CHAPTER - III

OBSERVATIONS

1. **MORTALITY**
2. **ULCER INDEX**
3. **GROSS MORPHOLOGY**
4. **HISTOLOGY**
5. **HISTOCHEMISTRY**

With respect to control, sialoadenectomized, cysteamine-HCl administered and sialoadenectomized, cysteamine-HCl treated male mice

1. MORTALITY

The male mice supplied with regular ration of food and water *ad libitum* did not show any mortality.

Mortality was observed in sialoadenectomized mice. It was nearly 20% and observed within ten days after the ablation of submandibular gland (Table No.1).

Mortality was also observed in cysteamine-HCl administered mice. The mortality was about 20% (Table No.2). After the administration of cysteamine-HCl, the mice become passive and found to be sitting idle at the corner of the cages. They were found with frequent scratching the skin at the region where cysteamine-HCl was subcutaneously administered. Such type of behaviour was not observed in sialoadenectomized mice but observed in sialoadenectomized, cysteamine treated mice.

In sialoadenectomized, cysteamine administered mice also mortality was observed. The mortality rate was increased to 30% in sialoadenectomized, cysteamine administered mice. The mortality was observed within ten days after the ablation of the submandibular gland. Sneezing was frequently noted in these mice (Table No.3).

2. ULCER INDEX

No sign of ulcerations or lesions were observed in the controlled mice, but it was observed in sialoadenectomized mice. The

incidence of ulcer was more in the proximal part of duodenum than the distal end. The duodenal ulcers were evaluated according to the method described in material and methods. The J score or ulcer index calculated. In sialoadenectomized mice the percentage incidence of duodenal ulcer was 80. The number of duodenal ulcers per mice was 4.5 ± 0.73; and severity of ulcer was 1.52 ± 0.08, the duodenal ulcer index was 3.52 (Table No.1 and Graph No.1).

The ulcerations were also observed in cysteamine, administered mice. The percentage incidence of duodenal ulcer was 100. Number of duodenal ulcer per mice was 2.3 ± 0.30, the severity of ulcer was 1.652 x 0.100, the duodenal ulcer index was 4.3 (Table No.2 Graph No.2).

The duodenal changes in sialoadenectomized, cysteamine-HCl treated mice were more remarkable. The ulcerations were observed. The percentage incidence of duodenal ulcer was 87.5 in all mice. The number of duodenal ulcer per mice was 1.55 ± 0.32, the severity was 1.58 ±0.14, the ulcer index was 3.33 (Table No.3, Graph No.3).

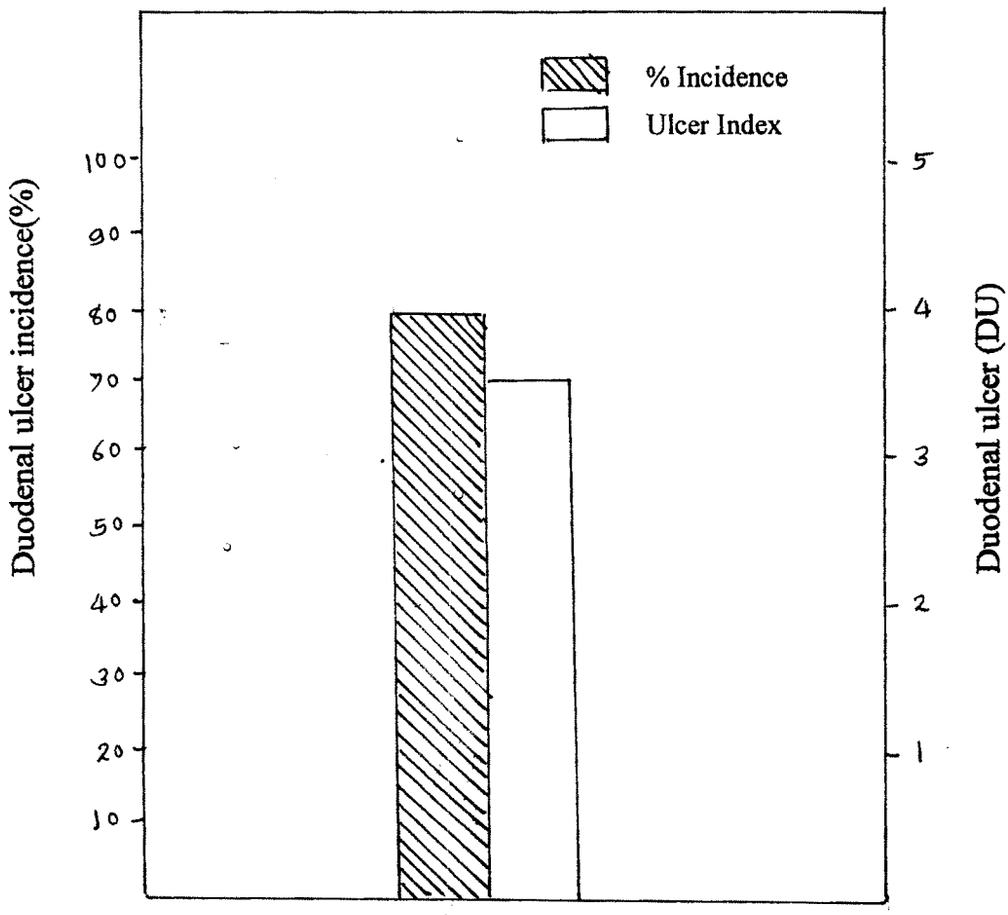
Table No. 1

SIALOADENECTOMIZED DUODENAL ULCER

Group	Mortality in %	% Incidence	Duodenal ulcer		
			Number/ mice	Severity	Ulcer index (UI)
A.Control (10)	-	-	-	-	-
B.Sialoadenec-tomized (10)	20	80	4.5 ± 0.73	1.52 ±0.08	3.52

* Values are mean, ± standard deviation.
* Number in paranthesis denotes the number of mice.

GRAPH – I



Sialoadenectomized Mice shows the % incidence of duodenal ulcer and ulcer index

Controlled mice do not show any ulcer

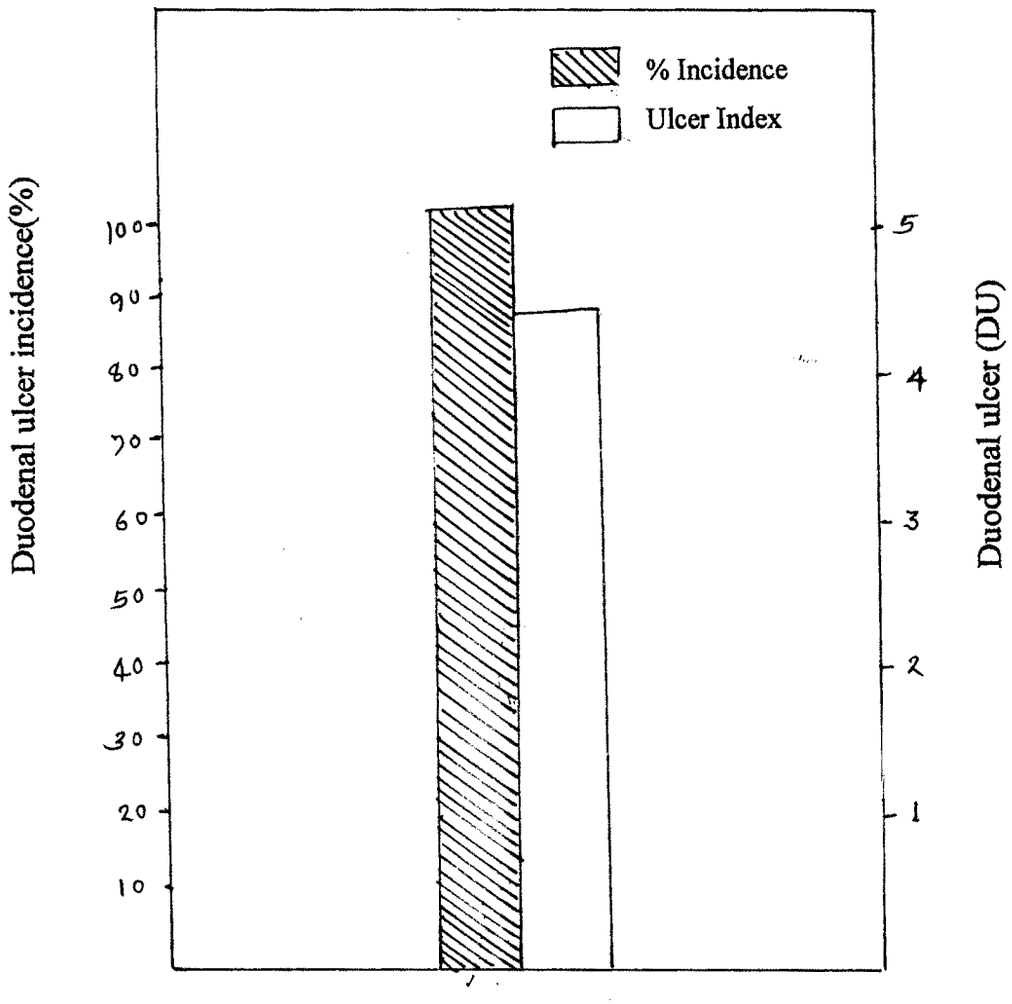
Table No.2

CYSTEAMINE ADMINISTRATED DUODENAL ULCER

Group	Mortality in %	% Incidence	Duodenal ulcer		
			Number/ mice	Severity	Ulcer index (UI)
A. Control (10)	-	-	-	-	-
B. Cysteamine administered (10)	20	100	2.3 ± 0.300	1.652 ±0.100	4.3

* Values are mean, ± standard deviation.
* Number in paranthesis denotes the number of mice.

GRAPH – II



Cysteamine administered mice showing the % incidence of duodenal ulcer and ulcer index

Controlled mice do not show any ulcer

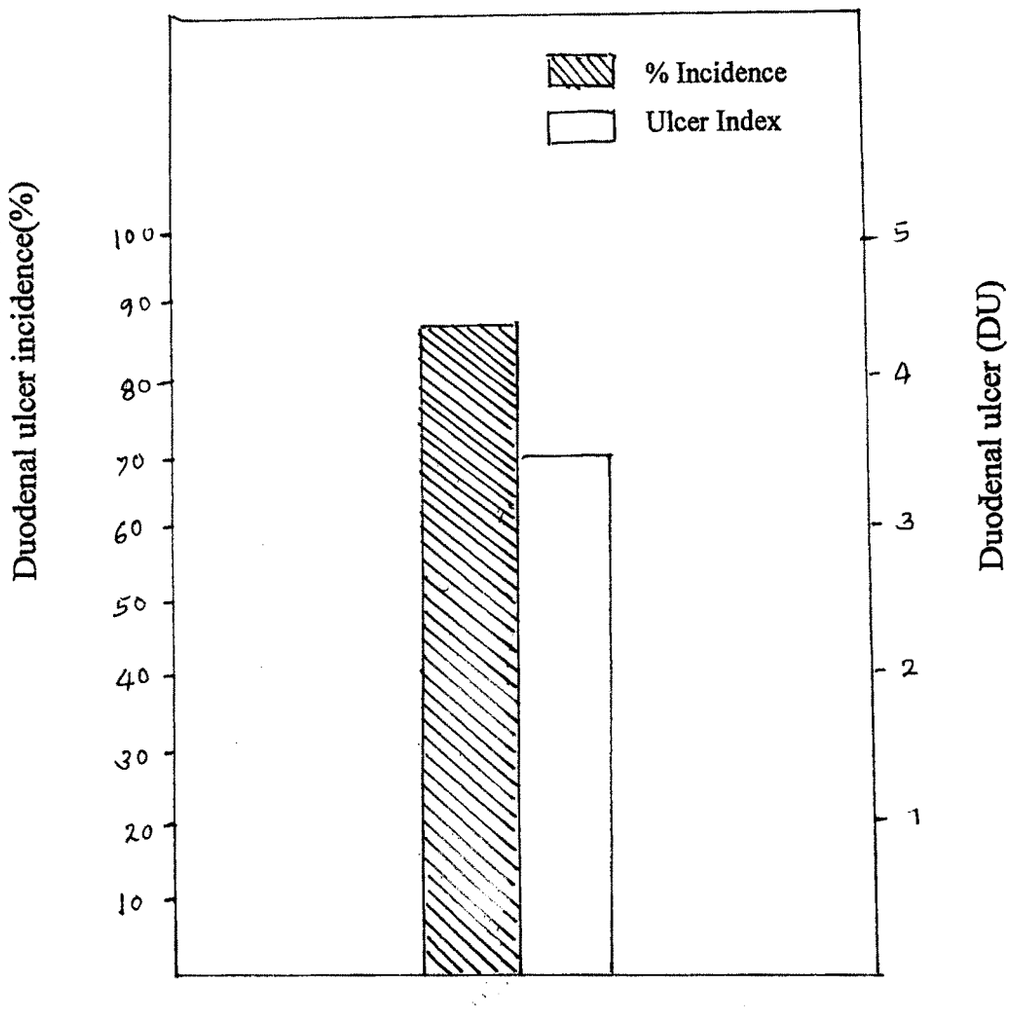
Table No.3

SIALOADENECTOMIZED AND CYSTEAMINE ADMINISTERED DUODENAL ULCER

Group	Mortality in %	% Incidence	Duodenal ulcer		
			Number/ mice	Severity	Ulcer index (UI)
A.Control (10)	-	-	-	-	-
B. Sialoadenec-tomized (10)	30%	87.5	1.55 ± 0.32	1.58 ±0.14	3.33

* Values are mean, ± standard deviation.
* Number in paranthesis denotes the number of mice.

GRAPH – III



Sialoadenectomy, cysteamine administered mice showing the % incidence of duodenal ulcer and ulcer index

Controlled mice do not show any ulcer

3. GROSS MORPHOLOGY :

Stereoscopic and occular magnifier observations showed that the duodenum of controlled mice had regular and leaf formed villi (Plate- I, Fig. 1). Height of villi is normal and can be very well visualised in the PAS staining technique (Plate II, Fig. 1).

The duodenum of sialoadenectomized mice developed ulcers. The ulcers were restricted towards the pyloroduodenal junction (Plate- I, Fig. 2). Comparatively the villi were short heighted with bulky appearance in the PAS staining technique (Plate-II, Fig. 2).

Cysteamine-HCl administration led to the formation of ulcer in duodenum. The ulcers were observed in the anterior part of duodenum (Plate I, Fig. 3). The villi were comparatively low in cysteamine-HCl administrated groups than that of control and sialoadenectomized group (Plate II, Fig.3).

More remarkable changes with ulcer formation were observed in the duodenum of sialoadenectomized, cysteamine-HCl administered mice. Deep ulcerations were observed which led damage in almost all parts of duodenum (Plate- I, Fig. 4). Majority of villi were not clearly observed. Villi, those were having intact form showed PAS positive staining while the villi, those were near to ulcer region showed less intense stain (Plate- II, Fig. 4).

CAPTIONS TO FIGURES : PLATE-I

Fig. 1

Gross photograph of Stomach and duodenum of controlled male mouse showing no ulcerogenic changes. X 3

Fig. 2

Gross photograph of stomach and duodenum of sialoadenectomized male mouse showing ulcerogenic changes. X 3

Fig. 3

Gross photograph of stomach and duodenum of male mouse after administration of a dose of cysteamine showing large number of ulcerated areas. X 3

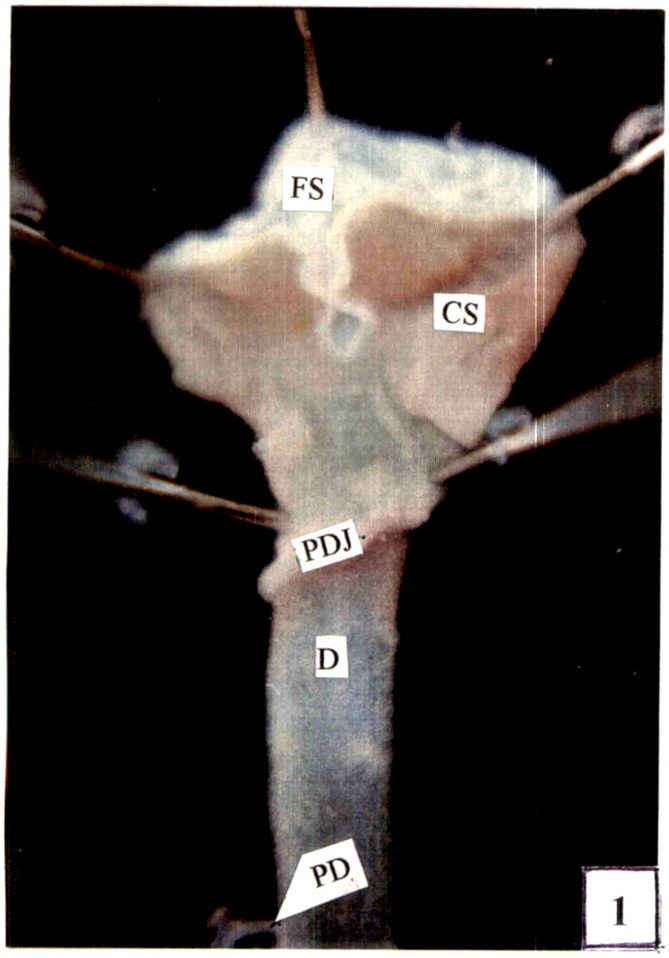
Fig. 4

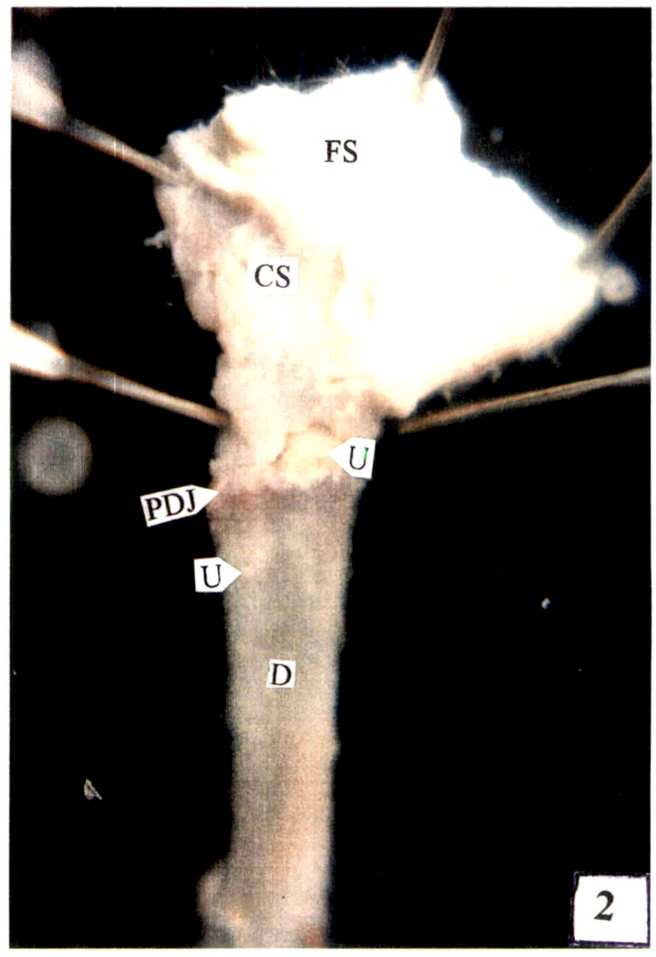
Gross photograph of stomach and duodenum of sialoadenectomized male mouse, receiving a dose of cysteamine showing perforated ulcers near PDJ. X 3

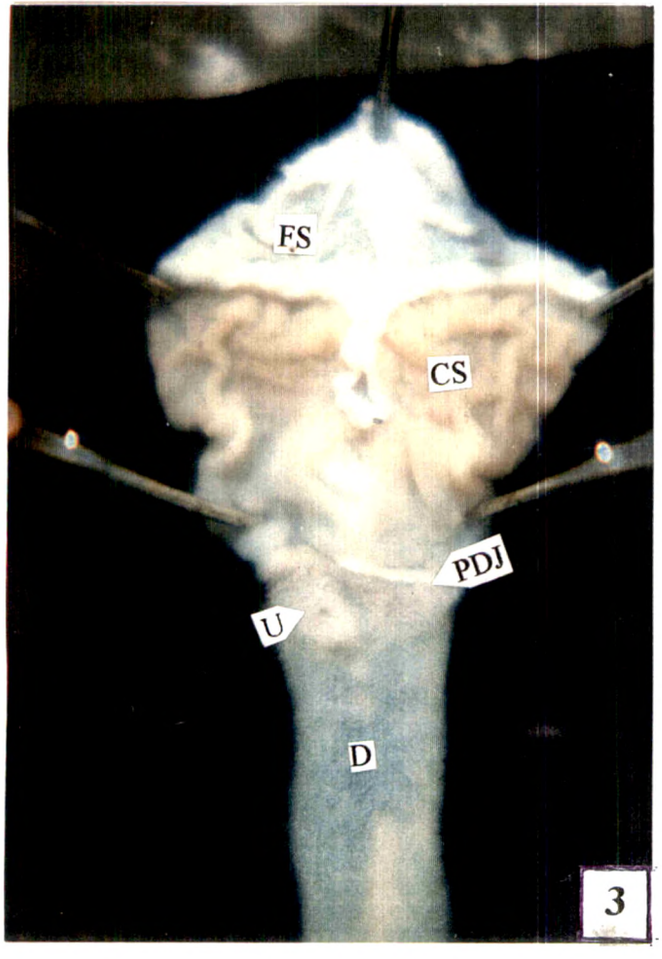
ABBREVIATIONS

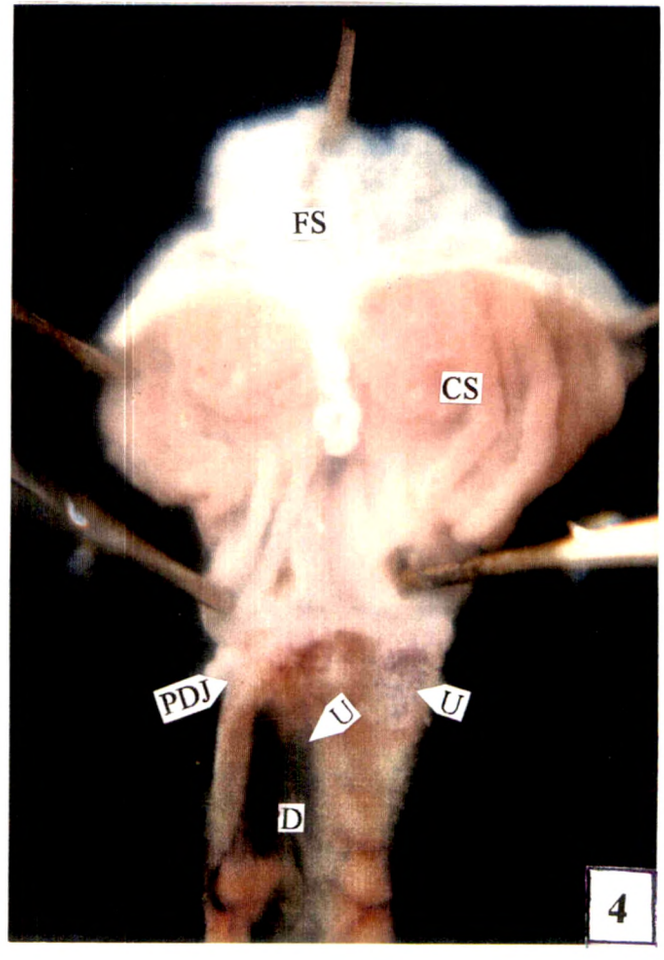
F.S. - Fore stomach
C.S. - Cardiac stomach
D - Duodenum
PDJ - Pyloro-duodenal junction
U - Ulcer
PD - Pancreatic duct
V - Villi

PLATE - I









CAPTIONS TO FIGURES : PLATE-II

Fig. 1

Stereoscopic microphotograph of duodenum of controlled male mouse stained by PAS, showing regular leaf formed villi. X 6.3

Fig. 2

Stereoscopic microphotograph of duodenum of sialoadenectomized male mouse stained by PAS, showing low and shortened villi. X 6.3

Fig. 3

Stereoscopic microphotograph of duodenum of cysteamine administered male mouse stained by PAS, showing erosion type of ulcer. X 6.3

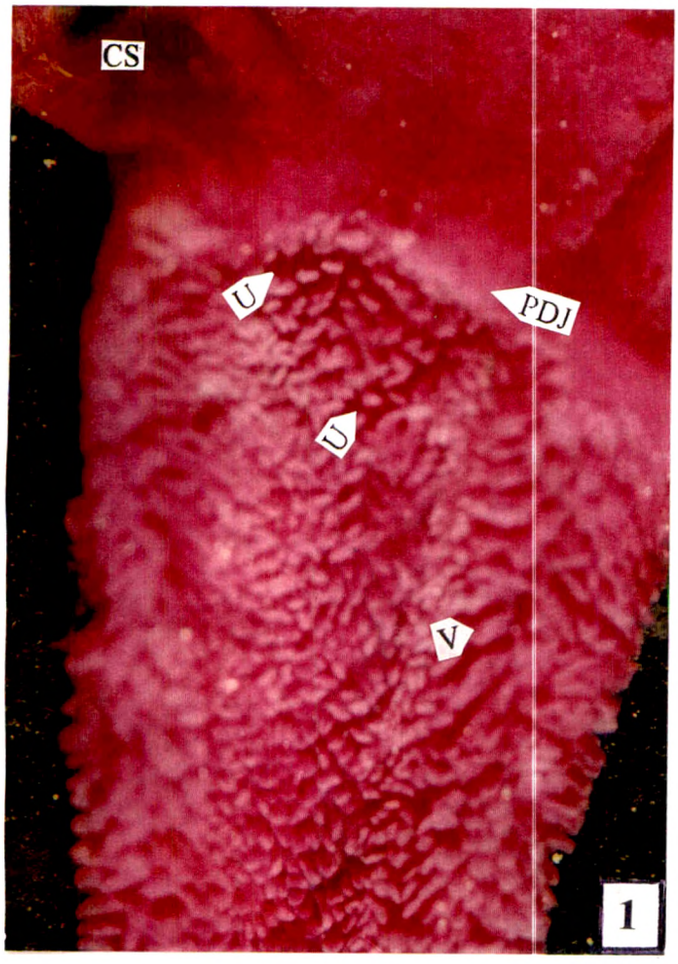
Fig. 4

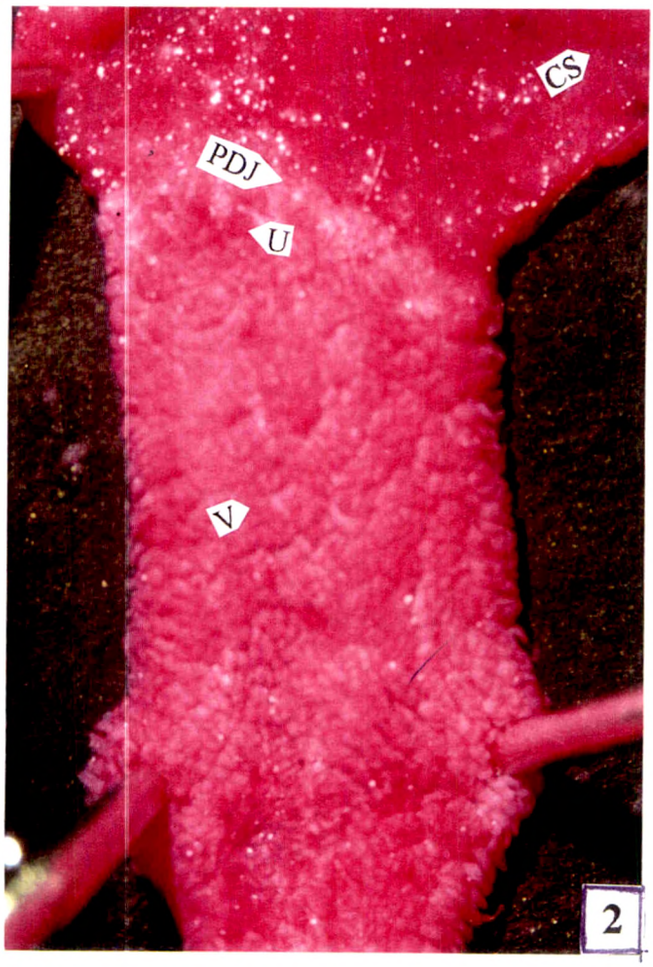
Stereoscopic microphotograph of duodenum of sialoadenectomized, cysteamine administered male mouse stained by PAS, showing least numbered and short sized villi. Duodenum also showing severe ulcer. X 6.3

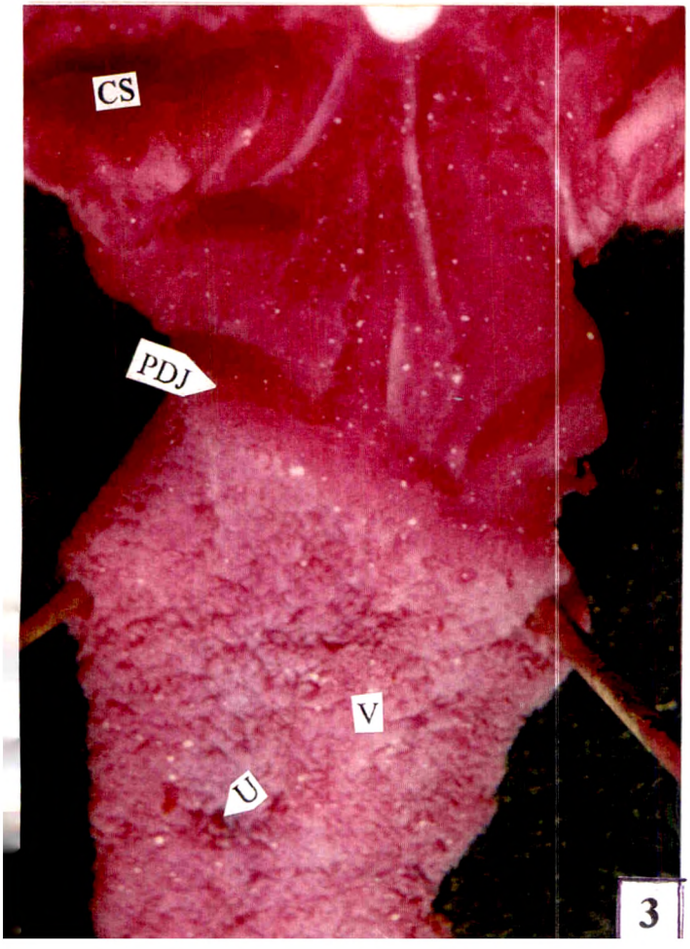
ABBREVIATIONS

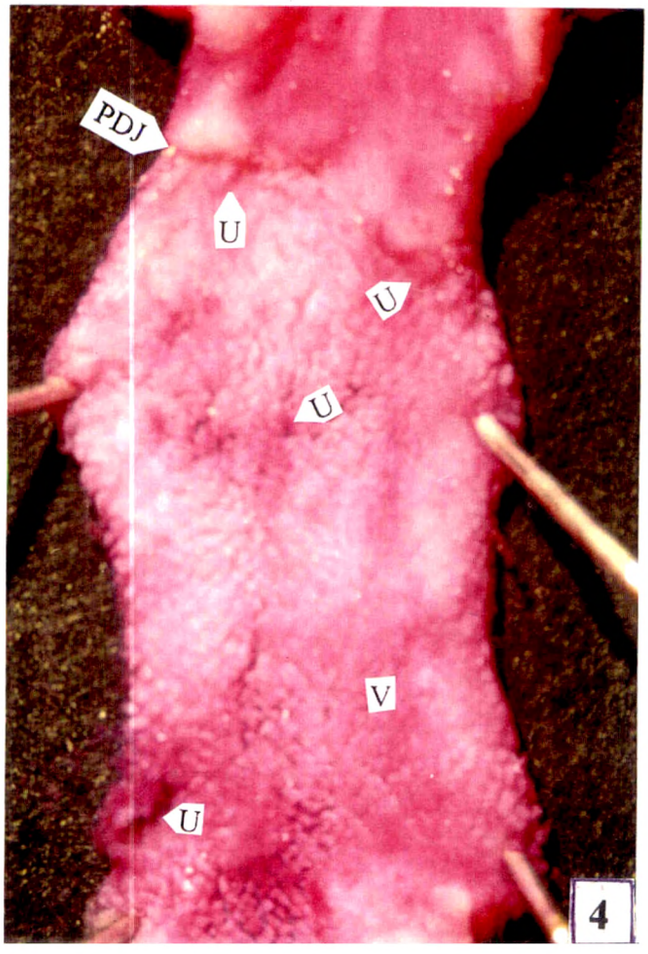
PDJ - Pyloro duodenal junction
U - Ulcer
V - Villi

PLATE - II









CAPTIONS TO FIGURES : PLATE-III

Fig. 1

T.S. of PDJ of controlled mouse, stained by H-E. showing transitional phase of pyloric stomach, sphinctor and villi with moderate H-E. stain. X 138

Fig. 2

T.S. of PDJ of sialoadenectomized male mouse, stained by H-E. showing lesions at villi and intense staining at Brunner's glands. X 138

Fig. 3

T.S. of PDJ of cysteamine administered male mouse, stained by H-E. showing damaged villi and unstained Brunner's glands by eosin. X 138

Fig. 4

T.S. of PDJ of sialoadenectomized, cysteamine administered male mouse, stained by H-E. showing damaged villi and increased stain of eosin at Brunner's glands. X 138

Fig. 5

T.S. of duodenum of controlled male mouse, stained H-E. showing slight low villi, intestinal glands and Brunner's glands. X 138.

Fig. 6

T.S. of duodenum of sialoadenectomized male mouse, stained H-E. showing ulcer eroding the villi. Intestinal glands and Brunner's glands were not damaged. X 138

Fig. 7

T.S. of duodenum of cysteamine administered male mouse, stained H-E. showing ulcer eroding the villi, Brunner's glands were intact. X 138.

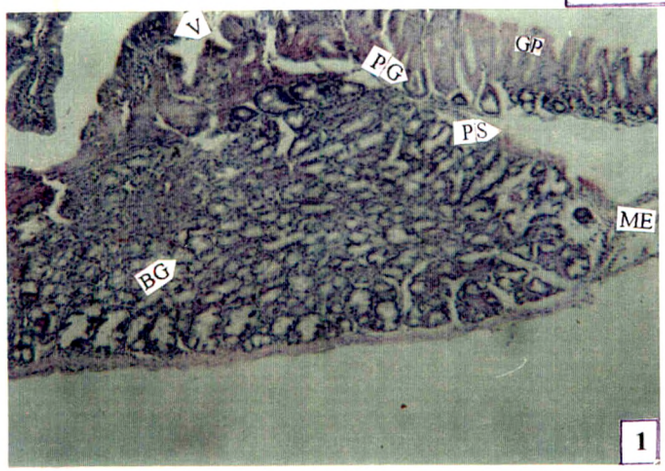
Fig. 8

T.S. of duodenum of sialoadenectomized, cysteamine administered male mouse, stained H-E. showing ulcer eroding villi. Brunner's glands were damaged. X 138.

ABBREVIATIONS

PDJ	-	Pyloro duodenal junction	GP	-	Gastric pits
PG	-	Pyloric gland	V	-	Villi
ME	-	Muscularis externa	PS	-	Pyloric spinchtor
BG	-	Brunner's glands	IG	-	Intestinal glands
GC	-	Goblet cells	PS	-	Pyloric sphinctor

PLATE - III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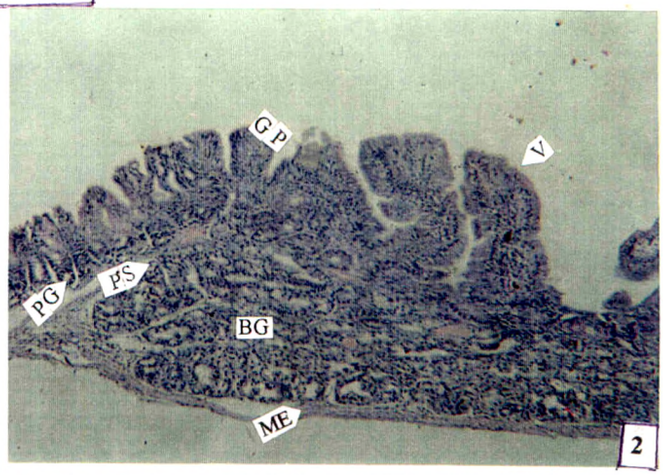
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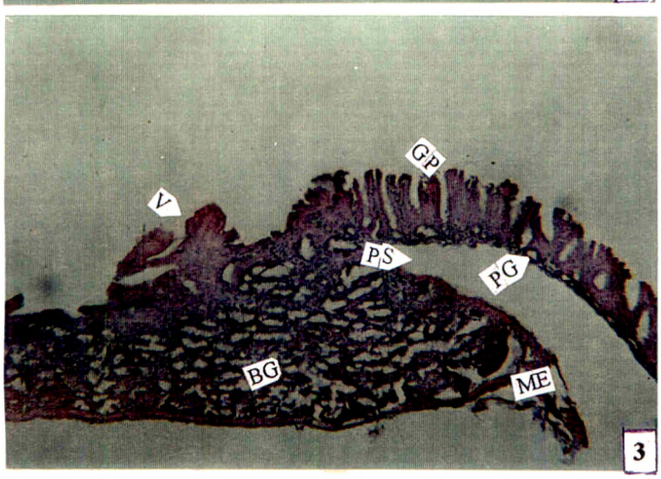
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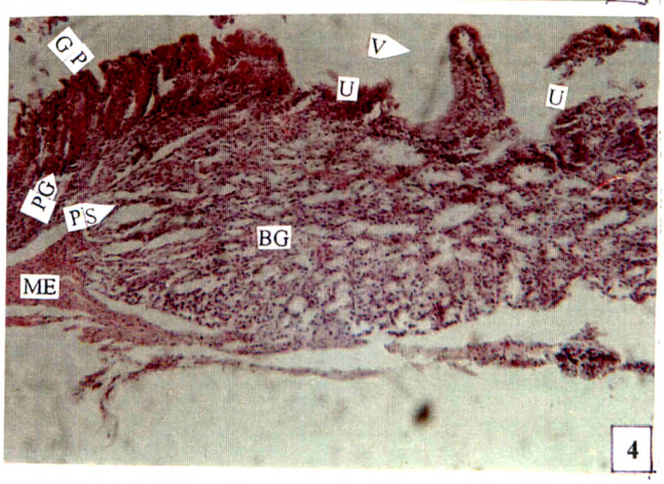
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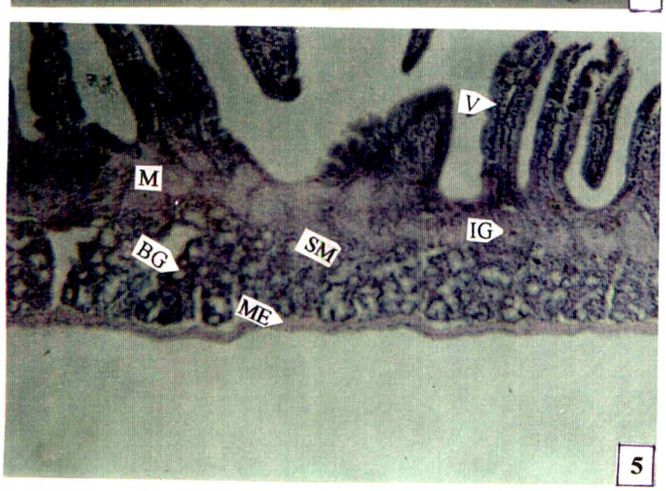
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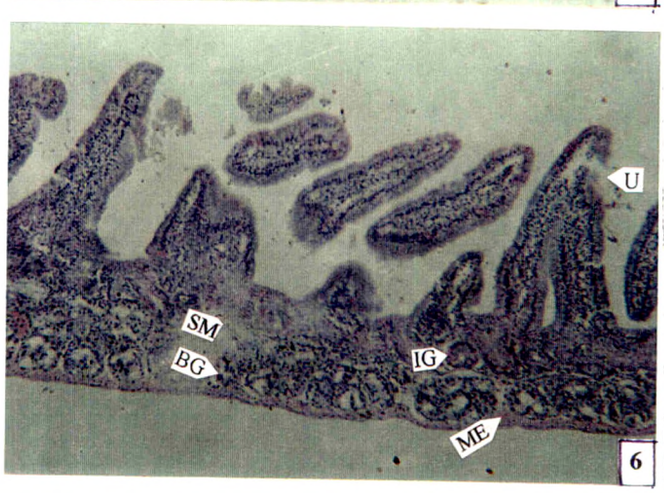
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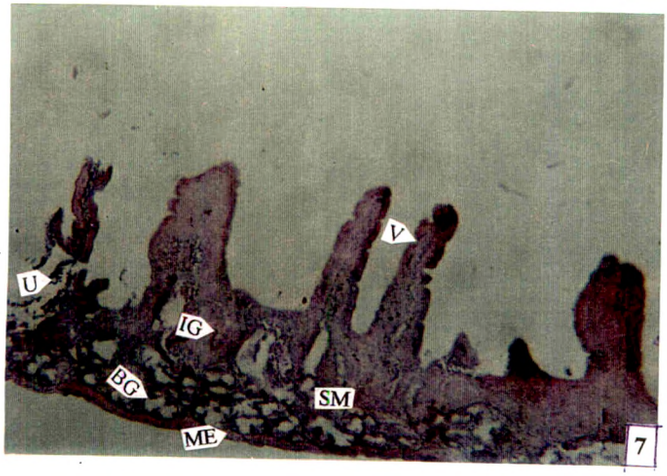
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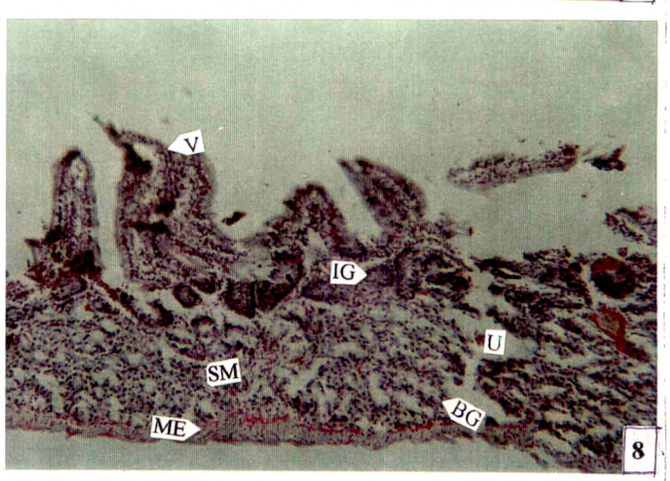
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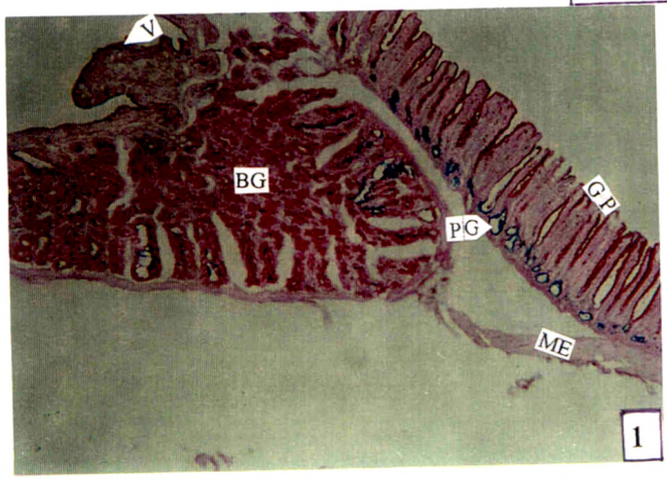
PLATE - V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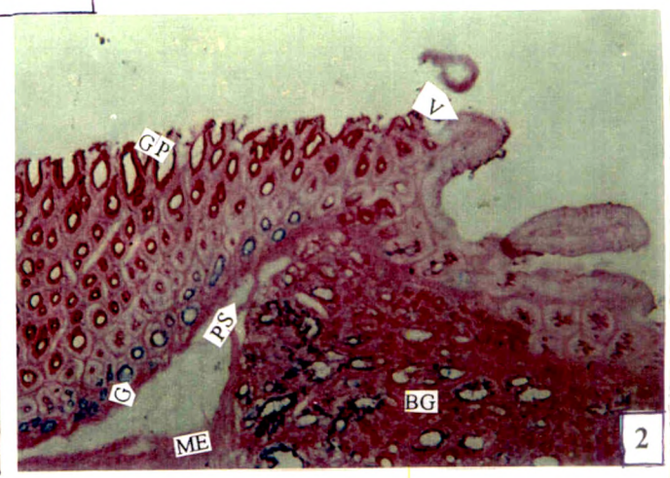
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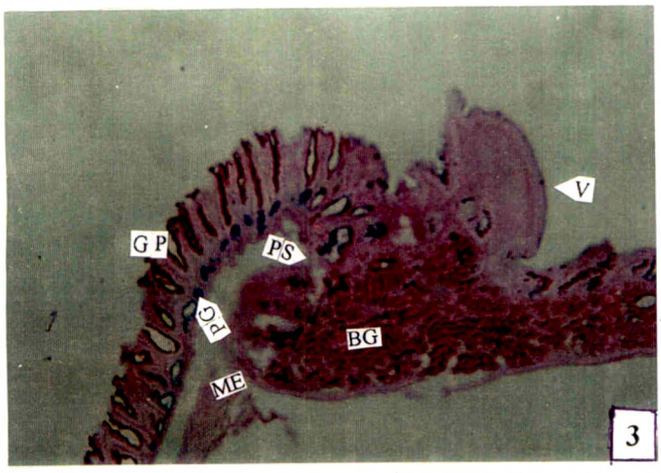
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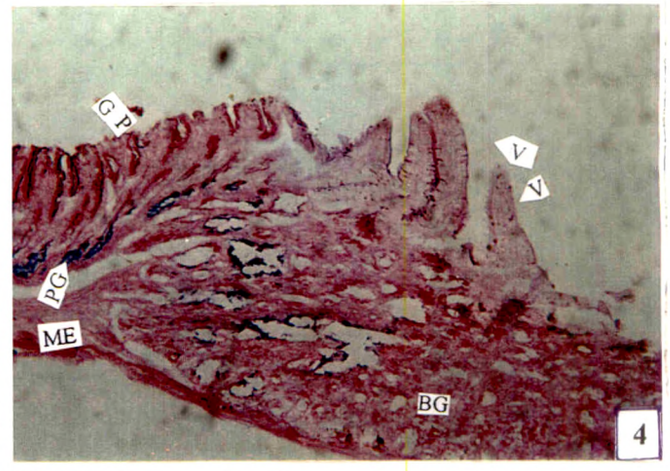
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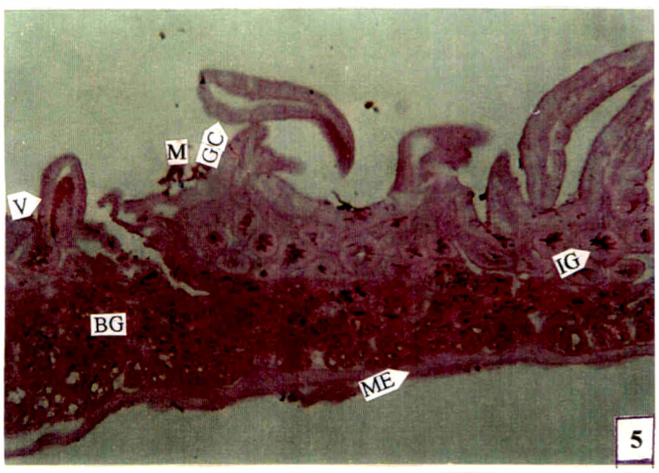
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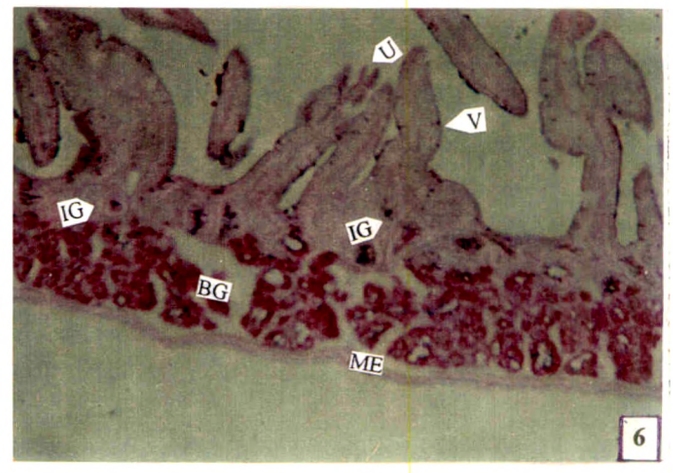
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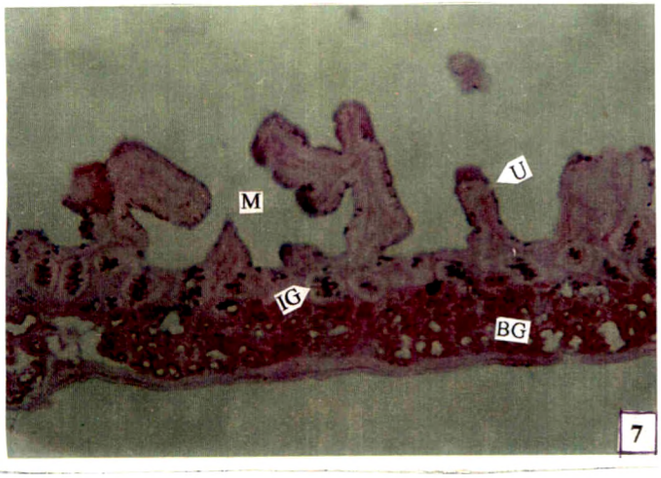
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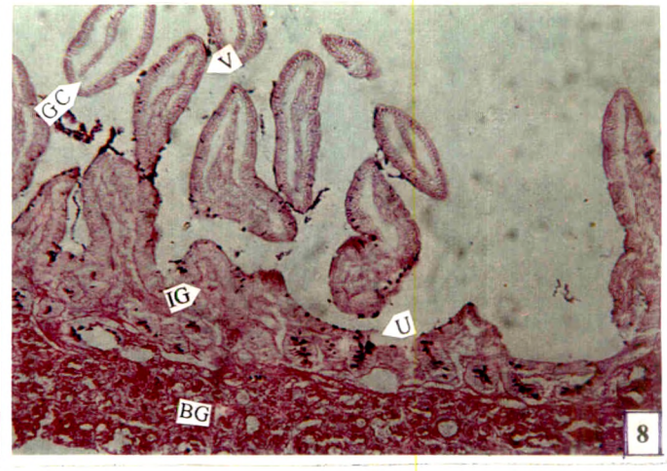
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CAPTIONS TO FIGURES : PLATE-V

Fig. 1

T.S. of PDJ of controlled male mouse, stained PAS + AB 2.5, showing moderate blue staining at pyloric glands and magenta stain at gastric pits. X 138

Fig. 2

T.S. of PDJ of sialoadenectomized male mouse, stained PAS + AB 2.5, showing moderate staining at pyloric gland. X 138

Fig. 3

T.S. of PDJ of cysteamine administered male mouse, stained PAS + AB 2.5, showing purple magenta staining at gastric pits and intense blue staining at pyloric glands. X 138

Fig. 4

T.S. of PDJ of sialoadenectomized, cysteamine administered male mouse, stained PAS + AB 2.5, showing magenta stain at gastric pits and moderate staining at pyloric glands. X 138

Fig. 5

T.S. of duodenum of cysteamine administered male mouse, stained PAS + AB 2.5, showing purple magenta staining at gastric pits and intense blue staining at pyloric glands. X 138

Fig. 6

T.S. of duodenum of controlled male mouse, stained PAS + AB 2.5, showing no staining at villi and blue colour at lumen of intestinal glands. X 138

Fig. 7

T.S. of duodenum of sialoadenectomized male mouse, stained PAS + AB 2.5, showing no staining at villi and purple magenta staining at lumen of intestinal glands. X 138

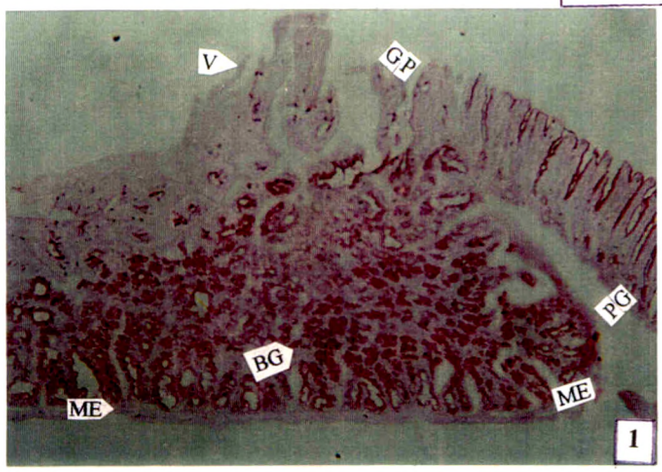
Fig. 8

T.S. of duodenum of sialoadenectomized, cysteamine administered male mouse, stained PAS + AB 2.5, showing magenta staining at gastric pits and moderate staining at pyloric glands. X 138

ABBREVIATIONS

PDJ	-	Pyloro duodenal junction	GP	-	Gastric pits
PG	-	Pyloric gland	V	-	Villi
ME	-	Muscularis externa	PS	-	Pyloric spinchtor
BG	-	Brunner's glands	IG	-	Intestinal glands
GC	-	Goblet cells			

PLATE - IV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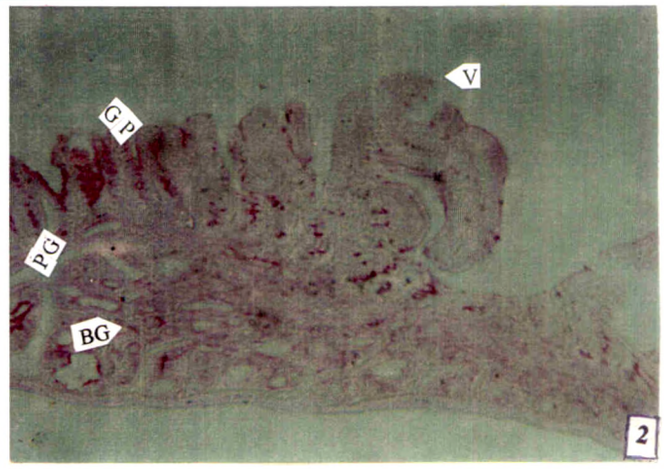
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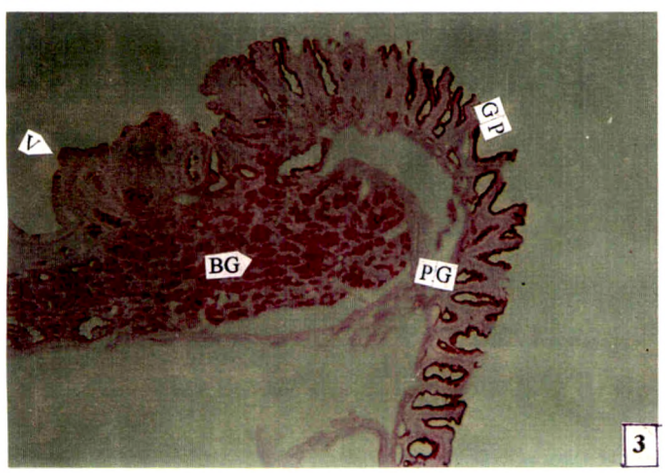
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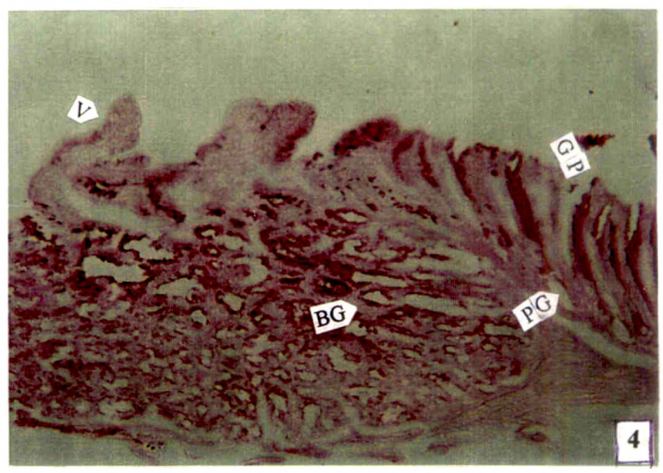
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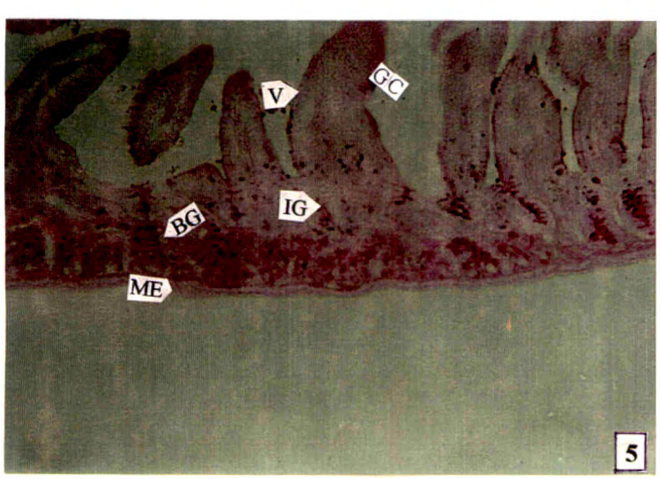
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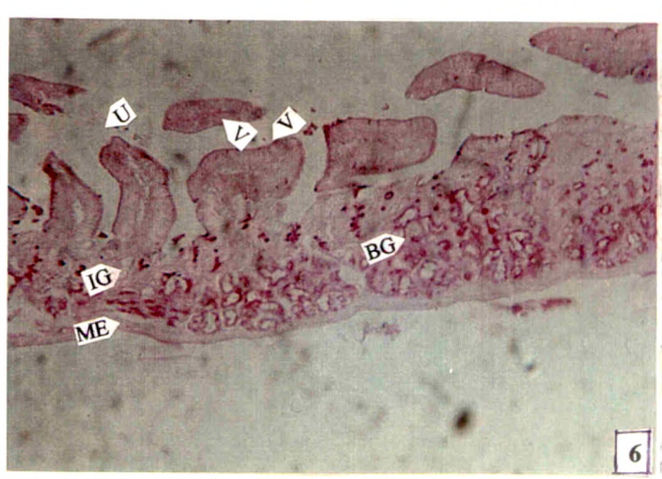
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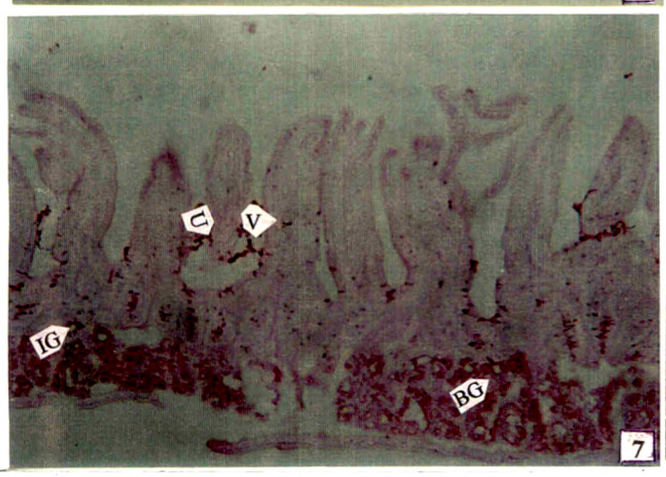
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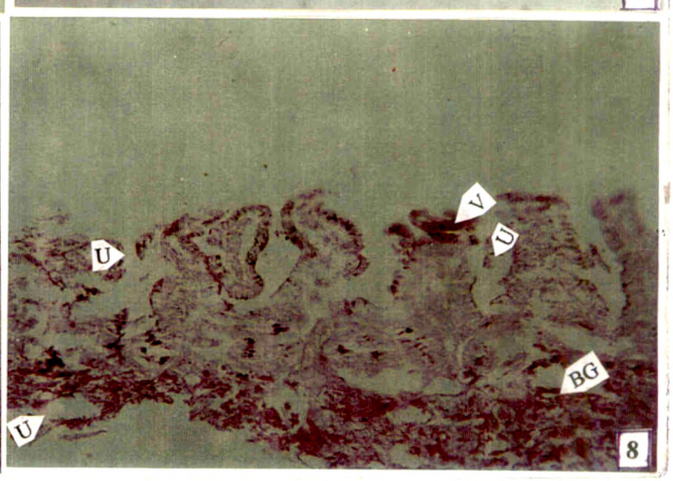
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CAPTIONS TO FIGURES : PLATE-IV

Fig. 1

T.S. of PDJ of controlled male mouse, stained PAS, showing with intense staining reactivity. X 138

Fig. 2

T.S. of PDJ of sialoadenectomized male mouse, stained PAS showing with decreased staining reactivity. X 138

Fig. 3

T.S. of PDJ of cysteamine administered male mouse, stained PAS, showing with intense staining reactivity. X 138

Fig. 4

T.S. of PDJ of sialoadenectomized and cysteamine administered male mouse, stained PAS, showing with no uniform staining reactivity and intense staining at luminal side of glands of Brunner. X 138

Fig. 5

T.S. of duodenum of controlled male mouse, stained PAS, positively found in villi, Brunner's glands shows PAS positive activity. X 138

Fig. 6

T.S. of duodenum of sialoadenectomized male mouse, stained PAS, showing decreased staining activity at goblet cells and no reactivity in intestinal glands. X 138

Fig. 7

T.S. of duodenum of cysteamine administered male mouse, stained PAS, showing decrease in PAS positive activity, in villi. Brunner's glands shows reducing PAS activity. X 138

Fig. 8

T.S. of duodenum of sialoadenectomized, and cysteamine administered male mouse, stained PAS, showing moderate staining activity X 138

ABBREVIATIONS

PDJ	-	Pyloro duodenal junction	GP	-	Gastric pits
PG	-	Pyloric gland	V	-	Villi
ME	-	Muscularis externa	PS	-	Pyloric spinchtor
BG	-	Brunner's glands	IG	-	Intestinal glands
			GC	-	Goblet cells

4. HISTOLOGY

Pyloric-duodenal Junction :

The alterations in pylaroduodenal junctions are described in Plate- III. The sections were taken longitudinally to show the histological structure of pyloric-stomach, the transitional part between pyloric stomach and duodenum and duodenum proper. The pyloric region of stomach was separated from the duodenum by a pyloric sphinctor (PS). The circular layer of muscularis externa (ME) formed a pyloric sphinctor (Fig.1).

The pyloric stomach showed four layers, these from inside outwards were mucosa, submucosa, muscularis externa and serosa. The simple columnar mucus epithelium lined the surface of the stomach extended into mucosa and lined the gastric pits (GP). The gastric pits were extended upto about half or more of its thickness. The pyloric glands (PG) were tabular and opened at the base of gastric pits. The submucosa contained connective tissue. The muscularis externa appeared as inner circular and outer longitudinal muscle layers. The outermost layer consisted of serosa (Fig.1).

The Brunner's glands (BG) were dispersed in the enlarged strands of circular muscle layer of muscularis externa. These glands occupied major part of the submucosa of duodenum. The duodenum at this region exhibited surface modification in the form of villi (V).

Each villus was leaf shaped urface projection with pointed end. The mucus secreting epithelium of stomach changed abruptly to the intestinal epithelium. This epithelium consisted of goblet cells (GC) and columnar (absorptive) cells. Intestinal glands (IG) were simple and tubular in the lamina propria. One or more intestinal glands were opening between the villi (Fig.1).

Gastric pits of mucosal layer of the pyloric stomach in sialoadenectomized mice were shorter and broader than the well organized deep gastric pits (GP) of the controlled mice (Fig. 2). Mucosal layer of pyloric stomach remained unstained by eosin in controlled and sialoadenectomized mice (Fig. 1 and 2). In cysteamine administered and sialoadenectomized, cysteamine treated mice, the gastric pits were shorter showing increased staining by eosin (Fig. 3 and 4). The gastric pits had wide opening towards the lumen in the cysteamine administered mice than those of sialoadenectomized mice and sialoadenectomized, cysteamine treated animals. (Fig. 2,3 and 4). The pyloric glands of the mucosa in sialoadenectomized mice (Fig.2) and cysteamine administered (Fig. 3) mice showed prominent lumen, whereas in sialoadenectomized, cysteamine treatment mice, these were narrow and elongated (Fig.4).

The Brunner's glands were remained unstained by eosin both in controlled and cysteamine treated mice (Fig. 1 and 3). The nuclei of the acinar cells were moderately stained in controlled mice by

haematoxylin, but these were intensely stained in sialoadenectomized one (Fig.2). In controlled and cysteamine administered mice (Fig. 1 & 3) the connective tissue in between the Brunner's gland stained moderately red, which was further increased in sialoadenectomized, cysteamine administered mice (Fig. 4).

The regular form of gastric mucosa changed into more irregular leaf shaped projections in the duodenal villi in cysteamine administered mice (Fig. 3) whereas these become short heighted, blunt and at some region lesions (U) were visualized in sialoadenectomized mice (Fig. 2). Mucosal damage with only few villi were observed in cysteamine administered (Fig.3) and more in sialoadenectomized, cysteamine administered individuals (Fig.4).

Muscularis externa of sialoadenectomized and cysteamine HCl administered mice (Fig. 2 and 3) resembled to that of controlled one (Fig. 1). In sialoadenectomized, cysteamine treatment mice it was disrupted but intensely stained by eosin (Fig. 4).

Duodenum :

The alterations in duodenal region are described in Plate No. III. The duodenum consisted of four layers, these were mucosa with its lining epithelium, lamina propria and muscularis mucosa; the underlying connective tissue submucosa with the Brunner's glands; the two smooth muscle layers of muscularis externa and the visceral peritonium serosa.

The inner lining was characterized by numerous finger like extensions called villi, which was formed by a lining epithelium of columnar cells, goblet cells and short tubular intestinal glands in lamina propria. The duodenum was characterized by presence of highly branched tubular Brunner's glands in the submucosa. The muscularis mucosa interrupted and Brunner's glands also penetrated into the lamina propria. The lining epithelium covered each villus and continued into the intestinal glands. Each villus had a core of lamina propria and a central lymphatic vessel lacteal (Fig. 5).

The submucosa was completely filled with highly branched tubular Brunner's glands. These glands delivered mucus secretory products to the bottom of the glands. The longitudinal section of duodenum showed gradual decrease in distribution of Brunner's glands in submucosa, from pyloric-duodenal junction and it tapered to the jejunum (Fig.5). The villi of duodenal mucosa of sialoadenectomized mice were short (Fig.6) blunt and few showed lesions at their tips in longitudinal sections passing through it as compared to elongated normal villi in controlled mice (Fig. 5). In cysteamine administered animals these were narrow as well as flat with remarkable initiations of lesions (Fig.7). Duodenal mucosa and submucosa were severely damaged and ulcers/ lesions were penetrated deep upto the muscularis externa and most of the villi were showing

discontinuity with lamina propria and submucosa in sialoadenectomized, cysteamine- HCl treated mice (Fig.8).

In heamatoxylin-eosin preparation the goblet cells and columnar cells remain unstained by eosin while intestinal glands showed moderate red staining in controlled as well as sialoadenectomized mice (Fig. 5 and 6). The nuclei of goblet cells and columnar cells in lining epithelium were regularly arranged at mid region of cells and stained deeply in controlled mice (Fig. 5), but they were displaced irregularly either toward apical or basal regions and intensely stained in sialoadenectomized mice (Fig. 6). In cysteamine administered animals columnar epithelial cells showed increased affinity towards eosin and the intestinal glands were uniformly stained. Goblet cells in the villi of these animal unstained by eosin, whereas their nuclei were lightly stained by haematoxylin (Fig.7). In sialoadenectomized, cysteamine treated mice, the goblet and columnar cells of villi were stained moderately by eosin and their nuclei were either blue or purple stained with haematoxylene (Fig. 8). The intestinal glands were stained more intensely by haematoxylin and eosin as compared to cysteamine administered and sialoadenectomized mice (Fig. 6 and 7).

The Brunner's glands were moderately stained by eosin in controlled mice (Fig. 5), but the staining intensity was increased in sialoadenectomized mice (Fig. 6). In cysteamine treated mice, eosin

reactivity was increased in Brunner's glands (Fig. 7) compared to control. Intensity of eosin staining was high in sialoadenectomized, cysteamine treated mice (Fig. 8).

Both in controlled and sialoadenectomized mice, the muscularis externa, stained red by eosin (Fig. 5 and 6), but there was increase in staining reactivity by eosin in cysteamine administered and sialoadenectomized, cysteamine administered mice (Fig. 8).

Alterations were not found in the serosa layer of all mice of different groups.

5. HISTOCHEMISTRY

Pyloro-duodenal Junction :

Alterations in glycoprotein content in the region of pyloro-duodenal junction are described in Plate No. IV.

Gastric pits in pyloric stomach of controlled and sialoadenectomized animals (Fig. 1 and 2) showed moderate and uniform PAS positive reaction, whereas there was intense reactivity to PAS in cysteamine administrated mice (Fig. 3). In gastric pits of sialoadenectomized, cysteamine treated mice the staining intensity was increased (Fig.4), than those of controlled and cysteamine administered mice. AB at pH 2.5 showed intense staining reactivity in the pyloric glands in controlled, cysteamine administered mice and sialoadenectomized, cysteamine treated animals, but it was moderate

in sialoadenectomized mice. The sequential staining with AB at pH 2.5 and PAS showed purple magenta staining reactivity in the gastric pits and intense blue staining at the pyloric glands in cysteamine administered mice (Plate-V, Fig. 3), whereas only magenta staining was observed in gastric pits and moderate blue staining in pyloric glands of controlled, sialoadenectomized mice and sialoadenectomized, cysteamine treated groups. (Plate-V, Fig. 1, 2 and 4).

Both controlled as well as cysteamine administered mice showed intense PAS staining in Brunner's glands. The acinar cells of Brunner's glands completely filled with the PAS positive secretory granules (Fig. 1 and 3). Decreased activity to PAS was observed in the Brunner's glands in sialoadenectomized mice (Fig.2), whereas the PAS activity was not uniformly observed and an intense staining was found towards the luminal sides in sialoadenectomized, cysteamine treated groups (Fig. 4). By AB at pH 2.5, the Brunner's glands were unstained but ducts were stained blue in all animals of different groups. At duct region in sialoadenectomized mice, the AB at 2.5 reactivity was intense as compared to controlled, cysteamine administered and sialoadenectomized, cysteamine treated mice. The sequential staining of AB at pH 2.5 and PAS showed blue staining to ducts of Brunner's glands and magenta to the acini of Brunner's

glands in all groups (Plate-V, Fig. 1,2,3 and 4). Some of the Brunner's acini in the sialoadenectomized mice stained purple with the sequential staining (Plate- V, Fig.2) whereas they were not stained in rest of three groups (Plate- V, Fig. 1,3 and 4).

Duodenum :

Alterations in duodenal region are described in Plate No. IV.

The surface epithelium of duodenal region including goblet cells and columnar cells were showed decreased PAS staining reactivity in sialoadenectomized (Fig.6) and cysteamine administered individuals (Fig. 7) than that of controlled one (Fig. 5), where it was moderate in sialoadenectomized, cysteamine treated mice (Fig. 8). The intestinal glands were stained with PAS reactivity at their luminal side exhibiting presence of secretory granules in controlled mice (Fig. 5), but they were not reactive to PAS in sialoadenectomized mice (Fig. 6) and also in sialoadenectomized, cysteamine treated mice (Fig. 8). The cysteamine administered mice showed less intense staining activity to PAS at the luminal sides of intestinal glands (Fig. 7). Both in controlled and cysteamine administered animals exhibited intense stain for PAS technique in Brunner's glands, (Fig. 5 and 7) but the staining activity was found decreased in sialoadenectomized mice (Fig. 6) and sialoadenectomized, cysteamine administered animals (Fig. 8). The lumenal region of intestinal glands of all groups were

stained blue for AB at pH 2.5. The sequential staining with AB at pH 2.5 and PAS showed purple magenta staining activity in the lumen of intestinal glands of sialoadenectomized (Plate-V, Fig. 5) and cysteamine administered mice (Plate-V, Fig. 7) whereas only blue staining activity was observed in controlled (Plate-V, Fig. 5) and sialoadenectomized, cysteamine treated animals (Plate-V, Fig. 8).

OBSERVATIONS AT GLANCE

Animal Groups → / Methods ↓	CONTROLLED				
	Gastric pits	**Pyloric glands**	**Brunner's glands**	**Villi**	**Intestinal glands**
Histology (H-E.)	Extended upto about ½ or more of the thickness of mucosa. - Unstained by eosin	Tubular and opened at the base of gastric pits	Dispersed regularly in submucosa with moderate stain by haematoxylene to acinar cells	**PDJ :** Modified surface epithelium. Villi leafshaped with pointed end. **Duo** : Goblet cells unstained by eosin. Arrangement of nuclei is regular and stain is deep. Columnar cells are unstained by eosin and nuclei deeply stained	Simple tubular. Moderate red by eos
PAS	Moderate and uniform		Intense staining with full of secretory granules in acinar cells	Intense staining reactivity	Intense stain. Luminal side exhibits secretory granules
AB 2.5 pH	Negative staining reactivity	Intense staining reactivity	Unstained glands, ducts blue stained with intense reactivity	Negative staining reactivity	Luminal region stained
AB 2.5 + PAS	Magenta staining	Moderate staining	Acinar cells are magenta and ducts are blue	Negative staining reaction	Lumens were purple magenta staining