CHAPTER V

EFFECT OF ESTROGEN ON CYSTEAMINE-INDUCED DUODENAL ULCERS AND BRUNNER'S GLANDS OF FEMALE MICE

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I) INTRODUCTION

One of the conspicuous features of duodenal ulcer is its common occurrence in adult male. In childhood the ulcer is uncommon and two sexes are equally affected. After puberty its frequency increases and a heavy male preponderance become apparent from the age of 20 years onwards. It has been shown 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, and further found that when a woman with a chronic peptic ulcer becomes pregnant, it is usually found that she is symptom-free during pregnancy although symptoms are likely to recover 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, these peculiarities of the ulcer were related to sex with the antiulcer effect of the circulating estrogens (Truelove, 1960). Manekar and Namaji (1977) observed that female sex hormones protect duodenal mucosa against ulceration induced by Histamine. The estrogen possibly diminishes the secretory activity of the stomach (Ojha and Wood,
Another possibility is that estrogens have a beneficial effect upon healing of lesions. The reason also seems that estrogens are general stimulators of mitosis (Truelove, 1960). The metabolic actions of estrogens in increasing protein anabolism and depressing blood cholesterol might be highly relevant to healing. It has been shown that steroids (naturally occurring human estrogens and synthetic stilbestrol which mimic the biological actions of estrogen) act as coenzymes and facilitate hydrogen in nuclear reactions of varieties of tissues. Truelove (1960) has interpreted duodenal ulcer as a 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 acids.

The binding site for the estrogen was found to be associated with female reproductive tissue. But in recent past estrogen binding proteins and/or physiological responses to estrogen have been observed in various organs reviewed by Grossman and his colleagues (Grossman et al., 1984). There are number of
reports describing control of estrogen on structure and secretion in salivary gland cells, specially in submandibular gland (Shafer and Muhler, 1953; Smith and Allson, 1966; Liu, 1967; Wilborn, 1968; Liu and Lin, 1969a,b; Mudd and White, 1975; White and Mudd, 1975; Rybakova, 1978; Flynn et al., 1983 and Mehansho, 1983). Secretory cells of the submaxillary glands can be compared with Brunner's gland cells which are also rich in glycoprotein secretion. Hence it may be possible that the glycoprotein secretion from Brunner's glands may be controlled by estrogen.

We have discussed in the introductory and 3rd chapter the role of glycoproteins in the protection of gastrointestinal tract. During induced ulceration the mucosa of polyduodenal junction must have been protected by glycoproteins secreted by Brunner's glands and other cells of the duodenum. Taking all this into consideration, it was decided to study the effect of estrogen on induced duodenal ulcers and Brunner's glands in female mice.

II) MATERIAL AND METHODS

A) Material

Female mice weighing 25 to 30 gm and about 2 months of age were used to study effect of estrogen on cysteamine-induced duodenal ulcers and Brunner's
glands. The females were overiectomised under mild ether anesthesia. The operated 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 the mice were injected (i.p.) consecutively for 3 days with 2 mg/100 gm BW Estradiol/17β (Sigma, Batch No.E 9505) in olive oil (F3). The remaining operated mice were injected with olive oil only (F2). The mice (F2 and F3) were then used for duodenal ulcer induction (CF2, CF3).

Out of different methods tried, cysteamine-induced duodenal ulceration method was used to induce ulceration. Initially, mice were starved for 24 hours during which only water was supplied ad libitum. The mice were injected with Cyhssteamine-HCl in water (40 mg/100 gm BW) subcutaneously twice at the interval of 4 hours. The remaining (F2 and F3) operated and hormone-injected mice were injected with distilled water only and were used as controls for the cysteamine-treated mice. Twenty-four hours after the second dose the animals were sacrificed, the pyloroduodenal junctions were dissected out opened along greater curvature of stomach and mesentery of duodenum and processed for different methods.
B) Methods:

1) Gross Morphology
   i) The mucosa of pyloroduodenal junction was stained for alkaline phosphatase, observed under stereomicroscope to critical evaluation of changes in mucosa due to ovariectomy and cysteamine injections.
   ii) Ulcer index was calculated by using Szabo's method (1979). Ulcer index were compared statistically to find out significance of 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 changes in duodenal mucosa, glycoproteins of crypts of Lieberkuhn, goblet cells, pyloric gland cells and Brunner's glands of cysteamine-treated and control mice following histochemical techniques were used.
   i) To study the glycoproteins in general PAS technique was used (McManus, 1946; Hotchkiss, 1948).
ii) Acidic glycoproteins were studied by using AB pH 2.5 technique (Mowry, 1956).

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

iv) To study the difference 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 sulphated and corboxy mucins acid hydrolysis technique was used (Quintarelli, et al., 1961).

4) Biochemistry

To study various constituents 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 Lieberkuhn and goblet cells but Brunner’s glands were isolated by using the method described by Smits et al. (1982) and glycoprotein was isolated by using method described by Satakopan 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-glucoronic 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. Crystalline N-aceyl neuraminic acid was used as a standard.

iv) Estimation of Protein (Lowry et al., 1951)
Protein from the glycoprotein was estimated by using Folin ciocalteu phenol, reagent and Bovine serum albumin as a standard.

III) Observations (Ovariectomised Mice F2)
1) Gross Morphology
A) Ovariectomised Mice (F2)
Fig.No.101, indicates pyloroduodenal mucosa of ovariectomised female, stained for alkaline phosphatase. The staining was almost uniform except certain patches of faintly stained areas. Villi from these areas were short desquamated (LV). Deep erosions and penetrating ulcers were not evident. Rest of the area showed normal, tall, leaf form villi (V).

B) Cysteamine-Treated Ovariectomised Female (CF2)
Cysteamine-HCl administered subcutaneously in double dose led to the formation of duodenal ulcers (U).
Normal villi stained dark red with alkaline phosphatase. The staining decreased with increase in severity and mucosal damage. Deep ulcerated areas did not stain at all (Fig.102).

2) Duodenal Ulcer Index

Table No.5 describes mean severity of ulcer and ulcer index of ovariectomised cysteamine treated (CF₃) mice. Duodenal ulcers were formed towards distal duodenum. Severity of ulcers increased as distance increased from pyloroduodenal junction. Ulcers were usually not formed at pyloroduodenal junction. The villi (LV) were short, desquamated. Erosions were deep forming deep ulcers, sometimes forming perforating ulcers. Percentage incidence was 100%. Superficial ulcers were 10%, 70% were deep ulcers and 20% were perforating ulcers. Mean severity was 3.1 ± 0.102 and ulcer index was 5.1. In normal cysteamine treated mice mean ulcer severity and ulcer index were 1.3 ± 0.2 and 3.9 respectively.

3) Histology

a) Ovariectomised Mice (F₂)

The figures 103, 104 and 107 describe histological structure of pyloroduodenal junction of ovariectomised female mice.
i) Pyloric Gland (PG) :

It showed similar structure as in the normal. The cells stained intensity with H-E. The glands were simple tubular situated deep in submucosa and opening into pyloric pit (Fig.103).

ii) Duodenal Villi (V) :

Majority of the villi (V) were tall, leaf like and uniformly arranged but at certain places the villi were not desquamated (LV). The villi stained intensely with H-E (Fig.103 and 104).

iii) Crypts of Lieberkühn (CL) :

Crypts of Lieberkühn showed similar structure as in the normal. The nuclei were situated basal in position, the cytoplasm stained deeply with eosin (Fig.103 and 107).

iv) Brunner's Glands (BG) :

When stained with H-E showed similar structure as in the normal. The cells of acini (AC) were pyramidal shape with nuclei basal in position and had a very narrow lumen (Fig.103, 107).

B) Cysteamine-Treated Ovariectomised Female Mice (CF₂):

i) Pyloric Glands (PG) :

In cysteamine treated ovariectomised mice some histological changes were observed. The lumen was dilated and nuclei were picnotic. Eosinophilia was
increased in both gland cells (PG) and cells of pyloric pit (PP) (Fig.105).

ii) Duodenal Villi (V):

Fig.106 indicates duodenal villi of cysteamine treated ovariectomised female mice. The villi in general were short, broad and desquamated, their apical surfaces showed fissures and ramifications. At certain regions villi were eroded to form avilous condition and at certain places epithelial barrier was broken and ulcers were formed. Number of goblet cells was reduced (Fig.105, 106).

iii) Crypts of Lieberkühn (CL):

In cysteamine treated ovariectomised female (Fig.105 and 108) showed changes in size and shape of the secretory cells of crypts of Lieberkühn (CL). The height was decreased and lumen dilated, the nuclei lost their normal shape and were picnotic.

iv) Brunner’s Glands (BG):

The cells of Brunner’s glands acini (AC) showed reduced height, dilated lumen and nuclei lost their normal size and shape. The eosinophilia was increased. The ducts (D) also showed dilated lumen and increased eosinophilia (Fig.105 and 108).

4) Histochemistry

Figures 109, 110, 113, 114, 117, 118, 121 and 122 describe histochemical nature of pyloroduodenal
junction of ovariectomised (F2) and cysteamine treated ovariectomised (CF2) mice. PAS, AB pH 2.5, AB pH 1.0, AB pH 2.5 + PAS and acid hydrolysis techniques were employed to study the histochemistry.

A) Ovariectomised Mice (F2)

i) Pyloric Glands (PG)

The fig.109 indicates structure of pyloric glands (PP) of ovariectomised mice. The gland cells gave strong PAS reaction, especially cells of pyloric pit (PP), pyloric glands (PG) stained slightly (Fig.109). With AB pH 1.0 cells of pyloric glands (PG) (Fig.115). Stained blue green, while pyloric pit (PP) cells did not stain at all. With AB pH 2.5 (Fig.117) the pyloric gland cells were strongly alcianophilic but pyloric pit cells showed mild alcianophilia. The pyloric gland cells when stained with AB pH 2.5 + PAS, the cells (PG) stained bluish green while cells of pyloric pit stained pink violet (Fig.121).

ii) Doudenal Villi (V)

PAS activity was seen in goblet cells (G) (Fig.109). Other cells were PAS negative. Alcianophilia was shown by the goblet cells at pH 2.5 and 1.0 (Fig.114 and 118). With AB pH 2.5 + PAS technique = some goblet cells stained purple some magenta and some blue green (Fig.122).
iii) Crypts of Lieberkühn

The cells of crypts of lieberkühn (Fig.10) showed strong PAS positive reaction. With AB pH 1.0 and 2.5 the cells stained blue green (Fig.114, 118). With AB pH 2.5 + PAS technique the cells stained dark purple (Fig.122).

iv) Brunner's Glands

Sections stained for PAS showed PAS positive reaction in acini (AC) and duct cells (Fig.109,110). The reaction was intense and observed only at 2/3 of the luminal cytoplasm. In the basal region PAS reaction was absent. Luminal side of the duct cells also showed dense PAS positive material. The Brunner's glands from distal part of duodenum showed less PAS positive activity. The Brunner's glands when stained for Alcian blue at pH 1.0 (Fig.113,114) the acini (AC and duct cells did not show alcianophilia. With AB pH 2.5 also did not show alcianophilia except the duct cells and some acini (AC) promixal part of duodenum (Fig.117,118). When AB pH 2.5 + PAS technique employed, majority of the acini and tubuloacini stained pink magenta but the duct cells and some acini (AC) from proximal part of the duodenum stained dark purple (Fig.121,122).

B) Cysteamine Treated Ovariectomised Mice (CF2)

Figures 111, 112, 115, 116, 119, 120, 123, 124
describe histochemical nature of cysteamine treated ovariectomised mice.

i) Pyloric Glands

All the cells of pyloric gland and pyloric pit showed reduction in PAS reactivity (Fig.111). The alcianophilia at pH 1.0 and pH 2.5 was found to be reduced (Fig.115 and 119). With AB pH 2.5 + PAS technique all the cells stained pink magenta except pyloric gland cells (PG) which stained blue green (Fig.123). With acid hydrolysis the alcianophilia was either reduced or lost completely.

ii) Duodenal Villi

In general the PAS reactivity for glycoproteins was found to be reduced in goblet cells (G) (Fig.112) or it was lost completely. The alcianophilia at pH 1.0 and 2.5 was also found to be reduced or lost completely (Fig.116 and 120). With alcian blue pH 2.5 + PAS technique the reactivity was reduced (Fig.124) and with acid hydrolysis the alcianophilia was reduced or lost.

iii) Crypts of Lieberkühn

Cysteamine treated ovariectomised mice showed in general reduction in staining with PAS technique. With AB pH 1.0 and pH 2.5 the alcianophilia was very much reduced (Fig.112,116,120). With AB pH 2.5 + PAS also the reactivity was found to be reduced (Fig.124).
iv) Brunner's Glands

The PAS activity was very much reduced and seen only on luminal side. The PAS activity was diffused instead of granular as in normal. The distal part duodenum showed Brunner's glands with less PAS activity than the glands at the proximal part (Fig. 111, 112). The alcianophilia at pH 1.0 and pH 2.5 decreased (Fig. 115, 116, 119 and 120). With AB pH 2.5 + PAS technique also the staining intensity was decreased (Fig. 123, 124).

5) Colorimetric Estimations

Glycoprotein was isolated from the Brunner's glands of ovariectomised (F_2) and cysteamine-treated ovariectomised (CF_2) mice by the method described in detail in Chapter II. The isolated glycoprotein was used for estimation of different constituents like sugars and protein. The detail of method is described in Chapter II.

The colorimetric estimations of sugars and protein are described in Table No. 6. The sugars are estimated in ug/mg glycoprotein. In ovariectomised (F_2) mice values were reduced as compared to normal female (F_1). The ovariectomised females when treated with cysteamine (CF_2) the values were further reduced.
i) **Hexose**: In normal female (F₁) the hexose content was 73.916 ± 0.83 which was reduced to 20.5 ± 0.25 in cysteamine treated normal female (CF₁). In ovariectomised (F₂) the hexose content was 63.00 ± 0.7427 and in cysteamine-treated ovariectomised mice (CF₂) it was reduced to 11.20 ± 0.2855. The reductions were statistically significant (F₁ : CF₁ = P < 0.005 , F₂ : CF₂ = P < 0.005).

ii) **Fucose**: In normal female (F₁) the fucose content was 4.070 ± 0.1229 and 1.486 ± 0.015 in cysteamine-treated normal female (CF₁). In ovariectomised cysteamine-treated mice (CF₂) the fucose value was 3.6816 ± 0.0367. The reductions were statistically significant (F₁ : CF₁ = P < 0.005 , F₂ : CF₂ = P < 0.001).

iii) **Sialic Acid**: In normal (F₁) and cysteamine treated female (CF₁) the sialic acid content was 0.3051 ± 0.008 and 0.0642 ± 0.0012 respectively. In ovariectomised (F₂) and cysteamine-treated ovariectomised mice (CF₂) the sialic acid content was 0.4275 ± 0.006 and 0.0383 ± 0.0011. The reduction in values were statistically significant (F₁ : CF₁ = P < 0.001 , F₂ : CF₂ = P < 0.001).

iv) **Protein**: The protein content in normal (F₁) and cysteamine-treated female (CF₁) was 20.1696 ± 0.0909 and 14.891 ± 0.329 respectively. In ovariectomised
(F₂) and cysteamine treated ovariectomised (CF₂) mice the protein content was 19.231 ± 0.037 and 12.447 ± 0.1437, respectively. The reductions in the values when compared were statistically significant (F₁ : CF₁ = P < 0.005 , F₂ : CF₂ = P < 0.005).

IV) Observations (Ovariectomised + Hormone Injected F₃)

Half the number of ovariectomised mice were further injected with estrogen, three days consecutively (F₃) and then half of them were used for ulcer induction by cysteamine injections (CF₃). The procedure is described in detail in Chapter II.

1) Gross Morphology

A) Ovariectomised Estrogen Injected Mice (F₃):

Pyloroduodenal junctions when stained for alkaline phosphatase, did not show any marked changes in mucosa. The staining was almost uniform with certain patches, less stained, the villi were rather short and broad (LV). Elsewhere the villi (V) were tall, leaf form and uniformly arranged like in normal mice (Fig.125).

B) Cysteamine Treated Ovariectomised Estrogen Injected Mice (CF₃):

The cysteamine-treated mice when stained for alkaline phosphatase, the mucosa showed differential staining. The affected part of the muosa showed less
staining the reduction of the staining increased with increase in severity of damage. The eroded regions did not show staining. The severity of ulcers was increased with increase in distance from pyloroduodenal junction (Fig. 126).

2) Ulcer Index

The percentage incidence of duodenal ulcer was 100%. The ulcers when critically evaluated by observing under stereo microscope, the deep ulcers involving submucosa were highest in number, i.e., 60%. The superficial ulcers involving erosion of mucosa were 30%, while 10% of the ulcers were perforating. The mean severity was 2.2 ± 0.058 and the ulcer index was 4.2.

3) Histology

A) Ovariectomised Estrogen Injected Mice (F3)

i) Pyloric Glands

These were simple, tubular glands which opened into the deep pyloric pits. Most of the pyloric gland (PG) cells were mucus secreting cells. The pyloric pits (PP) were lined by surface epithelial cells. The pyloric gland and pyloric pits stained intensely with H-E. Comparatively, pyloric glands were more eosinophilic than pyloric pit cells (Fig. 127).

ii) Duodenal Villi:

These were tall, leaf-form at many of the places, few were short showing desquamation. The surface
epithelial cells were tall with large nuclei at the base. Among these cells many goblet cells (G) were seen. The mucosal border was continuous and lamina propria was thin containing loose connective tissue (Fig.128).

iii) Crypts of Lieberkühn

They were simple tubular glands (Fig.131) located in the mucous membrane. They opened on the mucosal surface between the villi and extend through the lamina propria to the muscularis mucosae. The epithelium of the crypt was continuous with the surface epithelium of the villi. Some of the crypt cells were strongly eosinophilic.

iv) Brunner's Glands (BG)

Fig.127,131 describe Brunner's glands of ovariectomised estrogen injected female mice. The cells of acini (AC) and duct (D) were eosinophilic. The acinar cells were pyramidal in shape with nuclei at the base and had a very narrow lumen. The connective tissue also showed staining with eosin.

B) Cysteamine Treated Ovariectomised Estrogen Injected Mice (CF3)

i) Pyloric Glands

In cysteamine treated mice the pyloric glands (PG) showed increased eosinophilia (Fig.129). The pyloric mucosa show damage due to erosion.
ii) Duodenal Villi

In cysteamine treated mice the duodenal villi (V) (Fig.129,130) were fissured and there was aggregation of darkly staining cells in the connective tissue. Some villi were stout and broad and at certain places avillous conditions were formed. Further the surface epithelium was eroded along with mucosa.

iii) Crypts of Lieberkühn

The crypt cells showed strong eosinophilia (Fig.129,132). The cells were tall pyramidal in shape with large nucleus at the base.

iv) Brunner’s Glands

The cells of acini (AC) tubolo acini (TA) and duct were eosinophilic. The acinar cells were pyramidal in shape with large nuclei at the base and had a very narrow lumen. The connective tissue also showed eosinophilia (Fig.129,132).

4) Histochemistry

The sections were stained using various techniques such as PAS, AB pH 1.0, AB pH 2.5, AB pH 2.5 + PAS etc to visualize the nature of glycoproteins of pyloroduodenal junction. The figures 133, 134, 137, 138, 141, 142, 145, 146 describe the histochemical nature of the pyloroduodenal junction.
A) Ovariectomised Estrogen-Injected Mice (F3):

i) Pyloric Glands

The pyloric glands were mildly PAS positive but pyloric pit cell (PP) were strongly PAS positive (Fig. 133). With AB pH 1.0 only pyloric gland cells (PG) were mildly alcianophilic and pyloric pits cells (PP) were alcian blue negative (Fig. 137). With AB pH 2.5 the pyloric gland cells (PG) and pyloric pit cells (PP) were more alcianophilic (Fig. 141). With AB pH 2.5 + PAS technique the pyloric gland cells (PG) stained blue green while pyloric pit cells (PP) stained pink purple (Fig. 145).

ii) Duodenal Villi

Only goblet cells (G) on the duodenal villi were PAS positive (Fig. 133, 134). Similarly they were alcianophilic with AB pH 1.0 (Fig. 137, 138) and AB pH 2.5 (Fig. 141, 142). With AB pH 2.5 + PAS technique some goblet cells stained pink magenta, some were blue green and some were purple violet (Fig. 145, 146).

iii) Crypt of Lieberkühn: The crypt cells (CL) showed strong positiveness, towards luminal side with PAS (Fig. 134). The cells stained blue green with AB pH 1.0 but with AB pH 2.5 they stained more intensely (Fig. 137, 138, 141, 142). When AB pH 2.5 + PAS technique was employed the cells stained deep purple violet (Fig. 145, 146).

iv) Brunner's Glands: The
sections stained for PAS showed positive reaction in cells of acini (AC), duct (Fig.133, 134). The reaction was mainly towards luminal side and basal side was free of PAS positive material. The cells did not show alcianophilia with AB pH 1.0 (Fig.137,138). But with AB pH 2.5 duct cells and some acinar cells (AC) from duodenal bulb region stained intensely (Fig.141,142). With AB pH 2.5 + PAS technique all the acinar cells stained (AC) pink magenta except the duct cells and some acinar cells from duodenal bulb region stained blue and slightly purple violet (Fig.145,146). The alcianophilia was reduced when acid hydrolysis technique was employed.

B) Cysteamine-Treated Ovariectomised Estrogen Injected Mice (CF3)

Ovariectomised estrogen-injected mice were treated with two doses of cysteamine to induce duodenal ulcers and then the histochemistry of pyloroduodenal region was studied. The figures 133 to 148 describe histochemical nature.

i) Pyloric Glands

In cysteamine-treated mice in general the PAS activity was reduced (Fig.135), alcianophilia at pH 1.0 was absent (Fig.139) but with AB pH 2.5 the pyloric gland cells (PG) showed alcianophilia (Fig.143). With
AB pH 2.5 + PAS technique, pyloric gland cells (PG) stained blue green while pyloric pit cells (PP) stained purple violet (Fig. 147).

ii) Duodenal Villi

The Fig. 136 shows duodenal villi (V) with very few PAS positive goblet cells (G). With alcian blue technique also the number of alcianophilic goblet cells were reduced (Fig. 140, 144). With AB pH 2.5 + PAS technique the same thing was observed i.e. the number of goblet cells was reduced considerably (Fig. 148).

iii) Crypts of Lieberkühn

The Fig. 136 shows reduction in PAS positive staining. The alcianophilia at both pH 1.0 and pH 2.5 was found to be decreased considerably (Fig. 140, 144). The same thing was confirmed with the help of AB pH 2.5 + PAS technique (Fig. 148).

iv) Brunner's Glands

The Fig. 135, 136 shows Brunner's glands (BG) stained for PAS. The acini (AC) and duct cells showed reduction in PAS staining. With alcian blue at pH 1.0 there was no alcianophilia (Fig. 139, 140) at pH 2.5 the alcianophilia was reduced (Fig. 143, 144). The same thing was confirmed by using the AB pH 2.5 + PAS technique (Fig. 147, 148).
PLATE NO.13

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

Fig.53: Castrated = V - Uniform, tall, leafformed stained for ALP some times they are low (LV) degenerated at various sites.

Villie at PD are small, right angle to duodenal villi showing reduced staining

Ulcer induced.

Fig.54: Castrated mouse = V are disturbed, some times they are low (LV), degenerated at various sites, deep ulcers (U) lost ALP activity.
Figures 55, 56, and 59 are sections of pyloroduodenal junction and anterior part of the castrated mouse stained for H.E.

Figures 57, 58, and 60 are sections of pyloroduodenal junction.

**Fig.55**: Castrated mouse: It shows Brunner’s glands (BG) and crypts of Lieberkuhn (CL). The cells from these glands are normal and well organised. 50.4 x.

**Fig.56**: It shows a villus, Epithelium (E) is thick, goblet cells (G) are present well organised cells and their nuclei. The villus is blunt not leaf formed. 320 x.

**Fig.57**: Ulcer induced castrated mouse. It shows pyloric glands (PG), Pyloric pits (PP) intensley stained. Villi eroded (↑) cells of Brunner’s gland (BG) and crypts of Lieberkuhn (CL) disturbed. All the structures intensely stained. 50.4 x

**Fig.58**: A villus showing distruction, epithelial cells degenerating (E), goblet cells (G) very few in number, connective tissue (CT) show inflammation and degeneration 320 x.

**Fig.59**: The section shows crypts of Lieberkuhn (CL) and Brunner’s gland (BG) of castrated. The mouse cells of crypt Lieberkuhn (CL) acini (AC) and duct (D) are well arranged normal. 320 x.

**Fig.60**: The section showing crypts of Lieberkuhn (CL) acini (AC) and duct (D) of cysteamine treated castrated mouse. The cell arrangement is disturbed, nuclei picnotic lumen dilated. 320 x.
Fig. 61 and 62 are the sections of pyloroduodenal junction and anterior duodenum of castrated mouse stained for PAS.

Fig. 63 and 64 are the sections of pyloroduodenal junction and anterior duodenum of ulcer induced castrated mice stained for PAS.

Fig. 61: PP and PG are PAS positive, goblet cells, cells of crypts of Lieberkühn (CL) and Brunner's gland (BG) are also PAS positive. Staining intensity slightly less 50.4 x

Fig. 62: The section shows Goblet cells (G), crypts of Lieberkühn (CL) and Brunner's Glands of anterior duodenum. The cells are PAS positive. 50.4 x

Fig. 63: PAS staining only on luminal side of Brunner's glands (BG). Pyloric pit (PP) and Pyloric gland (BG) staining almost lost. 50.4 x

Fig. 64: The section shows erosion (↑) of the tissue. PAS staining very much reduced. Brunners gland (BG) degenerating. 50.4 x
Figures 65 and 66 are the sections of pyloroduodenal junction and anterior duodenum of castrated mouse, stained for AB pH 1.0.

Figures 67 and 68 are the sections of pyloroduodenal junction and anterior duodenum of ulcer induced castrated mouse, stained for AB pH 1.0

**Fig. 65:** It shows Pyloric pit (PP), Pyloric gland (PG) and Brunner's gland (BG). PG are AB pH 1.0 positive, PP and BG are AB pH 1.0 negative 50.4 x

**Fig. 66:** Goblet cells (G) and Crypts of Lieberkyuhn (CL) are AB pH 1.0 positive, while BG are AB pH 1.0 negative. 50.4 x

**Fig. 67:** AB pH 1.0 staining reduced (PG), PP and BG are AB pH 1.0 negative. 50.4 x

**Fig. 68:** AB pH 1.0 positive in G and CL is reduced and at certain places lost. 50.4 x
PLATE NO.17

Figures 69 and 70 are the sections of pyloroduodenal junction and anterior duodenum of castgrated mouse stained for AB pH 2.5.

Figures 71 and 72 are the sections of pyloroduodenal junction and anterior duodenum of ulcer induced castrated mice.

Fig. 69: Some of the acini (AC) and duct cells (D) and Ab positive at pH 2.5. 50.4 x

Fig. 70: Crypt of Lieberkuhn (CL) and goblet cells (G) are strongly AB pH 2.5 positive. 50.4 x

Fig. 71: AB pH 2.5 positive material in PP, PG, AC and D is reduced ( ) indicates tissue erosion. 50.4 x

Fig. 72: Brunner's glands are A?B pH negative, Crypt cells (CL) and goblet cells (G) degenerating. Their number decreased. 50.4 x
PLATE NO.18

The sections of pyloroduodenal junction and anterior duodenum castrated and castrated + cysteamine treated mice. Stained for AB pH 2.5 + PAS technique.

**Fig. 73**: Pyloric gland stained blue, Pyloric pits, stained purple, some acini (AC) and duct cells (D) stained purple and rest of the Brunner's gland pink magenta. 50.4 x

**Fig. 74**: Brunner’s gland (BG) stained pink magenta, crypt cells (CL) stained dark purple, some goblet cells stained pink, some green and some purple. 50.4 x

**Fig. 75**: PG stained blue, PP - Pink magenta, BG - staining diffused and granular. AC and D cells stained violet. 50.4 x

**Fig. 76**: Goblet cells (G), cells of crypt of Lieberkuhn (CL) and Brunner's gland cells PAS positive. Staining diffused. 50.4 x.
PLATE NO.19

Pyloroduodenal region and anterior part of the duodenum of castrated + hormone injected and ulcer induced mice, stained for alkaline phosphatase.

Abbreviations : PD - Poloroduodenal junction, LV - Low villi and V - Villi, ALP - Alkaline phosphatase.

Fig. 77 : Pyloroduodenal junction (PD) less stained, Few patches of low villi (LV) and rest of the area with normal villi (V).

Fig. 78 : PD - unstained, Patches of low villi (LV) and normal villi (V).
Histological sections of pyloroduodenal junctions of castrated + hormone injected (Figs.79,80,83) and ulcer induced mice (Figs.81,82,84) stained for H-E.

**Fig.79**: The section shows PP and PG stained normal, Villi (V) and crypts of Lieberkuhn stained normal. Acini (A) compactly arranged with narrow lumen. 50.4 x

**Fig.80**: A villus with thick epithelium (E) with goblet cells (G) and connective tissue. 320 x

**Fig.81**: Pyloric pit (PP), Pyloric gland (PG), Crypts of Lieberkuhn (CL) and Villi (V) darkly stained with H-E. Arrangement of cells of glands disturbed. Erosion (↑) but no deep ulcers. 50.4 x

**Fig.82**: Intensely stained villus with epithelium (E) goblet cells (G) and inflammation in connective tissue (CT) 320 x

**Fig.83**: Crypts of Lieberkuhn (CL) and Acini (AL). 320 x

**Fig.84**: Crypts of Lieberkuhn (CL) and Acini (AC), Cellular arrangement disturbed, intense staining. 320 x
PLATE NO.21

Histochemistry. PAS technique. Sections of pyloroduodenal junction and anterior duodenum of castrated + hormone injected mouse (Fig.85 and 86) and castrated + hormone injected + cysteamine treated (Fig.87 and 88).

**Fig.85**: PP and PG - PAS positive  
CL and D - PAS positive  
50.4 x

**Fig.86**: CL - PAS positive  
BG - PAS positive  
G - PAS positive  
50.4 x
PLATE NO. 22

Histochemistry, AB pH 1.0 technique.

Fig. 89 and 90 - Castrated + Hormone injected mouse
Fig. 91 and 92 - Castrated + Hormone injected + Cysteamine treated mouse.

Fig. 89: PG - Mild alcianophilia
PP - No alcianophilia
BG - No alcianophilia
50.4 x

Fig. 90: CL - Positive alcianophilia
G - Positive alcianophilia
50.4 x

Fig. 91: Degeneration of tissue (↑)
50.4 x

Fig. 91: Alcianophilia (CL and G) reduced
50.4 x
PLATE NO. 22

89

90

91

92
Histochemistry – AB pH 2.5 technique

Fig. 93 and 94 – Castrated + Hormone injected mouse.
Fig. 95 and 96 – Castrated + Hormone injected +
Cysteamine treated mouse.

Fig. 93: Pyloric gland (PG) strong alcianophilia.
Pyloric pig (PP) – Positive at certain places
only.
Brunner’s gland (BG) some acini (AC) 2.5 +ve
Duct cells (D) – AB 2.5 +ve.
50.4 x

Fig. 94: Brunner’s gland (BG) - AB pH 2.5 -ve
Crypt of Lieberkühn (CL) - AB pH 2.5 +ve
Goblet cells (G) - AB pH 2.5 +ve
50.4 x

Fig. 95: Intensity of staining decreased.
Pyloric gland (PG), Pyloric pig (PP),
Brunner’s gland acini (AC), Duct cells (D)

Fig. 96: Decrease in intensity of the staining.
Crypts of Lieberkühn (CL), Goblet cells (G),
number of cells reduced.
Brunner’s gland acini (AC) AB pH 2.5
negative. 50.4 x
PLATE NO. 24

Histochemistry - AB pH 2.5 + PAS technique

Fig. 97 and 98 - Pyloroduodenal junction of castrated
+ Hormone injected mouse.

Fig. 99 and 100 - Pyloroduodenal junction of castrated
+ Hormone injected + Cysteamine treated mouse.

**Fig. 97:** Section showing pyloric glands (PG) stained blue, green, pyloric pit (PP), purple, Brunner's gland acini (AC) - pink magenta and duct (D) - blue green. 50.4 x

**Fig. 98:** Section showing anterior part of the duodenum Brunner's gland (BG) stained pink magenta, Crypts of Lieberkühn (CL) purple and Goblet cell (G) - Some green, some purple and some pink. 50.4 x

**Fig. 99:** Pyloric gland (PG) - Blue green
Pyloric pit (PP) - Purple violet
Acini (AC) - Pink, some violet purple and some pink
50.4 x

**Fig. 100:** Brunner's gland (BG) - Pink magenta
Crypt of Lieberkühn (CL) - Purple and Goblet cell (G) - Some pink, some green and some purple
50.4 x
Pyloroduodenal region and anterior part of ovariectomised and ovariectomised + cysteamine treated mice, stained for alkaline phosphatase.

Abbreviations: Villi - V; Pyloroduodenal junction -PD; Low villie (LV), Ulcer - (U), Alkaline phosphatase (ALP)

Fig. 101: Ovariectomised mouse
Villi (V) - tall, leaf form, Uniform. Some are low, degenerated villi (LV), showing reduced staining.

Fig. 102: Ovariectomised + Cysteamised-treated. Most of the villi are affected. They are low degenerated (LV), deep ulcers are seen (U) where ALP activity is lost.
PLATE NO.26

Histology of pyloro duodenal region of ovariectomised and overiectomised and cysteamine treated ulcer.

**Fig.103**: Ovariectomised mouse, Pyloric glands (PG), Pyloric pig (PP) and brunner’s glands (BG) cells are normal and well organised. 50.4 x

**Fig.104**: It shows a single villus of ovariectomised mouse, Epithelium (E) is thick, goblet cells (G) present. 320 x.

**Fig.105**: Ulcer induced ovariectomised mouse Pyloric glands (PG), Pyloric pits intensely stained, Villi eroded (♀) Brunner’s glands (BG) and crypts of Lieberkuhn (CL) intensely stained.

**Fig.106**: A single villus of ulcer induced ovariectomised mouse showing degeneration of epithelial lining (E), goblet cells (G) very few in number. Connective tissue show inflammation. 320 x

**Fig.107**: The section of ovariectomised mouse shows crypts of Lieberkuhn (CL), Acini (AC) of Brunner’s gland and Duct (D). 320 x

**Fig.108**: The section of ulcer induced ovariectomised crypts of Lieberkuhn (CL), Brunner’s gland acini (AC) and Duct (D). 320 x
PLATE NO.27

PAS technique.

**Fig.109**: Ovariectomised mouse. Pyloric glands (PG) and Pyloric pig (PP), PAS positive. Goblet cells (G), Crypts of Lieberkuhn (CL) and Brunner’s gland (BG) also positive. 50.4 x

**Fig.110**: Ovariectomised mouse. Section of anterior duodenum with villi (V), Goblet cells (G), Crypts of Lieberkuhn (CL) and Brunner’s gland (BG). All are PAS positive. 50.4 x

**Fig.111**: Ovariectomised + Cysteamine treated mouse. The section shows pyloric pigs (PP), Pyloric glands (PG), Goblet cells (G), Crypts of Lieberkuhn (CL) and Brunner’s gland (BG). All PAS positive but staining intensity less and diffused.

**Fig.112**: Ovariectomised + Cysteamine treated mouse. Anterior duodenum. The section shows villi (V), Goblet cells (G), Crypts of Lieberkuhn (CL) and Brunner’s glands (BG). Erosion of tissue (↑) staining very much reduced. 50.4 x
AB pH 1.0 technique.

**Fig.113**: Ovariectomised mouse. The section shows, Pyloric glands AB pH 1.0 positive. Pyloric pit and Brunner's glands are AB pH 1.0 negative. 50.4 x

**Fig.114**: Ovariectomised mouse. Anterior duodenum. Goblet cells (G) and Crypts of Lieberkühn (CL) AB pH 1.0 positive. Brunner's glands (BG) AB pH 1.0 negative. 50.4 x

**Fig.115**: Cysteamine treated ovariectomised mouse. Alcianophilia reduced (PG). 50.4 x

**Fig.116**: Cysteamine treated ovariectomised mouse. Alcianophilia reduced (CL), (G). 50.4 x
AB pH 2.5 Technique.

**Fig.117**: Ovariectomised mouse. Pyloric gland cells (PG) AB pH 2.5 positive. Pyloric pit cells (PP) AB pH 2.5 negative. Some acini (AC) and duct (D) of Brunner's glands are AB pH 2.5 positive. 50.4 x

**Fig.118**: Section of anterior duodenum of ovariectomised mouse. Crypt of Lieberkühn (CL) and Goblet cells (G) strongly AB pH 2.5 positive. Brunner's glands (BG) AB pH 2.5 negative. 50.4 x

**Fig.119**: Cysteamine treated ovariectomised mouse. Alcianophilia very much reduced (PG), (AC) and (D). 50.4 x

**Fig.120**: Cysteamine treated ovariectomised mouse. Alcianophilia very much reduced (CL), (G) Number of (G) cells were reduced. 50.4 x
AB pH 2.5 + PAS Technique.

**Fig. 121**: Ovariectomised mouse. The section of showing Pyloric glands (PG), blue green, Pyloric pit (PP) purple, Brunner's gland acini (AC) pink magenta and duct (D) blue green. 50.4 x

**Fig. 122**: The section of anterior part of duodenum of ovariectomised mouse. Brunner's gland - pink magenta. Cypts of Lieberkuhn (CL) - purple and (G) goblet cells - some green, some pink and some purple. 50.4 x

**Fig. 123**: Ovariectomised + Cysteamine treated mouse. The pyloroduodenal junction shows pyloric gland (PG), Pyloric pit (PP), Acini (AC) and Duct (D) staining reduced. 50.4 x

**Fig. 124**: Ovariectomised + Cysteamine treated. The section shows reduction in staining (BG), (G) and (CL). 50.4 x
PLATE NO. 31

Pyloroduodenal region and anterior part of ovariectomised + hormone injected and ovariectomised + hormone injected + cysteamine treated mice.

**Fig. 125**: Ovariectomised + hormone injected mouse. Most of the villi (V) are tall, leaf form and uniform. Only few are low (LV) and degenerated. Showing reduced staining.

**Fig. 126**: Ovariectomised + hormone injected + cysteamine treated. Majority of the villi are affected. They are low degenerated (LV). Deep ulcers (U) are seen where (ALP) activity is lost.
PLATE NO.32

Histology of pyloroduodenal region of ovariectomised + hormone treated and ovariectomised + hormone injected and cysteamine treated mice.

Fig.127: Ovariectomised + hormone injected mice, Pyloric gland (PG), Pyloric pit (PP) and Brunner’s gland (BG) cells normally stained and well organised. 50.4 x

Fig.128: It shows a single villus. Epithelium (E) is thick, goblet cells (G) present. Connective tissue (CT) without inflammation.

Fig.129: Ulcer induced, ovariectomised hormone injected mouse. Pyloric gland (PG), Pyloric pit (PP) intensely stained. The villi eroded (↑). Brunner’s gland(BG) and Crypts of Lieberkuhn (CL) are intensely stained. Lumen dilated. 50.4 x

Fig.130: A single villus showing degeneration of epithelium (E), goblet cells (G) reduced very much in number. Connective tissue (CT) showing inflammation. 320 x

Fig.131: The section show crypts of Lieberkuhn (CL), Acini (AC) of Brunner’s gland and Duct (D). 320 x

Fig.132: The section of ulcer induced showing crypts of Lieberkuhn (CL), Brunner’s gland acini (AC)and Duct (D) intensely stained and lumen dilated. Arrangement of the cells disturbed.320 x
PLATE NO.33

Histochemistry PAS technique.

**Fig.133**: Ovariectomised + hormone—injected mouse. Pyloric gland (PG), Pyloric pit (PP), Brunner’s glands (BG) and Crypts of Lieberkuhn all are PAS positive. 50.4 x

**Fig.134**: Ovariectomised + hormone—injected mouse. Section of anterior duodenum showing normal villi (V) goblet cells (G), crypts of Lieberkuhn (CL) and Brunner’s gland (BG). All are PAS positive. 50.4 x

**Fig.135**: Ovariectomised + hormone—injected + cysteamine treated mouse. Pyloric glands (PG), Pyloric pit (PP), Crypts of Lieberkuhn (CL) and Brunner’s gland (BG) show reduction in staining. 50.4 x

**Fig.136**: The section of anterior duodenum. The villi (V), goblet cell (G), crypts of Lieberkuhn (CL) and Brunner’s gland (BG) show reduced staining. Erosion of epithelium (↑). 50.4 x
PLATE NO.34

Histochemistry AB pH 1.0 technique.

Fig.137: Ovariectomised mouse. The section shows pyloric glands AB pH 1.0 positive. Pyloric pit (PP) and Brunner’s gland (BG) AB pH 1.0 negative. 50.4 x

Fig.138: Anterior section of duodenum shows goblet cells (G) and crypts of Lieberkuhn (CL) AB pH 1.0 positive while Brunner’s gland AB pH 1.0 negative. 50.4 x

Fig.139: Cysteamine-treated ovariectomised hormone injected mice shows reduction in staining (PG). 50.4 x

Fig.139: Section of anterior duodenum shows reduction in staining (CL) and (G). 50.4 x
PLATE NO. 34

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PLATE NO.35

Histochemistry AB pH 2.5 technique

**Fig.140**: Ovariectomised + hormone injected mouse. Pyloric gland (PG), AB pH 2.5 positive, Pyloric pit (PP) negative. Some acini (AC) and duct (D) AB pH 2.5 positive. 50.4 x

**Fig.141**: Section of anterior duodenum of ovariectomised hormone injected mouse - Crypts of Lieberkühn (CL) and goblet cells strongly positive to AB pH 2.5. 50.4 x

**Fig.142**: Cysteamine treated, ovariectomised + hormone injected mouse. The alcianophilia very much reduced PG, AC and D. 50.4 x

**Fig.143**: Section of anterior duodenum of cysteamine treated hormone injected mice alcianophilia reduced CL,G and D. 50.4 x
PLATE NO. 36

AB pH 2.5 + PAS technique

**Fig. 144**: Ovariectomised + hormone injected mouse. Pyloric gland (PG) - blue green, Pyloric pit - purple, Brunner’s gland acini (AC) - pink magenta and duct (blue green) 50.4 x

**Fig. 145**: The section of anterior part of duodenum. Crypt of Lieberkuhn (CL) purple, goblet cells (G) green, pink and purple and Brunner’s gland pink magenta. 50.4 x

**Fig. 146**: Ovariectomised + hormone injected + cysteamine treated mouse - Pyloric gland (PG), Pyloric pit (PP), Brunner’s gland acini (AC) and Duct (D) the staining intensity reduced. 50.4 x

**Fig. 147**: Ovariectomised + hormone treated + cysteamine treated mouse. The section shows BG, G and CL where the staining intensity is reduced. 50.4 x
5) Colorimetric Estimations

Brunner's glands were separated from pyloroduodenal junctions and were used to isolate glycoprotein to estimate protein and constituent sugars such as hexose, fucose and sialic acid. The protein content was found to be $21.981 \pm 0.4683$ ug/mg glycoprotein. The sugars were estimated in terms of ug/mg glycoprotein. The hexose was found to be $81.25 \pm 0.236$. Fucose $3.9728 \pm 0.0713$, sialic acid $0.3141 \pm 0.0033$ and protein $21.98 \pm 0.1846$.

When ovariectomised estrogen injected females were injected with cysteamine two doses, the values were found to be in general reduced. The protein was reduced to $15.441 \pm 0.328$, hexose $35.66 \pm 0.9$, sialic acid $0.0425 \pm 0.0022$ and fucose $2.1774 \pm 0.0262$. The reduction in values when compared were statistically significant. (Hexose $F_3 : CF_3 = P < 0.005$, Fucose $F_3 : CF_3 = P < 0.005$, sialic acid $F_3 : CF_3 = P < 0.001$, protein $F_3 : CF_3 = P < 0.01$).

V) DISCUSSION

In the present investigation, it has been observed that the damage to the duodenal mucosa was maximum in cysteamine-treated ovariectomised mice, which was prevented in the injections of the estrogen. The ulcer severity was reduced significantly in estrogen-treated
females. Histological and histochemical changes that took place in goblet cells, pyloric gland cells and Brunner's gland cells in ovariectomised cysteamine-treated mice were not observed in overiectomised estrogen-injected, cysteamine-treated mice. Sugars from glycoprotein of Brunner's gland which were depleted in cysteamine-treated females were at a normal level in estrogen-treated females. These findings indicated that the estrogen is able to protect the duodenal mucosa from damaging effect of cysteamine. Hanekar and Namaji (1977) suggested that female sex hormones have protective role against ulcer formation. Nadar and Pillai (1991, 92) showed that the incidence and number of duodenal ulcers were always more in males than females. Nazar Ahemed (1977) reported that forestomach ulcer formation was more in male than female rats. Sex difference in peptic ulcer disease in human is described by Truelove (1960); Kronberger and Hafner (1965); Kurata et al. (1985). It has been shown by Doll and Avery Jone that chance of women developing duodenal ulcer remains relatively low throughout the whole of her active reproductive life than men, but increases sharply at menopause (Truelove, 1960). Not only is duodenal ulcer much less common in women than men, but it also runs a less severe course at any rate judged by the liability to the perforation and male to female
ratio was found to be 4 : 1 (Kronberger and Hafner 1965). Another feature of the duodenal ulcer in women is the protective effect of pregnancy. Beneficial effect of pregnancy and lactation have been reported on histamine and steroid induced ulcers in rats (Kahlson et al., 1964; Kelly and Robert, 1969). Nine ulcer patients were treated with an estrogen and claimed good results (Truelove, 1960). Truelove (1960) working with dog found that ulcerations in gastric pouch were healed up after the administration of an estrogen. Recurrence of peptic ulcer formations were also relieved by estrogen therapy. Induced duodenal ulcers in dogs with Cinchophen and observed 100% prevention by stilboestrol. Our observations from present investigation and present knowledge described above, we realized that estrogen helps in the protection of duodenal mucosa from ulcers. But how it assists? One obvious thing is that it may inhibit gastric acid secretion from the stomach or it may be involved in the synthesis of protective agents like glycoprotein, bicarbonates, epidermal growth factor and prostaglandin. All these protective agents except prostaglandin are synthesized and secreted by Brunner's gland. Ojha and Wood (1950) found that administration of Stilboestrol to cats caused gastric diminution of acid secretion. But in pregnancy (in which circulating
estrogen levels are high) the acid secretion of stomach shows no fall. Recently it has been suggested that EGF inhibit the gastric acid secretion. EGF is secreted by Brunner's glands (Kirkegaard et al., 1983; SkovOlsen et al., 1984, 1985).

It has been fully established that glycoprotein containing bicarbonates is mainly responsible for the protection of duodenal mucosa. The glycoprotein and bicarbonates are mainly secreted by Brunner's glands. Humoral (Florey et al., 1935; Kirkegaard et al., 1984) and nervous control (Wright et al., 1940, Stach and Hung, 1978; Kirkegaard et al., 1981; SkovOlsen et al., 1985) over the secretion of Brunner's gland has already discussed and it can be concluded that estrogen may be assisting the secretion of Brunner's glands. Increase in the secretion of glycoprotein from Brunner's gland acini and Brunner's gland duct cells has been shown in the present investigation in estrogen treated mice. Secretion of glycoprotein of other exocrine cells of the duodenum is also influenced by the estrogen.
Table 5
Effect of Oestrogen on Cysteamine-Induced Duodenal Ulcers in Female Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage</th>
<th>Ulcers (%)</th>
<th>Mean Ulcer Incidence</th>
<th>Ulcer Severity</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Superficial</td>
<td>Deep</td>
<td>Perforating</td>
</tr>
<tr>
<td>CF₁ Normal Q + Cysteamine</td>
<td>100</td>
<td>20</td>
<td>70</td>
<td>10</td>
<td>1.90</td>
</tr>
<tr>
<td>CF₂ Operated Q + Cysteamine</td>
<td>100</td>
<td>10</td>
<td>70</td>
<td>20</td>
<td>3.10</td>
</tr>
<tr>
<td>CF₃ Operated Q + Hormone Injected + Cysteamine</td>
<td>100</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>2.20</td>
</tr>
</tbody>
</table>
Table 6
Carbohydrates and Protein Contents of Soluble Glycoprotein Isolated from Brunner’s Glands of 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>F1 Normal</td>
<td>73.916</td>
<td>4.070</td>
<td>0.3051</td>
<td>20.1696</td>
</tr>
<tr>
<td>F1 Normal</td>
<td>± 0.83</td>
<td>± 0.1229</td>
<td>± 0.008</td>
<td>± 0.0909</td>
</tr>
<tr>
<td>CF1 Normal</td>
<td>20.5</td>
<td>1.486</td>
<td>0.0642</td>
<td>14.891</td>
</tr>
<tr>
<td>CF1 Normal</td>
<td>± 0.25</td>
<td>± 0.0154</td>
<td>± 0.0012</td>
<td>± 0.329</td>
</tr>
<tr>
<td>F2 Operated</td>
<td>63.00</td>
<td>3.6816</td>
<td>0.4275</td>
<td>19.231</td>
</tr>
<tr>
<td>F2 Operated</td>
<td>± 0.7427</td>
<td>± 0.0367</td>
<td>± 0.0006</td>
<td>± 0.0375</td>
</tr>
<tr>
<td>CF2 Operated</td>
<td>11.20</td>
<td>0.5474</td>
<td>0.0388</td>
<td>12.447</td>
</tr>
<tr>
<td>CF2 Operated</td>
<td>± 0.2855</td>
<td>± 0.0081</td>
<td>± 0.0011</td>
<td>± 0.1437</td>
</tr>
<tr>
<td>F3 Operated</td>
<td>81.25</td>
<td>3.9728</td>
<td>0.3141</td>
<td>21.981</td>
</tr>
<tr>
<td>F3 Operated</td>
<td>± 0.236</td>
<td>± 0.0713</td>
<td>± 0.0033</td>
<td>± 0.1846</td>
</tr>
<tr>
<td>CF3 Operated</td>
<td>35.66</td>
<td>2.1774</td>
<td>0.0425</td>
<td>14.441</td>
</tr>
<tr>
<td>CF3 Operated</td>
<td>± 0.965</td>
<td>± 0.0262</td>
<td>± 0.0022</td>
<td>± 0.328</td>
</tr>
</tbody>
</table>

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

Hexose
F1 : CF1 = P < 0.005
F2 : CF2 = P < 0.005
F3 : CF3 = P < 0.005

Fucose
F1 : CF1 = P < 0.005
F2 : CF2 = P < 0.001
F3 : CF3 = P < 0.005

Sialic acid
F1 : CF1 = P < 0.001
F2 : CF2 = P < 0.001
F3 : CF3 = P < 0.001

Protein
F1 : CF1 = P < 0.005
F2 : CF2 = P < 0.005
F3 : CF3 = P < 0.001