positive while pyloric glands (PG) cells were less reactive to PAS (Fig. 21 and 25) in both male and female mice. When the sections were stained with AB pH 1.0, the pyloric pits and pyloric glands both were unreactive (Fig. 29 and 33), with AB pH 2.5 the pyloric pits cells remained unreactive but the pyloric gland cells showed strong alcianophilia (Fig. 37 and 41). With AB pH 2.5 + PAS technique the pyloric pits stained pink violet while the gland cells stained bluish-green (Fig. 45 and 49). In acid hydrolysis the alcianophilia was lost partially or totally at some places in pyloric gland cells.

ii) Deodenal villi

Goblet cells (G) seemed to be absent or sporadically distributed in villi located at the pyloroduodenal junction. But goblet cells of duodenal villi located away from the pyloroduodenal junction were evenly distributed (Fig. 22 and 26). The goblet cells stained strongly with PAS in both the sexes. With AB pH 1.0 goblet cells (Fig. 30 and 34) showed alcianophilia, especially in the distal duodenum. These cells were strongly reactive with AB at pH 2.5 showing strong alcianophilia (Fig. 38 and 42). At the proximal
part of duodenum the alcianophilia was less. When AB pH 2.5 + PAS technique was employed some goblet cells stained purple violet while some bluish green, (Fig.46 and 50). When sections were treated for acid hydrolysis the alcianophilia was partially or totally lost in goblet cells. Observations were similar in both male and female mice.

iii) Crypts of Lieberkühn

The cells of crypts of Lieberkühn (CL) stained dark magenta showing strong reaction with PAS in both male and female (Fig.22 and 26). When Alcian blue technique was employed the cells showed alcianophilia in both the sexes at pH 1.0 as well as at pH 2.5 (Fig.30,34 and 38,42). With AB pH 2.5 + PAS technique some cells stained dark purple while others were bluish green (Fig.46 and 50). In acid hydrolysis there was partial loss of alcianophilia. The difference between the glycoprotein histochemistry of crypts of Lieberkühn of male and female mice was not noticed.

iv) Brunner's glands

The sections stained for PAS showed PAS-positive material in cells of Brunner's glands from proximal and distal site in both male and female mice (Fig.21,22,25 and 26). The reaction was intense and present at the luminal 2/3 of the cytoplasm. The Brunner's glands from distal part of duodenum showed less PAS reactivity. (Fig.22 and 26).
Brunner’s glands from proximal and distal sites when stained with AB pH 1 the acini and duct cells did not show alcianophilia (Fig. 29, 30, 33 and 34). But AB at pH 2.5 did show alcianophilia in duct cells (D) and in some acini (AC) from proximal part of duodenum (Fig. 37 and 38). The acini from distal part of duodenum did not stain at all (Fig. 41 and 42).

With Alcian Blue pH 2.5 + PAS technique majority of the acini (AC) stained pink magenta, while the duct cells and some acini (AC) from proximal duodenum stained purple violet in both the sexes. The reaction was rather stronger in male (Fig. 45, 46) than female (Fig. 49, 50).

B) Cysteamine-Treated Male (CM₁) and Female (CF₁) Mice

1) The Pyloric Glands

In cysteamine-treated male and female mice the pyloric pit (PP) and pyloric glands (PG) showed decrease in PAS reactivity (Fig. 23 and 27). With Alcian Blue at pH 1 mild alcianophilia appeared in the cells (Fig. 31) while at pH 2.5 the alcianophilia was very mild in male and completely lost in female (Fig. 43). AB pH 2.5 + PAS-reactive material was reduced in pyloric pits (PP) as well as pyloric glands (PG). The pyloric pits appeared purple violet while pyloric glands appeared blue in male (Fig. 47) in female the
alcianophilia was lost (Fig.51). With acid hydrolysis the alcianophilia was further reduced and was lost in some glands.

ii) The villi

In cysteamine-treated the goblet cells (G) of duodenal villi showed reduction in PAS-positive material. In severely affected villi the number of goblet cells were reduced (Fig.24 and 28). With alcian blue at pH-1 the goblet cells showed decrease in alcianophilia (Fig.32,36) in both male and female mice, at pH 2.5 also there was observed marked reduction in alcianophilia and the number of goblet cells also appeared to be reduced considerably (Fig.40 and 44). With AB pH 2.5 + PAS technique the reactivity of the goblet cells was reduced (Fig.48 and 52). In acid hydrolysis the alcianophilia was further reduced.

iii) Crypts of Lieberkühn

The cysteamine-treated mice, the cells of crypts of Lieberkühn (CL) showed decrease in reactivity with PAS (Fig.24,28), AB pH-1 (Fig.32,36), AB pH 2.5 (Fig.40,44) and AB pH 2.5 + PAS (Fig.48,52).

iv) Brunner's glands

In cysteamine-treated male and female mice, the Brunner's glands PAS activity was reduced considerably. The activity was seen as thin border on luminal side only. It was instead of granular as in normal.
PLATE NO.1

Pyloroduodenal region and anterior part of the duodeum of mice stained for alkaline phosphatase.

Abbreviation used are:

Villi - V; Pyloroduodenal junction - PD;
Alkaline Phosphatase - ALP

Fig.1: Male mouse = V - Uniform, tall, leaf formed,
stained with ALP.

Fig.2: Female mouse = V - Uniform, tall, leaf formed, stained with ALP.

Villi at PD are small, right angle to duodenal villi ALP +ve.
PLATE NO.2

Pyloroduodenal region and anterior part of the duodenum of ulcer induced mice stained for alkaline phosphatase.

**Fig. 3:** Male mouse - V are disturbed degenerated at various site. Deep ulcer (U) at PD; V from PD lost ALP activity.

**Fig. 4:** Female mouse - V are disturbed, some times there are low (LV), degenerated at various cites, deep ulcers (U) at PD formation of low villi.
FIGURES 5, 6, 7 and 8 are cross sections of pyloroduodenal junction and anterior part of the duodenum of male mice.

Figures 9, 10, 11 and 12 are cross sections of pyloroduodenal junction and anterior part of the duodenum of ulcerated male mice.

Abbreviations:
- Pyloric pit (PP)
- Pyloric gland (PG)
- Brunner's gland (BG)
- Acinar cell (AC)
- Duct cell (D)

Fig. 5: It shows pyloric pit (PP), pyloric gland (PG) and Brunner's gland (BG) and Crypts of Lieberkuhn (CL). Cells from all these structures are normal and well organised. 50.4 x

Fig. 6: It shows villi of normal mouse. Epithelium (E) is thick; Goblet cells (G) are present in well organised cells and their nuclei. 320 x

Fig. 7: It shows crypts of Lieberkuhn (CL) and little part of the Brunner's gland acini (AC). CL are normal with large amount of cytoplasm, nuclei are situated at the basal region, stained very well with eosine hematoxylin. 320 x

Fig. 8: This figure shows Brunner's gland acini (AC) with ducts (D), nuclei are uniformly arranged and darkly stained with hematoxylin. 320 x

Fig. 9: Section passing through pyloroduodenal junction, PP and PG are degenerated. Some part of the PG also degenerated. Increase in the lumen of acini. 50.4 x

Fig. 10: Epithelial erosions (↑) at several places. Erosions (↑) are in general near to goblet cells. 320 x

Fig. 11: Villi become flat, epithelial living is reduced considerably; mixing of two villi, 320 x

Fig. 12: Crypts of Lieberkuhn (CL) are elongated. Structure is disturbed; nuclei of acini (AC) are faintly stained compared to normal (Fig. 8, AC). Dilation of the lumen of AC is seen. 320 x
Figures 13, 14, 15 and 16 are cross sections of pyloroduodenal junction and anterior part of duodenum of normal female mice.

Figures 17, 18, 19 and 20 are cross sections of pyloroduodenal junction and anterior part of duodenum of ulcerated female mice.

**Abbreviations:** Pyloric pit - PP; Pyloric gland - PG; Brunner's gland - BG; Crypts of Lieberkuhn - CL.

**Fig. 13:** Cross section of pyloroduodenal junction and anterior part of the duodenum is stained for E + H, PP, PG and CL are normally arranged, the structure is normal. 50.4 x

**Fig. 14:** Villi are well formed with thick epithelium; cells are well arranged; nuclei are basally situated in the mucosa, well arranged goblet cells (G) are seen. 320 x

**Fig. 15:** It shows the section passing through crypts of Lieberkuhn (CL), cells of crypts of Lieberkuhn are well arranged with basally situated nuclei. 320 x.

**Fig. 16:** It shows the section passing through Brunner's gland area, cells of BG are well arranged with basally situated nuclei; nuclei are well stained with hemotoxylin. 320 x.

**Fig. 17:** The section is of pyloroduodenal junction and anterior part of the duodenum. PP and PG are degenerated (U) ulcerated (U) area are seen. 50.4 x

**Fig. 18:** It shows structure of villi of ulcerated duodenum; nuclei of columnar epithelial region were swollen and irregularly arranged, goblet cells (G) show degeneration and thinning of mucosa. 320 x

**Fig. 19:** The section is passing through crypts of Lieberkuhn (CL) of ulcerated duodenum. Size of CL is reduced. It is degenerating. 320 x

**Fig. 20:** This is cross section of Brunner's gland of ulcerated duodenum. Cells are not properly stained with eosin hematoxylin. Nuclei of BG stained faint. 320 x
Pyloroduodenal region and anterior part of the duodenum of normal and ulcerate mice stained with PAS.

**Abbreviation**: Pyloric pit - PP, Pyloric gland - PG, Brunner's gland - BG, Duct - D, Goblet cell - G.

**Fig. 21**: Cross section of pyloroduodenal region. 50.4x
- PAS +ve - PP, PAS -ve - PG,
- PAS +ve - BG, PAS +ve - D

**Fig. 22**: Cross section of anterior part of the duodenum. 50.4x
- PAS +ve - G, PAS +ve - CL, PAS +ve - BG

**Fig. 23**: Cross section of pyloroduodenal region of ulcerated mice. 50.4x
- BG - PAS activity is reduced
- PP, PG are not seen in the figure but in observation in PP PAS activity reduced.

**Fig. 24**: Anterior part of the duodenum of ulcerated mice. 50.4x
- BG - Reduced PAS activity
- CL - Considerable reduction in PAS
- G - Reduction in the PAS activity as well as number of goblet cells.
PLATE NO.6

Pyloroduodenal region and anterior part of the duodenum of normal and ulcerated mice stained with PAS.

**Abreviation:** PP - Pyloric pit, PG - Pyloric gland
BG - Brunner’s gland, G - Goblet cells

**Fig.25:** Cross section of pyloroduodenal region. 50.4 x
- PP - Pyloric pit PAS +ve
- PG - Pyloric stand cells - weakly PAS +ve

**Fig.26:** Cross section of anterior part of duodenum 50.4 x
- G - PAS activity reduced
- CL - Considerable reduction in PAS

**Fig.27:** Cross section of pyloroduodenal region of the duodenum of ulcerated mice. 50.4 x
- G - Number is reduced, PAS activity is reduced in PP and G and lost in PG.

**Fig.28:** Cross section of anterior part of the duodenum of ulcerated mice. 50.4 x
- Number of G is reduced, PAS activity is reduced in G, cells of CL and BG.
PLATE NO.7

Pyloroduodenal region and anterior part of the duodenum of normal and ulcerated mice stained with AB pH 1.0.

**Fig.29**: Cross sections of pyloroduodenal region of normal male mice. 50.4 x

- PP - Pyloric pit AB pH 1.0 -ve
- PG - Pyloric stand AB pH 1.0 +ve
- G - Goblet cell AB +ve
- CL - Cells of crypts of Lieberkuhn AB +ve
- BG - Cells of Brunner's gland AB -ve

**Fig.30**: Cross section of anterior part of the duodenum of normal male mice. 50.4 x

- Goblet cells (G) AB +ve
- Crypts of Lieberkuhn (CL) AB +ve

**Fig.31**: Cross section of pyloroduodenal region of ulcerated mice. 50.4 x

AB pH 1.0 staining from pyloric stand (PG) and Goblet cell (G) is lost.

**Fig.32**: Cross section of anterior part of the duodenum of ulcerated mice. 50.4 x

AB pH 1.0 reacting from crypt cells and goblet cell is lost.
PLATE NO. 8

Pyloroduodenal region and anterior part of the duodenum of normal and ulcerated O mice stained with AB pH 1.0

Fig. 33: Cross section of pyloroduodenal region and normal female. 50.4 x

G - Goblet cells are AB pH 1.0 -ve

Fig. 34: Cross section of anterior part of normal female. 50.4 x

Cell of CL AB pH 1.0 +ve
Goblet cells (G) AB +ve
Brunner's gland (BG) AB -ve

Fig. 35: Cross section of pyloroduodenal region of ulcerated female. 50.4 x

Some of the goblet cells (G) showed slight alcianophilia.

Fig. 36: Cross section of anterior of region duodenum. 50.4 x

Some of the goblet cells (G) and cells of crypts of Lieberkühn (CL) showed slight alcianophilia.
PLATE NO. 9

Pyloroduodenal region and anterior part of one duodenum of normal and ulcerated O mice stained with AB pH 2.5

**Fig. 37**: Cross section of pyloroduodenal region of normal O mouse. 50.4 x

- Pyloric gland cells AB pH 2.5 +ve (Fig. 45) and pyloric pit cells are AB pH 2.5 -ve.
- Some of the alcini (AC) and Ducts (D) of Brunner's gland (BG) are AB +ve
- Goblet cells (G) are AB +ve

**Fig. 38**: Cross section of anterior part of the duodenum of normal mouse. 50.4 x

- Cell of CL and Goblet cells (G) intensely stained with AB.
- Slight activity is in AC of BG

**Fig. 39**: Cross section of pyloroduodenal region of ulcerated O mouse. 50.4 x

- AB positivity is reduced duct cells (D) and acinar cells (AC)

**Fig. 40**: Cross section of anterior of region duodenum of ulcerated mouse. 50.4 x

- AB positivity is reduced considerably in goblet cells (G) and cells of crypt (CL)
PLATE NO. 10

Pyloroduodenal region and anterior part of one duodenum of normal and ulcerated O mice stained with AB pH 2.5

**Fig. 41**: Cross section of pyloroduodenal region of normal mouse. 50.4 x

Cells of PG and PP showed very little staining reactivity.

Ducts and anci of BG are AB +ve

Goblet cells (G) are AB +ve

**Fig. 42**: Cross section of anterior part of the duodenum of ulcerated mouse. 50.4 x

Cell of CL and Goblet cells (G) are AB pH 2.5 +ve

**Fig. 43**: Cross section of pyloroduodenal region of ulcerated mouse. 50.4 x

AB pH 2.5 positivity from all cells is lost

**Fig. 44**: Cross section of anterior part of duodenum of ulcerated mouse. 50.4 x

AB pH 2.5 positivity from cells of crypts of Lieberkuhn and goblet cell is lost
Pyloroduodenal region and anterior part of duodenum of normal and ulcerated 0 mice stained with AB pH 2.5 followed by PAS.

Fig.45: Cross section of pyloroduodenal region of duodenum of normal 0. 50.4 x

Pyloric pit cells (PP) are PAS +ve
Pyloric gland cells (PG) are AB +ve blue.

Some of the acinar cells (AC) of Brunner’s gland (BG) are purple(AB & PAS +ve) and some are pink.
Duct cells of BG stained purple

Fig.46: Cross section of anterior duodenum of normal 0 50.4 x

Some goblet cells (G) are blue, some are purple; cells of CL purple; Brunner’s gland acini PAS +ve

Fig.47: Cross section of pyloroduodenal region of the duodenum of ulcerated 0. 50.4 x

From all sides i.e. PP, BG, Duct cells PAS activity is lost

Some reduction in AB staining

Fig.40: Cross section of anterior part of the duodenum PAS reactivity from BG, CL and G is lost; but AB pH 2.5 is not completely abolished. 50.4 x
PLATE NO.12

Pyloroduodenal region and anterior part of one duodenum of 0 normal and ulcerated mice stained for AB + PAS

**Fig.49 :** Cross section of pyloroduodenal region of normal 0. 50.4 x

Pyloric pit cells - PAS +ve  
PG - slight AB +ve  
AC of Brunner's gland - AB +ve  
Duct cells (D) of BG are blue purple  
Goblet cells - purple

**Fig.50 :** Cross section of anterior duodenum normal female. 50.4 x  

G - AB PAS positive, some G are purple  
BG - PAS positive  
CL - purple

**Fig.51 :** Cross section of pyloroduodenal region of ulcerated 0. 50.4 x

AB positivity is completely lost.

**Fig.52 :** Cross section of anterior duodenum of ulcerated 0. 50.4 x

Cells of CL reduced its purple colour  
G - lost both blue and purple colour  
AC of BG lost PAS positivity.
PLATE NO. 5
PLATE NO. 7
PLATE NO.8
PLATE NO. 9
Connective tissue also showed some PAS activity (Fig. 23, 24, 27 and 28). With Alcian Blue at pH 1 there was no alcianophilia (Fig. 31, 32, 35 and 36) and at pH 2.5 the alcianophilia was reduced but it was not completely lost (Fig. 39, 40, 43 and 44). With AB pH 2.5 + PAS the reactivity was found to be reduced and was present only on luminal side (Fig. 47, 48, 51 and 52) with acid hydrolysis further loss of activity was noticed.

5) Colorimetric Estimations of Sugars and Protein from Glycoprotein of Brunner’s Glands of Male (M₁) and Female (F₁) Mice

Values of colorimetric estimations of sugars and protein from Brunner’s glands glycoprotein of normal and cysteamine-treated male and female mice are given in Table No. 2. In general, the values are found to be decreased in cysteamine-treated mice.

1) Hexose: Hexose content is expressed as ug/mg glycoprotein. In normal male and female mice hexose content was 80.125 ± 0.7212 and 73.916 ± 0.83 respectively. In cysteamine-treated male and female mice the hexose content was 31.3503 ± 0.4142 and 20.5 ± 0.25 respectively. The values were found to be decreased in cysteamine-treated mice. The decrease, as compared to normal values, was highly significant in both male (M₁ : CM₁ = P < 0.005) and female (F₁ : CF₁ = P < 0.005) mice. Reduction in hexose content was
significant in both the sexes. It was nearly or more than 30%.

ii) Fucose: Fucose content was measured in terms of ug/mg glycoprotein. The fucose content in normal male mice was $3.8474 \pm 0.0074$ and $4.070 \pm 0.1229$ in female mice. In female it was slightly greater than in male. In cysteamine-treated mice the fucose content in both male and female was found to be $1.2538 \pm 0.0111$ and $1.486 \pm 0.0154$, respectively. The decrease in fucose content was $M_1 : CM_1 = P < 0.0001$ and $F_1 : CF_1 = P < 0.001$) highly significant.

iii) Sialic Acid: Sialic acid was estimated using Warren's (1959) method from glycoprotein isolated from Brunner's glands of normal and cysteamine-treated male and female mice. The sialic acid was measured in terms of ug/mg glycoprotein. The sialic acid content in normal male and female was $0.2609 \pm 0.0016$ and $0.3051 \pm 0.008$, respectively. The female had slightly more sialic acid content. In cysteamine-treated mice the sialic acid content was $0.0409 \pm 0.0013$ in male and $0.0642 \pm 0.0012$ in female. The decrease in sialic acid content was found to be highly significant ($M_1 : CM_1 = P < 0.001$, $F_1 : CF_1 = P < 0.001$) in both male and female mice.

iv) Protein: Protein content was estimated from glycoprotein isolated from the Brunner's glands of
### Table 2

**Carbohydrates and Protein Contents of Soluble Glycoprotein Isolated from Brunner's Glands of Normal and Cysteamine-Treated Male and Female 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>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₁</td>
<td>Normal</td>
<td>80.125</td>
<td>3.8474</td>
<td>0.2609</td>
</tr>
<tr>
<td></td>
<td>± 0.7212</td>
<td>± 0.0074</td>
<td>± 0.0016</td>
<td>± 0.1437</td>
</tr>
<tr>
<td>CM₁</td>
<td>Normal + Cysteamine</td>
<td>31.3583</td>
<td>1.2538</td>
<td>0.0409</td>
</tr>
<tr>
<td></td>
<td>± 0.4142</td>
<td>± 0.0111</td>
<td>± 0.0013</td>
<td>± 0.1835</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>Normal</td>
<td>73.916</td>
<td>4.070</td>
<td>0.3051</td>
</tr>
<tr>
<td></td>
<td>± 0.83</td>
<td>± 0.1229</td>
<td>± 0.008</td>
<td>± 0.0909</td>
</tr>
<tr>
<td>CF₁</td>
<td>Normal + Cysteamine</td>
<td>20.5</td>
<td>1.486</td>
<td>0.0642</td>
</tr>
<tr>
<td></td>
<td>± 0.25</td>
<td>± 0.0154</td>
<td>± 0.0012</td>
<td>± 0.329</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Error.

P > 0.05 is non-significant.

**Hexose**

- M₁ : CM₁ = P < 0.005 HS
- F₁ : CF₁ = P < 0.005 HS

**Fucose**

- M₁ : CM₁ = P < 0.0001 HS
- F₁ : CF₁ = P < 0.001 HS

**Sialic acid**

- M₁ : CM₁ = P < 0.001 HS
- F₁ : CF₁ = P < 0.0001 HS

**Protein**

- M₁ : CM₁ = P < 0.001 HS
- F₁ : CF₁ = P < 0.005 HS
normal and cysteamine treated male and female mice. The protein content was estimated as ug/mg glycoprotein.

The protein content in normal male and female estimated was $20.4845 \pm 0.1437$ and $20.1696 \pm 0.0909$ respectively. The content was found to be almost equal in male and female. In cysteamine treated mice the values were found to be reduced. The treated male had $14.899 \pm 0.1835$ and the female had $14.891 \pm 0.329$. The reduction in protein content in cysteamine treated male and female mice was highly significant ($M_1 : CM_1 = P < 0.001, F_1 : CF_1 = P < 0.005$).

IV) DISCUSSION

Duodenal mucosa of male and female mice was observed under stereo microscope and stained for alkaline phosphatase. Villi of pyloro-duodenal mucosa and duodenal mucosa were normal and selectively stained for alkaline phosphatase. With the help of these techniques it has made possible to estimate ulcer index as well as to observe degeneration of villi, during ulceration. Mice of both the sexes starved for 48 hours, receiving water ad libitum, did not show any sign of destruction of villi. Anterior and posterior regions of duodenum have regular leaf form villi were stained very well for alkaline phosphatase. A band of microvilli at the pyloro-duodenal junction was also
intensely stained. But the introduction of cysteamine of two doses (40 mg/100 gm BW) to 24 hours starved mice which were sacrificed after 24 hours produced severe damage to the mucosa at the junction and posterior to it. Continuation of the process results in desquamation of the epithelium on the apical part of the villi, as a result they became short and broadened and ultimately avillus surface was formed, which showed several lesions. Differences in the structure was not noticed between male and female. The incidence of lesions was 100% and ulcer severity and ulcer index were more or less same in both the sexes. Histological picture showed very low, broad villi, nearly flat at the top with mild inflammation. Necrotic changes were observed. Epithelial barrier was broken and erosions were formed. Several lesions at the anterior and posterior walls of proximal duodenum were observed. Similar changes were observed in cysteamine-induced duodenal ulcers in rat (Selye and Szabo, 1973; Szabo, 1978). Cysteamine-induced duodenal ulcers in rat and pathophysiological changes during ulceration closely resemble duodenal ulcers in man (Szabo, 1978; Poulsen and Szabo, 1977).

Though apparently, sex difference was noticed in the formation of ulcers; statistically no significant difference was noticed in the severity of ulcers and
ulcer index. Earlier in human beings, it has been reported that the prevalence of duodenal ulcer is common in men that in women (Culmer et al., 1939; Gray et al., 1939; Truelove, 1960; Dey and Dey, 1974). Not only do duodenal ulcers appear much less common in women than in men but it also appears to run less severe course, at any rate judged by the liability to perforation. It was shown that chance of a man developing duodenal ulcer remains remarkably constant between ages of 20 and 68 (Doll and Jones, 1951). By contrast, chance of a woman developing duodenal ulcer remains relatively low throughout the whole of her reproductive life but increases sharply at the time and after the menopause (Truelove, 1960). Sex difference in experimentally induced gastroduodenal ulcers in albino rats was shown by Nadar and Pillai (1992). They induced gastroduodenal ulcers by various methods— starvation, duodenal ligation and intragastric HCl administration. Percentage incidence of ulcer in the forestomach of male and female of starved rats was 80 and 70%, in ligated rats it was 37.5% and 40%, in HCl administrated rats it was 25% and 11.11%, respectively. In all above methods, ulcers were either located in forestomach or in duodenum and in the forestomach but not in the duodenum. But cysteamine-induced duodenal ulcers developed only in duodenum and incidence was 100% in
both male and female. The ulcer index was 4.3 and 3.888 for male and female, respectively. The contractile report was made in 1971 (Robert et al., 1971). According to them, female rats were more sensitive than males to cysteamine-induced ulcers. At a dose of 350 mg/kg, only 12% of the males developed ulcers as compared to 72% of the females. At 475 mg/kg the incidence was about the same for both the sexes, but severity of the ulcers, as judged by the incidence of the animals with duodenal perforation, was much lower in male rats (15%) vs 64% in females. The differences in incidences and severity between males and females were statistically significant at the 1% level for both the doses, except for the incidence at a dose of 425 mg/kg which was similar in both the sexes.

Induction of ulcers in rats with the help of cysteamine is described by Selye and Szabo (1973) and later on by several workers (Poulsen, 1973; Landboe and Christensen and Paraport, 1974; Robert et al., 1974; Ishii et al., 1976; Poulsen and Szabo, 1977; Szabo, 1978; Szabo et al., 1979; Szabo et al., 1980; Kirkegaard et al., 1980; Adler et al., 1982; Nadar & Pillai, 1989).

Mode of induction of ulcers used was different by different investigators. Some have used small dose but
introduced 3 to 4 times at the interval of 3 to 5 hours. Some have small dose 3 time a day for 3 to 4 days, some have used large single dose. All these doses were adequate enough for the formation of duodenal ulcers (Parmar and Desai, 1993).

All these observations do not provide significant clue as to the mechanism by which cysteamine produces duodenal ulcers; we know that the passage of acid contents over the duodenum is necessary for the formation of ulcers. But in case of cysteamine however no secretogogue action is responsible for the development of ulcers, since cysteamine is an antisecretory (Robert et al., 1974). This statement is contradictory because there are reports describing enormous increase in gastric acid after the administrating cysteamine (Poulsen and Szabo, 1977) and cimetidine (Szabo et al., 1979), antacid and antisecretory agents (Poulsen and Szabo, 1977) as well as vagotomy (Haith et al., 1975) prevent duodenal ulcers caused by cysteamine. Whatever may be the reason, it is fact that cysteamine induces only duodenal ulcers. Cysteamine seems to prepare the duodenum for damage produced by gastric contents. It may weaken the natural defence of duodenal mucosa against intraluminal irritants to such a degree that, in spite of a decrease in gastric secretion, whatever
acid pepsin is still present in the lumen is sufficient to damage duodenal mucosa. How? such a weakening in the local resistance is accomplished, either cysteamine may reduce duodenal blood flow or reduces mucus secretion or it may have a direct cytotoxic action on duodenal cells present at the gastroduodenal area. The gastroduodenal mucus secreted by these cells along with bicarbonate forms a water-insoluble gel adherent to the mucosal surface and also present as a viscous, soluble form in the lumen. The adherent gel covering the mucosal surface is considered to have a protective role against acid ejected from the stomach acting as a stable mixing barrier (Hollander, 1954; Heatley, 1959; Allen and Garner, 1980; Flemstorm and Garner, 1982), and luminal pepsin by forming diffusion barrier (Allen, 1981 a;b), mechanical damage by acting together with soluble mucus as a lubricant (Florey, 1955).

The main cells contributing mucus secretion in the gastroduodenal lumen are pyloric glands, goblet cells, cells of Lieberkühn and a thick band of Brunner's glands located in the submucosal region of the anterior portion of the duodenum. The Brunner's gland seems to be the major source of mucus secretion in lumen. Histochemically it has been observed that Brunner's glands were rich in PAS +ve material. At the extreme
anterior region some of the Brunner's glands and duct cells consisted of neutral and acid mucopolysaccharides. The other cells like goblet cells and crypts of Lieberkühn also consisted of neutral and acid mucopolysaccharide. No sex difference in distribution of these cells as well as mucopolysaccharide content was noticed. For biochemical study it was difficult to separate pyloric glands, goblet cells and cell of crypts of Lieberkuhn, but Brunner's glands can easily be isolated. Glycoprotein from the Brunner's glands was isolated and various sugars and protein were estimated. It has been observed that the sugars like hexose and fucose were in high concentration in male Brunner's glands whereas sialic acid is more in female. There was tremendous loss of glycoproteins from Brunner's glands as well as from other mucus-secreting cells described above. Reduction in the glycoprotein secretion of the Brunner's glands in cysteamine-administered rat was described already by Nadar and Pillai (1989). Enzyme β-glucuronidase is involved in the glycoprotein secretion. The decrease in β-glucuronidase activity in cysteamine-administered rat has also been observed by Nadar and Pillai (1989). Inrepairement of Brunner's gland function in cysteamine-treated rat is also observed (Poulsen et al., 1981; Nadar, 1988).
Except Brunner's glands, as described earlier, other cell types also contribute to the gastrointestinal glycoprotein secretion; these cells are pyloric glands, goblet cells, and cells of crypts of Lieberkühn. These cells not only secrete neutral glycoproteins but also acidic mucopolysaccharides. Histologically and histochemically, in the present investigation it has been observed that the number of goblet cells is decreased during ulceration as well as there was a decrease in intensity of staining for glycoproteins in the ulcerated conditions in both the sexes. Tober and Gerok (1987) reported that biosynthesis of mucus glycoprotein is decreased during gastric ulceration, while breakdown is enhanced.

In conclusion it has been clearly indicated that cysteamine induces constant and precisely located ulcers both in male and female mice. Sex difference observed in the formation of ulcers is not significant. The various cells like pyloric gland cells, goblet cells, cells of crypt of Lieberkühn and Brunner's gland cells located in the gastroduodenal area are responsible for the secretion of glycoprotein. Glycoprotein secreted by these cells is of neutral and acidic in nature. Both the types of glycoproteins were reduced in ulcerated condition. Loss of glycoproteins
may be due to degeneration of these cells during ulceration. Apparently there is no loss of Brunner’s gland cells; their secretion is inhibited in ulceration. Various sugar isolated from Brunner’s glands showed quantitatively tremendous decrease both in male and female.

Thus, one of the main reasons of formation of duodenal ulcer is decrease in glycoprotein secretion in the duodenum. There is no sex difference in the increase or decrease in glycoprotein synthesis, and thus in the formation of duodenal ulcers.