CHAPTER THREE

OBSERVATION AND DISCUSSION ON MALE RAT

Florey and Harding (1935), Write et al. (1940) and Sonnenschein et al. (1947) established that duodenal glands of Brunner have a hormonal mechanism of control but the nature of this hormone is unknown. Cooke and Grossman (1966) studied secretion and motility of Brunner's gland pouches after administration of hormone-like gastrin and secretin. Their studies indicate that neither gastrin nor secretin is the regulating hormone. In other exocrine cells like salivary glands and pancreas secretion is regulated by steroid hormone and glucocorticoid which is very well discussed by Palade (1975), Grossman (1984), where secretion of ready secretory granules is stimulated by these hormones.

The above review indicates the significant paucity of literature on the hormonal control over Brunner's glands. To establish the fact that the synthesis and secretion of the products from Brunner's glands regulated by hormone like androgen, an experimental approach is involving creation of artificial deficiency of hormone by castration and administration of hormone in desired concentration and dose is essential. The laboratory white rat was found to be ideal experimental animal for such studies. The work on hormonal control over Brunner's gland thus, aims at i) studying the histology and histochemistry of Brunner's glands of mature male rat; ii) studying the histology and histochemistry of Brunner's glands of castrated male rats; iii) studying the histology and histochemistry of

enzymes from Brunner's glands of testosterone injected rats.

MATERIAL AND METHOD

For the study of distribution of Brunner's glands gross anatomical preparations were carried out using 15 male rats as described in Chapter Two. Six mature male adults were within the age group 160 to 180 days and weighing about 225 to 250 gm. were sacrificed by cervical dislocation and their duodenum were pulled out and fixed in 10% neutral formalin and 5% calcium formal for the study of histology and histochemistry of enzymes respectively.

About 10 mature males within the age group of 160 to 180 days and weighing about 225 to 250 gm were selected and kept separately at least four days before they were operated. They were anesthesized with ether anesthesia and the skin was shaved and an incision was made at the tip of the scrotum, large enough to permit extrusion of the testicles. Single ligature was made around the spermatic cord, spermatic vein and differential vessels. Both the testes were excized and sutures were made to the scrotal sac. The day on which orchiodectomy was performed was taken as zero day of orchiodectomy. Thirty days after the operation five rats were sacrificed and their duodenums were excised and employed for histology and histochemistry.

To observe the effects of administration of male hormone a batch of five rats, 30 days orchiodectomized, was used. These rats were injected with testosterone propionate 4 mg in 1 ml of corn oil/100 gm of body weight, at an interval of 24 hrs. consecutively for 3 days. On 4th day at 10 a.m. they were sacrificed for further study.

Observations

Gross Anatomy

The segment of gut containing pyloroduodenal junction showed mucosal depressions concentrated on the duodenal side of the junction. The pyloric mucosa became broken up into small villus-like structure covered by gastric epithelium. These structures were replaced after a short interval by intestinal villi of duodenum. There was abrupt change from gastric to intestinal epithelium at the junction washing of fresh specimen for approximately for five days and subsequent clearing in 3% acetic acid removed villi and most of the secretory product of Brunner's glands. Upon removal of muscularis mucosa, a collar of lobed glandular tissue of considerable amount was exposed. The staining with hematoxylin this cleaned material showed presence of a number of glands in submucosal region. Glands were crowded below the junction forming thick color. At the posterior end the number of glands reduced considerably.

The amount of glandular tissue present was subject to considerable variation but did not extend less than 4 cm and more than 5 cm beyond the pyloroduodenal junction. It was upto the opening of pancreatic duct (Fig. 1,D).

Histology

The sections taken from anterior part of the duodenum of normal, castrated, and castrated + hormone injected rats were stained for eosin and hematoxylin. . . Brunner's glands were arranged in aggregates mainly within submucosa (Fig. 2 inset). Parenchyma of the glands was divided into lobules which were separated from one another by connective tissue septa (Fig. 2 ↑). The lobular parenchyma consisted of secretory end pieces acini (AC), tubulo acini (TC) and ducts (D) (Fig. 2, AC, TC and D). The term acini is used wherever an approximately spheroidal terminal expansion is described, but expansion of the axis is two to three times the diameter, then end pieces are referred as tubulo acini. In the male Brunner's glands the number of acini was very small, which were sporadically distributed in tubular acini. Cells of tubulo acini and acini were pyramidal in shape, nuclei of tubulo acini were basally situated whereas that of acini were situated in the central region of the cells (Fig. 2). Cells of ducts (D) were low cuboidal in shape, large nuclei were situated in the centre.

Captions to Figures
Figs. 1 to 8

Fig.1: The distribution of Brunner's glands in an adult male rat prepared by the Landboe-Christensen method. From pyloroduodenal junction the Brunner's glands extend upto the opening of the pancreatic duct (D)

Fig.2: The Brunner's glands of normal male rat stained for Haematoxylin-Eosin (H-E) showing acini (AC), tubulo acini (TC), duct (D) and connective tissue septum (↑). Inset showing submucosal position of Brunner's glands x 240.

Fig.3: The Brunner's glands of castrated male stained for H-E, showing acini (AC), tubulo acini (TC) and increased area of intralobular septa (↑) x 240.

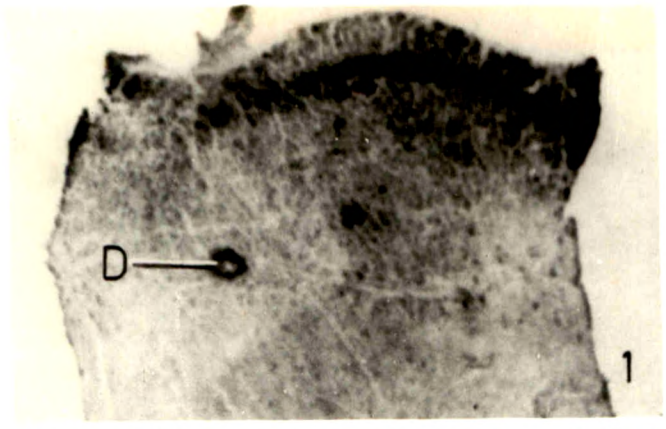
Fig.4: The Brunner's glands of castrated and hormone-injected male rat stained for H-E showing acini (AC) and tubulo acini (TC) with increased dimensions, and intralobular space reduced x 240.

Fig.5: The Brunner's glands of normal male rat stained for PAS, showing PAS positive acini (AC) and tubulo acini (TC) at the anterior 2/3 of the cells. Connective tissue weakly PAS positive (↑) x 240.

Fig.6: The Brunner's glands of castrated male rat stained for PAS, showing reduced PAS activity in acini (AC) and tubulo acini (TC). Connective tissue (↑) x 240.

Fig.7: The Brunner's glands of castrated and hormone injected male rat showing increased PAS activity in acini (AC), tubulo acini (TC). The arrow (↑) indicates connective tissue x 240.

Fig.8: The Brunner's glands of normal male rat stained for β-glucuronidase, showing granular reaction all over the cytoplasm in acinar (AC) and tubulo acinar cells. In duct (D) cells activity was found only at luminal side. Connective tissue (↑) x 540.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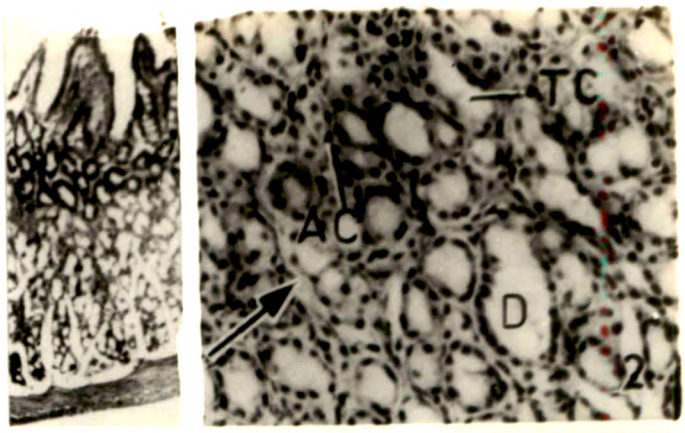
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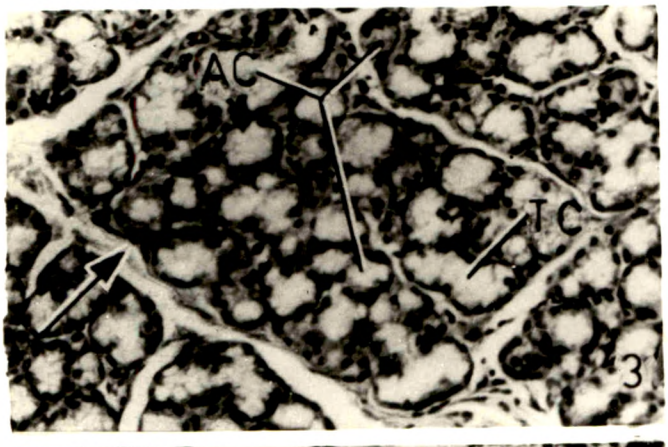
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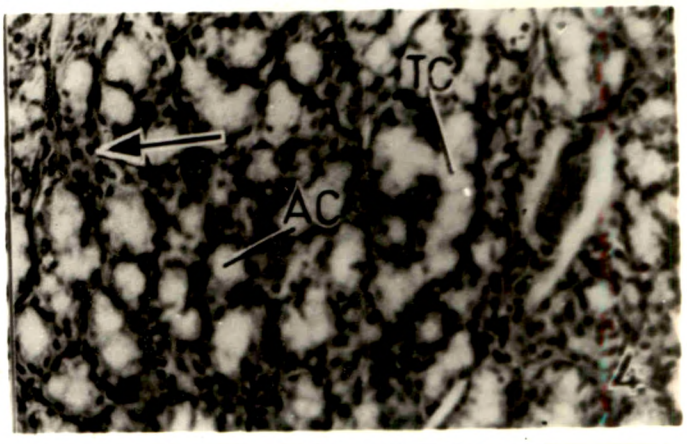
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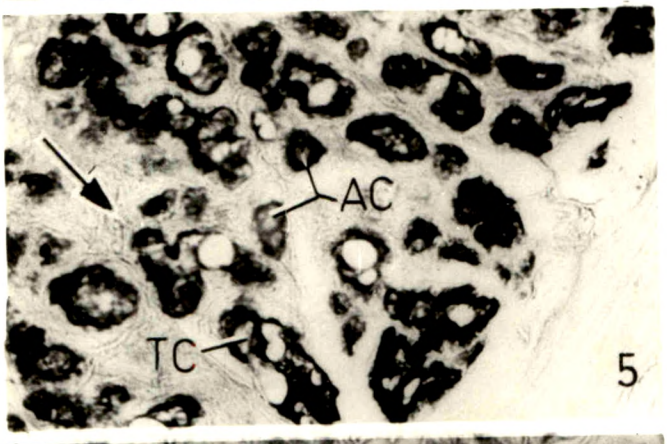
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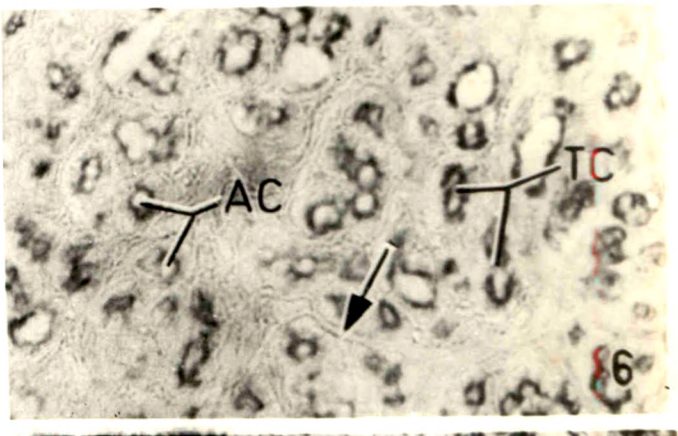
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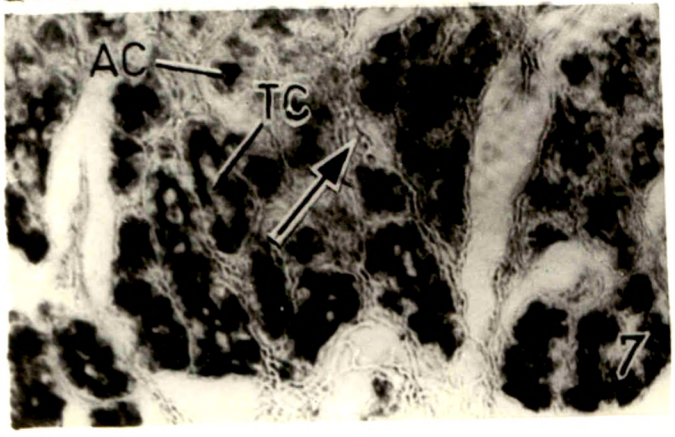
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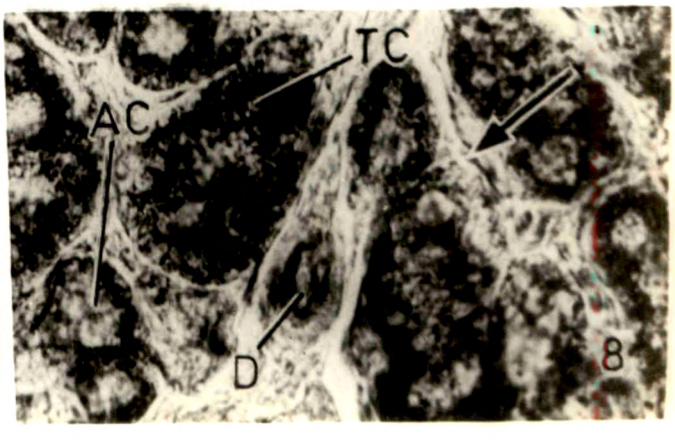
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In the eosin hematoxylin technique, cells of some acini were stained with eosin (AC) but cells of tubulo acini did not stain with eosin. Only slight one fourth portion of tubulo acini was stained with eosin. Duct cells were also stained with eosin.

Brunner's gland secretory end pieces (Fig. 3 AC and TC) apparently did not seem to be altered in their size or shape in castrated male rat. But dimensions of intralobular septa were increased all over the gland indicating possible decrease in size of secretory end pieces. But these intralobular septa did not completely fill with connective tissue but they looked like empty space (Fig. 3 ↑). There was no apparent change in the location of nuclei from both the acini but little more part of the cytoplasm of tubulo acini cells was stained with eosin. There was no change in the staining of eosin in the cells of acini. Both the acini and tubulo acini of Brunner's glands of castrated and then hormone injected male rats seemed to be altered. Length of tubulo acini was increased all over and size of acini was also increased (Fig. 4 AC and TC). Intralobular space was reduced compared to normal and castrated Brunner's glands of male rats (Fig. 4).

Histochemical Observation

i) Mucosubstances

Sections of duodenum stained for PAS technique showed

PAS positive reaction both in tubulo acini and acini. Reaction was intense and totally present at anterior 2/3 of the cytoplasm, in the basal region of the cells PAS reaction was absent (Fig. 5 AC and TC). Luminal side of the duct cells also showed dense PAS positive reaction (Fig. 5 ↑). Connective tissue septa was also stained with PAS. The PAS activity did not alter from the acini, tubulo acini, duct cells and connective tissue in malt diastase treated sections. The cells of these acini tubulo acini and ducts did not stain with Alcian Blue (pH 2.5). Reaction could not appear even after treating sections with pepsin and then staining with Alcian Blue (pH 2.5). But connective tissue did stain with AB 2.5 after pepsin digestion.

In castrated males PAS activity from acini and tubulo acini of Brunner's gland was reduced considerably (Fig. 6 AC and TC), but there was no reduction in the PAS activity from cells of duct. In castrated hormone injected males, cells of acini and tubulo acini were stained intensely for PAS. A number of PAS-positive clusters of granules were observed in the cytoplasm of these cells (Fig. 7 AC and TC).

ii) β-glucuronidase

With the naphthol AS-BI post-coupling technique, the azo-dye end product of this histochemical reaction, was found to be localized all over the cytoplasm of cells of acini and

tubulo acini (Fig. 8 AC and TC). But in the duct cells enzyme reaction was observed only in the luminal side of the cells (Fig. 8D). Connective tissue was also stained for β-glucuronidase. The reaction produce in both the acini and duct cells was in the form of very distinct sharply delimited granules spread all over the cytoplasm, but it was diffused and non-granular in connective septa (Fig. 8↑).

Thirtieth day after orchiodectomy, acini and tubulo acini cells exhibited remarkable change in their β-glucuronidase activity. As in normal male, β-glucuronidase activity in acinar cells was completely granular in nature but population of β-glucuronidase positive granules from these cells decreased considerably and granules were not present all over the cytoplasm. 1/3 luminal border of acinar cells did not show β-glucuronidase-positive granules (Fig. 9 AC and TC). In hormone-treated rats there was increase in the intensity of staining reaction for β-glucuronidase in the granular forms, but these granules were localized at the basal side of the cells. β-glucuronidase-positive granules were smaller compared to the normal and in the form of clusters (Fig. 10 AC and TC). There was no change in the enzyme activity from connective tissue cells (Fig. 10↑).

Captions to Figures
Figs. 9 to 17.

Fig.9: The Brunner's glands of castrated male, stained for β-glucuronidase, showing acinar (AC) and tubulo acinar (TC) cells with reduced number of β-glucuronidase positive granules. Connective tissue (↑) x 540.

Fig.10: The Brunner's glands of castrated and hormone-injected male rat, stained for β-glucuronidase, showing acinar (AC) and tubulo acinar (TC) cells with increased activity restricted towards basal side. The granules comparatively smaller in size. Connective tissue (↑) shows no change in activity x 540.

Fig.11: The Brunner's glands of normal male rat, stained for Esterase, showing granular activity all over the cytoplasm, but more towards the basal side, of acinar (AC) and tubulo acinar (TC) cells. In connective tissue (↑) the activity is diffused nongranular x 540.

Fig.12: The Brunner's glands of castrated male rat, stained for Esterase, showing acinar (AC) and tubulo acinar (TC) cells with diffused, nongranular activity all over the cells, but a granular belt at the basal side of the cells. Connective tissue (↑). x 540.

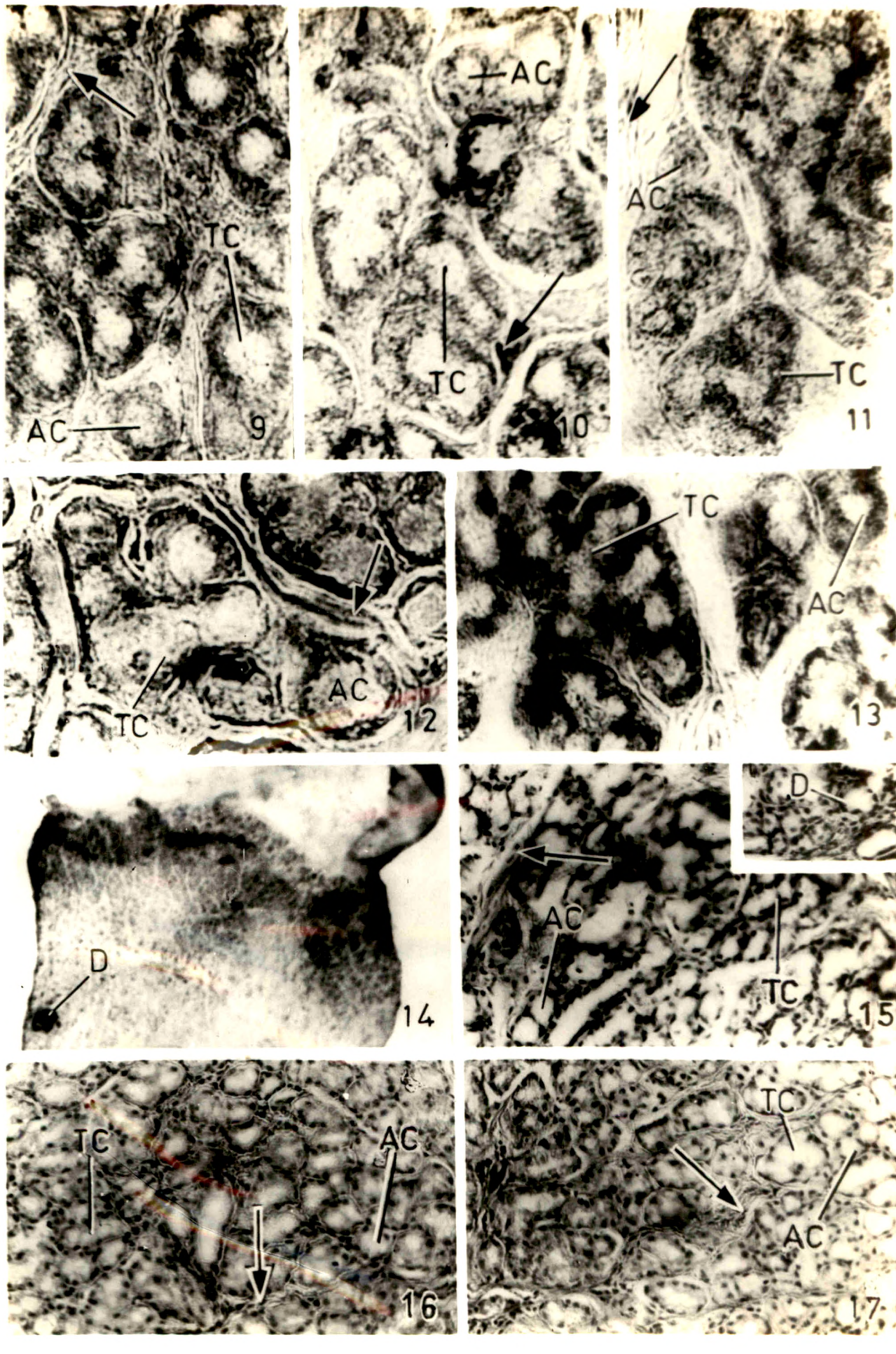
Fig.13: The Brunner's glands of castrated and hormone-injected male rat, stained for Esterase, showing intense activity at the basal 2/3 of the acinar (AC) and tubulo acinar (TC) cells x 540.

Fig.14: The distribution of Brunner's glands in an adult female rat prepared by the Landboe-Christensen method. From pyloroduodenal junction the Brunner's glands are extending upto the opening of the pancreatic duct.

Fig.15: The Brunner's glands of normal female rat, stained for H-E, showing many tubulo acini (TC) and a few acini (AC). Both weakly staining with eosin. Inset showing a duct with cuboidal cells with nucleus at the centre. Connective tissue (↑) x 240.

Fig.16: The Brunner's glands of overiectomised female rat stained for H-E, showing more positively staining acinar (AC) and tubulo acinar (TC) cells with Eosin. Connective tissue (↑) increased considerably x 240.

Fig.17: The Brunner's glands of overiectomised and hormone-injected female rat, stained for H-E, showing acini (AC) and tubulo acini (TC) weakly stained with Eosin. Connective tissue (↑) x 240.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iii) Esterase

With 5-bromoindoxyl acetate technique esterase activity was revealed in all cells of Brunner's glands of normal rat. The localization and morphology of esterase activity in acinar cells of both the types did not show any difference. But it was not localized throughout the cytoplasm but esterase-positive granules were populated towards the basal side of the cells. Enzyme activity was localized in large particulate granules in the cells of acini and tubulo acini (Fig. 11 AC & TC) but it was diffused non-granular in connective tissue cells (Fig. 11↑). Due to castration, enzyme activity was greatly affected in the cells of Brunner's glands. Granular cytoplasmic activity of secretory cells which was observed in normal male, was completely abolished where basal cytoplasm of the cells showed intense diffused non-granular activity at the basal region. The fine granular esterase activity could also be observed at basal side of the cytoplasm (Fig. 12↑). Enzyme activity increased in Brunner's glands acini, tubuloacini of castrated, hormone injected male. Activity was localized in 2/3 of the basal region of all secretory end pieces. It was in the form of more granular and less diffused (Fig. 13 AC and TC).

Discussion

Gross anatomical structure of Brunner's glands indicates that the glands of Brunner begin at the transition from gastric to intestinal mucosa. Population of the glands is more towards pyloroduodenal junction. Thick belt-like structure is formed due to these glands at the junction which is reduced posteriorly. Histologically secretory end pieces are of two types, speroidal and tubular. Speroidal end pieces are called as acini whereas those of long axis are called as tubulo acini (Young and Van Lennep, 1978). Acini are stained with eosin but tubulo acini are hollow only at basal region of the acinar cells stained with eosin. Generally acini which stain with eosin are called as serous and which do not stain with eosin are called as mucous or seromucous; of course, this is not accurate description, but rather convenient way of identifying cell types, because there are a number of reasons for this. First it is clear that there exists a virtually continuous series of carbohydrate protein complexes in secretory substances, ranging from serous secretions with an almost negligible amount of carbohydrate to mucous secretions of acid glycoproteins with a very high carbohydrate to protein ratio. In order to arrive at a proper histochemical definition of a gland, a whole battery of histochemical reactions has to be applied and, apart from the fact that the correct interpretation of some of the reactions is still not well established, strict

adherence to histochemical definition of the term serous and seromucous may lead to great practical difficulties. If a serous secretion were defined as one containing no carbohydrate, the term serous might have to be dropped altogether, since nearly all secretions contain some glycoprotein. And hence Young and Van Lennep suggested that terms serous, mucous and seromucous be used as much as possible in the classical sense, based on cell morphological characteristics, without prejudice to the histochemical definition of the secretory product. In the male Brunner's glands acini are stained with eosin but tubuloacini are hollow only in basal region of these cells stained with eosin and hence acini may be of serous nature and tubulo acini are seromucous. But acini and tubulo acini do stain in PAS technique indicating presence of mucus secreting material in them and hence it is not possible to classify acinar cells using only eosin hematoxylin PAS staining technique. The mucous material stained with PAS is not glycogen because PAS activity is retained even after diastase digestion. Both acino do not stain with Alcian blue (pH 2.5). Even after pepsin digestion cells are not stained with AB (pH 2.5), which indicates that cells are devoid of acid mucopolysaccharides in them, possibility of protein masked acid mucopolysaccharides is also ruled out with pepsin digestion technique. These observations lead to conclude that the cells of acini of Brunner's glands contain neutral glycoproteins.

In male secretion of neutral glycoprotein may be controlled by male hormone because there is tremendous alteration in the secretory end pieces of Brunner's glands after castration and hormone injection. In normal rats, space occupied by connective septa is very less, but in castrated animals area occupied by connective septa is increased conspicuously. This area is reduced in castrated hormone injected animals. Increase in size of both the acini of Brunner's glands is also markedly observed in castrated hormone-injected animals compared to normal and castrated animals. PAS positive material which was observed in the acini and tubulo acini is also markedly reduced in castrated but in castrated-hormone injected animals PAS-positive material is increased considerably.

In normal male an enzyme β-glucuronidase is present in the form of clusters of granules all over the cytoplasm. But in castrated animals activity for β-glucuronidase is decreased considerably from the apical border of the cells which is increased after administration of androgen. Similarly, esterase activity which is present in the clusters of small granules present in acini and tubulo acini is decreased in castrated male. Increase in enzyme activity is noticed after introduction of male hormone. Increase in esterase activity after testosterone injection is also demonstrated in other exocrine glands by Angeletti and Angeletti (1967), Calissano and Angeletti (1968).

These studies indicate that testosterone affects the Brunner's glands. Increase in space between lobules in castrated rats indicates decrease in size of acini and tubulo acini. The apace is reduced in hormone injected rats. PAS activity is decreased in operated male rat which can be restored after hormone injection. An enzyme β-glucuronidase which is mainly localized at the basal side of the cell is also affected due to less androgen in the body of rat. Androgen level from the body of rat is reduced due to castration. Condition is recovered after the injection of androgen. Similarly, esterase morphology and intensity is also changed due to androgen. In castrated male activity is completely abolished from cytoplasm and very little enzyme activity at the basal region of acini and tubulo acini is observed, which was again increased in androgen treated rats.