CHAPTER THREE

OBSERVATIONS

OBSERVATIONS

(I) Normal Prostate Gland

A) Histological Observations:

The cut sections of the normal prostate showed presence of glandular elements in the form of acini and ducts or tubules, smooth muscles and fibrous connective tissue. The acini and ducts are invested by a limited fibromuscular stroma propria. Immediately outside the stroma propria the extensive supporting framework of the prostate is continued as fibrous tissue containing irregular interlacing strands and bundles of smooth muscles.

B) Histochemical Observations:

The results of the histochemical reactions for normal and BPH are similar, except that they slightly differ at acini only and hence photomicrograph of PAS reaction at normal acini is only shown in PMC Plat No. I (Fig. 2). Histochemical results are recorded in Table No. 1.

i) Acini:

The acini with inner layer of tall columnar cells and with a basal layer of elliptical cells showed the following results with the various histochemical reactions. The epithelium revealed moderate to less intense PAS reactivity which was resistant to diastase or

TABLE NO. 1

Histochemical observations on mucosubstances found at various cellular sites of the normal prostate

Sr. No.	Histochemical Reactions	Normal acini	Acinar Secretion	Stroma	Muscles	Ducts
1	PAS	+++P	++++P	+++P	++P	++P
2	D-PAS	+++P	++++P	++P	+P	+P
3	P-PAS	+P	+P	+P	-	-
4	AB pH-1.0	+B	++B	±B	-	+B
5	AB pH-2.5	++B	+++B	+B	+B	++B
6	AB pH-1.0-PAS	++PB	+++PB	+P	+P	++PB
7	AB pH-2.5-PAS	++BP	+++BP	++PB	+PB	++BP
8	CI	++B	+++B	+B	+B	++B
9	AF	+P	+P	±P	-	±P
10	M 37-AB pH-2.5	-	-	-	-	-
11	DM 37°c-AB pH-2.5	+B	++B	±B	-	±B
12	M 60°c-AB pH-2.5	-	-	-	-	-
13	DM 60°c-AB pH-2.5	-	±B	-	-	-
14	Acid hydrolysis	+B	+B	-	-	-

α-amylase digestion, (Plate No. 1, Fig. 2) but the PAS reactivity of the epithelium was partially abolished with prior phenylhydrazine-PAS reaction. These reactions showed presence of neutral and acidic mucins in moderate concentration and absence of glycogen. Alcianophilia at AB (pH-1.0) was very weak but at AB (pH-2.5) it was moderate to intense. Acid hydrolysis partially abolished the alcianophilia showing presence of carboxy mucins as well as sulfated acidic mucins. With combined sequential AB (pH-1.0 and 2.5)-PAS staining technique the acinar epithelium revealed blue purple colour showing presence of acidic and neutral mucins. Reactions with CI technique confirmed the presence of acidic mucins and AF technique confirmed the presence of weak sulfated mucins especially in the active cells with apocrine secretion. The mild methylation abolished the alcianophilia while it was partially restored after saponification. Thus, the above histochemical reactions showed that the epithelial lining of the acini of normal prostate contained neutral mucins (moderate), acidic mucins (sialomucins) in weak concentration and traces of sulfated mucins.

ii) Secretion:

The secretion observed in the acinar lumen showed intense PAS reactivity which was not affected by diastase digestion (Plate No. 1, Fig. 5), but it was partially abolished with the prior phenylhydrazine treatment, indicating the presence of neutral and acidic

mucins and the absence of glycogen in the secretion. The secretion revealed intense alcianophilia with AB (pH-2.5) and with CI, while weak alcianophilia was observed with AB (pH-1.0) indicating the presence of carboxy mucins, the fact was further confirmed after acid hydrolysis, in which the alcianophilia was partially abolished. With combined AB (pH-2.5)-PAS staining the secretion exhibited intense bluish purple colour, whereas with AB (pH-1.0)-PAS staining the secretion revealed magenta to purple colour, indicating the presence of neutral mucins and acidic mucins containing carboxyl and sulfated groups. The presence of sulfated group was further confirmed by moderate purple colour with AF technique. The mild and active methylation abolished basophilia, while it was partially restored after saponification only at low temperature, indicating the presence of carboxyl groups. Thus, the various histochemical reactions indicated that the secretion of the normal prostatic acini contained neutral mucins (intense) sialomucins (moderate to less) and sulfomucins (traces).

iii) Ducts:

The duct system found in the normal prostate showed moderate to intense reaction with PAS technique. The PAS reactivity of the duct was resistant to diastase or α-digestion and labile to prior-phenylhydrazine PAS treatment. Similarly, with AB (pH-2.5) alcianophilia in this region was intense, while AB (pH-1.0) gave very weak alcianophilia. The CI technique showed moderate to intense reaction for both epithelium and secretion. In a combined sequential AB (pH-

1.0 and 2.5)-PAS technique the secretion and epithelium of the duct showed blue purple colour, whereas duct wall revealed magenta colour. AF staining technique revealed only faint purple colour in the above regions. Partial restoration of alcianophilia after saponification at 37°c indicated presence of both sialomucins and weak sulfated mucins. Thus the epithelial lining of duct and luminal secretion revealed presence of neutral mucins (moderate concentrated) carboxymucins (Moderate to weak) and the sulfated mucins (weak).

iv) Stroma:

The stroma with connective tissue found in the intra-acinar region revealed moderate PAS-reactivity which was resistant to diastase digestion while it was partially retained with prior phenylhydrazine-PAS reaction. The reactions with AB (pH-2.5) and AB (pH-1.0) showed moderate and weak alcianophilia respectively. Acid hydrolysis abolished the alcianophilia. In a combined sequential AB (pH-2.5)-PAS staining technique the stroma showed moderate bluish purple colour and with AB (pH-1.0)-PAS weak purple colour. The AF technique gave weak reaction, while CI showed moderate blue colour. The active methylation and mild methylation abolished the basophilia, while it was restored only after saponification at low temperature. Thus the series of above histochemical techniques revealed that the stroma and connective tissue contained high concentration of neutral mucins, less concentration of acidic mucins and traces of sulfomucins.

v) Muscle Fibres:

The smooth muscle fibres found in the stroma showed moderate PAS reactivity which was partially abolished after diastase digestion. Basophilia at AB (pH-2.5) was very weak and was negative for AB (pH-1.0). With the combined sequential AB (pH-1.0 and 2.5)-PAS staining technique the muscle fibres showed poor reactivity. The basophilia was lost after active and mild methylation while it was restored only after saponification at low temperature. CI technique showed faint blue colour and AF technique gave negative reaction. The above histochemical reactions showed that the muscle fibres showed the presence of neutral mucins, acidic mucins and also glycogen.

vi) Blood Vessels:

The wall of the blood vessel showed moderate to intense PAS reactivity which was not altered after diastase or α-amylase digestion but partially abolished with prior phenylhydrazine treatment, indicating the presence of neutral and acidic mucins. Weak alcianophilia was observed with AB (pH-1.0) and AB (pH-2.5), while the combined sequential staining with AB (pH-1.0 and 2.5)-PAS showed magenta colour in which traces of purple coloured material were distributed, showing the presence of neutral mucins and sialomucins. The acid hydrolysis abolished alcianophilia. The methylation, demethylation and also AF technique gave negative reactions indicating absence

of sulfomucins. Thus the above reactions indicated the presence of neutral mucins (moderate) and sialomucins (weak to traces) in the wall of the blood vessels.

(II) Benign Prostatic Hyperplasia with Prostatitis (BPH)

A) Gross Description of the Tissue:

Five irregular greyish-white, soft prostate tissue pieces, firm in consistency, were received after operation. The largest one measured about 3.5 x 2.5 x 1 cm^3 and the smallest one measured about 0.8 cm in diameter. The cut surfaces of the tissues were greyish-white showing lobules of varying sizes and have hemorrhagic areas at certain places.

B) Histopathological Observations:

(Plate No. 1, Fig. 1; Plate No. 2, Figs. 1,2 & 5)

The sections studied for histopathology consisted of only glandular prostate tissue. The acini were hyperplastic and were lined with tall columnar epithelium, some of them were dilated and cystic in nature and were lined with flattened epithelium, whereas some of the acini containing secretion showed formation of corpora amylacea. Some prostatic nodules showed an area of infarction adjacent to which the acini were lined with metaplastic squamous epithelium (Plate No. 2, Figs. 1 & 5). The stroma of the tissue containing connective tissue and smooth muscle fibres was normal but reduced

because of dilated acini. Cut sections also showed interacinar spaces filled with blood, mainly distributed in the region of acini with metaplastic squamous epithelium (Plate No. 2, Fig. 2).

C) Histochemical Observations:

The results of the various histochemical techniques are illustrated in the Plate No. 1 (Figs. 1 to 8) and Plate No. 2 (Figs. 1 to 8) and recorded in the histochemical Table No. 2.

i) Hyperplastic Acini:

The hyperplastic acini with tall columnar epithelium and dilated acini with flat epithelium showed more or less similar pattern of histochemical reactions. The epithelium revealed moderate to less intense PAS reactivity which could not be altered after diastase or α-amylase digestion (Plate No. 1, Fig. 3), but partially abolished with prior phenylhydrazine-PAS reaction. Thus it is observed that these reactions revealed the presence of neutral and acidic mucins in moderate concentrations and absence of glycogen. Alcianophilia at AB (pH-1.0) was very weak but at AB (pH-2.5) it was moderate to intense, especially in the acini lined with flat epithelium (Plate No. 1, Figs. 4 & 7). Acid hydrolysis partially abolished the alcianophilia showing presence of carboxy mucins as well as sulfated acidic mucins. With combined sequential AB (pH-1.0 & 2.5)-PAS staining technique the acinar epithelium revealed blue purple colour showing presence of acidic and neutral mucins. Reactions with CI technique

TABLE NO. 2

Histochemical observations on mucosubstances found at various cellular sites of the Benign prostatic hyperplasia with prostatitis.

Sr. No.	Histochemical Reactions	Normal appearing acini	Hyperplastic acini	Metaplastic squmous epithelium	Acinar secretion	Corpora amylacea	Stroma	Muscles	Ducts
1	PAS	+++P	++to+++P	++P	++++P	+++P	+++P	++P	++P
2	D-PAS	+++P	++P	+P	+++P	++P	++P	+P	+P
3	P-PAS	+P	+P	±P	+P	+P	+P	-	-
4	AB pH-1	+B	+B	±B	++B	++B	±B	-	+B
5	AB pH-2.5	++B	++B	+B	+++B	+++B	+B	+B	++B
6	AB pH-1.0-PAS	++PB	++PB	+P	+++PB	++PB	+P	+P	++PB
7	AB pH-2.5-PAS	++PB	++PB	+PB	+++BP	+++PB	+PB	+PB	++PB
8	CI	++B	++B	+B	+++B	++B	+B	+B	++B
9	AF	+P	+P	+P	+P	+P	±P		±P
10	M 37°c-AB pH-2.5	-	-	-	-	-	-	-	-
11	DM 37°c-AB pH-2.5	+B	+B	+B	++B	++B	±B	-	+B
12	M 60°c AB pH-2.5	-	-	-	-	-	-	-	-
13	DM 60°c AB pH-2.5	-	-	-	±B	±B	-	-	-
14	Acid hydrolysis AB pH-2.5	+B	+B	-	+B	+B	-	-	-

confirmed the presence of acid mucins and AF technique confirmed the presence of weak sulfated mucins (only in the active cells with apocrine secretion). The mild methylation abolished the alcianophilia while it was partially restored after saponification. Thus, the above histochemical reactions showed that the epithelial lining of the hyperplastic acini contained neutral mucins (moderate), acidic mucins that to sialomucins (weak) and traces of sulfated mucins.

ii) Secretion:

(Plate No. I, Figs, 5,6 & 8; Plate No. 2, Figs. 6,7 & 8)

The secretion of hyperplastic acini showed intense PAS reactivity which was not affected by diastase digestion while it was partially abolished with prior phenylhydrazine treatment, indicating the presence of neutral and acidic mucins and the absence of glycogen in the secretion. The secretion exhibited intense alcianophilia with AB (pH-2.5) and CI and weak alcianophilia with AB (pH-1.0) indicating the presence of carboxy mucins; this fact was further confirmed after acid hydrolysis, in which the alcianophilia was partially abolished. With combined AB (pH-2.5)-PAS staining the secretion showed moderate to intense bluish purple colour (Plate No. 1, Fig. 8), whereas with AB (pH-1.0)-PAS staining technique the secretion revealed magenta to purple colour, indicating the presence of neutral mucin, and acidic mucins containing carboxyl and sulfated groups. The presence of sulfated group was further confirmed by moderate purple colour with AF technique. The mild and active methylation abolished basophilia, while it was partially

restored after saponification only at low temperature, indicating the presence of carboxyl groups. Thus the series of above histochemical reactions indicated that the secretion of the hyperplastic acini contained neutral mucins (intense), sialomucins (moderate to less) and sulfomucins (traces).

iii) Corpora Amylacea:

The corpora amylacea occasionally found in some of the prostatic acinar lumen revealed moderate PAS reactivity which was resistant to diastase or α-amylase digestion and labile to the prior phenylhydrazine treatment - PAS reaction, indicating presence of neutral and acidic mucins. Alcianophilia with AB (pH-2.5) was also moderate, whereas with AB (pH-1.0) it was weak, indicating the presence of acidic mucins. Presence of acidic mucins was further characterized by the partial loss of alcianophilia after acid hydrolysis. With combined AB (pH-2.5)-PAS staining technique amylacea showed purple blue colour but it revealed only weak magenta colour with AB (pH-1.0)-PAS reaction, showing presence of neutral and acidic mucins. AF technique failed to stain amylacea indicating absence of sulfomucins. The presence of carboxy mucins could be confirmed, as mild methylation abolished the total basophilia which was restored after saponification. Thus the aforementioned results indicated the presence of only neutral mucins in moderate concentration and carboxymucins (sialic acid) in weak quantities in the corpora amylacea.

iv) Metaplastic Squamous Epithelium:
(Plate No. 2, Figs. 3,4,6 to 8)

In some of the regions of affected prostate gland the hyperplastic acini had lost their original structure and the columnar epithelial lining had changed to metaplastic squamous epithelium. This modification in the cellular structure led to the loss of functional structure of prostate. The metaplastic squamous epithelium when stained with PAS showed moderate to weak reactivity which could be partially blocked with α-amylase or diastase-digestion, such partial loss was also evident with prior phenylhydrazine treatment. These histochemical reactions indicated presence of neutral mucins, carboxy mucins and glycogen. With AB (pH-2.5) there was weak alcianophilia but some of the cells showed strong AB reaction, whereas alcianophilia at AB (pH-1.0) was negative towards most of the cells and moderate in the case of cells which were intensely stained with AB (pH-2.5). Further, in a combined sequential staining technique with AB (pH-1.0 and 2.5)-PAS (Plate No. 2, Fig. 4):

i) some cells showed only magenta colour,

ii) others appeared purple blue, while

iii) still others showed only blue colour,

indicating three different types of cells. This was further supported by acid hydrolysis technique, where alcianophilia was lost in the case of majority of the cells but retained in some of the cells. Similarly, active and mild methylation abolished the basophilia and

was partially restored in the above first two types of cells, whereas third type of cells failed to restore the basophilia. AF showed strong reaction only for the third type of cells. Thus, from the above histochemical reactions it could be concluded that metaplastic epithelial cells could be differentiated into three categories on the basis of presence of different mucosubstances:

a) cells elaborating neutral mucins only,

b) cells elaborating mixture of neutral and acidic mucins,

c) cells elaborating only sulfated acidic mucins.

v) Duct:

(Plate No. 2, Figs, 3 to 5)

The duct system found in the various regions of affected prostatic tissue showed degeneration in the wall and also in the epithelial lining leading to dilation of lumen. Such ducts when stained with PAS technique showed moderate to intense reaction in their epithelium and luminal secretion (brush border of the epithelium was strongly PAS positive). This PAS reactivity was resistant to diastase or α-amylase digestion and labile to prior phenylhydrazine-PAS treatment. Similarly with AB (pH – 2.5) alcianophilia, in this region, was intense; on the other hand, with AB (pH-1.0) alcianophilia was very weak while CI technique showed moderate to intense reaction for both epithelium and secretion. In a combined sequential AB (pH-1.0 and 2.5)-PAS technique the secretion and epithelium of the duct showed blue purple colour, whereas duct wall revealed magenta

colour. AF staining technique revealed only faint purple colour in the above regions. Partial restoration of alcianophilia after saponification at 37°c indicated the presence of both sialomucins and weak sulfated mucins. Thus the epithelial lining of duct and luminal secretion revealed presence of neutral mucins (intense to moderate concentration), carboxymucins (moderate to weak) and sulfated mucins (moderate).

vi) Stroma:

(Plate No. 1, Figs. 5,6,8; Plate No. 2, Figs. 3 & 4)

The connective tissue and stroma present in the intra-acinar region revealed moderate PAS reactivity which was resistant to diastase digestion, while it was partially retained with prior phenylhydrazine-PAS reaction. Reactions with AB(pH-2.5) and AB (pH-1.0) showed mnoderate and weak alcianophilia respectively. Loss of alcianophilia was found after acid hydrolysis. In a combined sequential AB (pH-2.5)-PAS staining technique the stroma showed moderate bluish-purple colour and with AB (pH-1.0)-PAS weak purple colour was noted. The AF technique showed no staining while CI showed moderate blue colour. The basophilia was totally abolished after active methylation and mild methylation, while it was restored only after saponification at low temperature. Thus the series of above histochemical techniques revealed that the stroma and connective tissue contained high concentration of neutral mucins, less concentration of acidic mucins and traces of sulfomucins.

vii) Muscle Fibres:

The smooth muscle fibres showed moderate PAS reactivity which was partially abolished after diastase digestion. Basophilia at AB (pH-2.5) was very weak and was negative for AB (pH-1.0). With the combined sequential AB (pH-1.0 and 2.5)-PAS staining technique the muscle fibres showed poor reactivity. Mild and active methylation abolished the basophilia while it was restored only after saponification at low temperature. CI technique showed moderate blue colour and AF technique gave negative reaction. The results of above discussed histochemical reactions showed that the muscle fibres showed the presence of neutral mucins, acidic mucins and also glycogen as in the case of muscle fibres of normal prostate.

(III) Chronic Prostatitis

A) Gross Description of the Tissue Received:

Two pieces of prostate specimen were received. The large piece measured about 5.5 x 3.5 x 0.3 cm^3. The cut surface showed grey white appearance with small area of necrosis.

B) Histopathological Observations:

The microscopic observations of this prostatic tissue showed the presence of acini and ducts. Acini showed focal hyperplasia (Plate No. 3, Fig. 1) and were lined with cuboidal epithelium. The

acini and ducts were destroyed at places (Plate No. 3, Fig. 2) and were replaced by exudate of plasma cells, lymphocytes, few microphages and eosinophils. Large area of remaining tissue showed proliferating fibrous stroma with smooth muscle fibres (Plate No. 3, Fig. 2). The cells with hypertrophid nuclei were observed in the hypertrophid acini. Another important feature of the above region was destruction and obliteration of blood vessels (Plate No. 4, Figs. 2 & 3). Such condition of the prostate tissue in pathology is diagnosed as chronic prostatitis.

C) Histochemical Observations:

The results of the histochemical reactions are illustrated in Plate No. 3 (Figs. 1 to 8) and Plate No. 4 (Figs. 1 to 7) and are recorded in the histochemical Table No. 3.

i) Hyperplastic Acini:

The hyperplastic acini show weak to moderate PAS reactivity (Plate No. 3, Fig. 3) in the basement membrane and the epithelium. The PAS reactivity was found to be resistant to diastase or α-amylase digestion and sensitive to prior phenylhydrazine treatment. Weak to moderate alcianophilia was observed weith AB (pH- 2.5) in the basement membrane (Plate No. 3, Fig. 5), whereas negative alcianophilia in the epithelium. Staining reactivity with AB (pH- 1.0) was negative in both the above acinar structures. The weak alcianophilia

TABLE NO. 3

Histochemical observations on the mucosubstances at various cellular sites of the Chronic Prostatitis.

Sr. No.	Histochemical Reactions	Normal appearing acini	Hyperplastic acini	Destroyed acini	Acinar secretion	Stroma	Muscles	Ducts	Blood vessels
1	PAS	++P	++P	+P	+++P	++P	++P	+P	++to+++P
2	D-PAS	++P	++P	+P	+++P	+P	+P	+P	++to+++P
3	P-PAS	+P	+	–	++P	+P	+P	–	++P
4	AB pH-1	-	-	-	±B	+B	+B	-	+B
5	AB pH-2.5	+B	+B	±B	++B	++B	+B	+B	+B
6	AB pH-1.0-PAS	+P	+P	±P	+++P	+PB	+PB	+P	++P
7	AB pH-2.5-PAS	++P	++P+B	±P	+++P	++PB	+PB	+P	+++PB
8	CI	+B	+B	±B	++B	++B	+B	+B	+B
9	AF	–	–	–	–	+	–	–	+
10	M 37°c-AB pH-2.5	-	-	-	-	-	-	-	-
11	DM 37°c-AB pH-2.5	±B	±B	±B	++B	++B	+B	+B	+B
12	M 60°c AB pH-2.5	-	-	-	-	-	-	-	-
13	DM 60°c AB pH-2.5	-	-	-	±B	±B	-	-	-
14	Acid hydrolysis AB pH-2.5	-	-	-	±B	-	-	-	±B

CAPTIONS TO FIGURES

Plate No. 1, Figs. 1 to 8

Fig. 1: T.S. of BPH prostate showing Hyperplastic acini (HA), Secretion (SE) and Stroma (S). H.E. Staining x 200.

Fig. 2: T.S. of normal prostate stained with PAS. Note intense PAS reaction at epithelial lining and basement membrane of normal acini (NO) and Duct (D), and moderate PAS reaction at stroma. x 200.

Fig. 3: T.S. of BPH prostate stained with PAS. Note moderate to intense PAS reaction at epithelium and basement membrane of hyperplastic acini (HA) and ducts (D). x 200.

Fig. 4: Hyperplastic acini of BPH stained with AB pH 2.5-PAS. Note moderate bluish-purple colour at epithelium and basement membrane (HA) and Ducts (D). Stroma (S) showing moderate magenta-purple colour. x 200.

Fig. 5: BPH prostate stained with PAS showing intense staining at Secretion (SE) and brush-border and moderate PAS reactivity at epithelium, Stroma (S) and Duct (D) x 300.

Fig. 6: BPH prostate stained with AB pH-2.5 showing moderate to intense alcianophilia at Secretion (SE), weak alcnaiophilia at Degenerated epithelium (DE) and Stroma (S). x 300.

Fig. 7: BPH prostate stained with AB pH-2.5. Note moderate alcianophilia at hyperplastic acini (HA) with strong AB +ve reaction at apocrine region. Stroma (S) and Muscles (M) are weakly alcianophilic x 200.

Fig. 8: Hyperplastic acini of BPH stained with AB pH 2.5-PAS, showing intense magenta-blue and moderate blue colour at Secretion (SE). Note moderate magenta-blue colour at Duct (D) and Stroma (S). x 300.

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CAPTIONS TO FIGURES

Plate No. 2, Figs. 1 to 8

Fig. 1: T.S. of BPH prostate showing reduced acini with metaplastic epithelium (MS) increased Stroma (S), Smooth muscles (M) and Duct (D). H-E staining, x 300.

Fig. 2: T.S. of BPH prostate showing increased amount of Stroma (S) few groups of Metalpastic acini (MS) and Hemorrhagic areas near the metaplastic area (B). H-E staining, x 200.

Fig. 3: T.S. of BPH prostate stained with PAS, showing moderate to weak PAS reactivity at Metaplastic acini (MS) and Stroma (S) and weak reactivity at (D). x 300.

Fig. 4: A similar region as above (Fig. 3), stained with AB pH-2.5-PAS Note intense blue colour (AB) (some cells), while other cells showing moderate purple colour (P) and weak purple-magenta at remaining metaplastic epithelium (MS), weak bluish purple colour at Duct (D) and Stroma (S). x 300.

Fig. 5: T.S. of BPH prostate showing degeneration of hyperplastic acini with inner active epithelium (AE) and outer layer of metaplastic epithelium (MS). Section also shows area of infarction (I) and small Ducts (D). H-E staining, x 200.

Fig. 6: T.S. of BPH prostate as above (Fig. 5) stained with PAS. Note moderate PAS reactivity at the Secretion (SE), intense PAS +ve material at brush border (arrow) of active epithelium, also note weak to moderate PAS reactivity at metaplastic epithelium (MS) and Hyperplastic acini. x 300.

Fig. 7: T.S. of BPH prostate, same as above, stained with AB pH 2.5. Note intense alcianophilia at the Secretion (SE) and brush-border of active epithelium (arrow), weak alcianophilia at Metaplastic epithelium (MS). x 300.

Fig. 8: T.S. of BPH prostate same as above (Figs. 6 & 7), stained with AB pH-1 PAS showing moderate bluish-magenta at Secretion (SE) in Lumen (RL) of degenerating acini. Note weak magenta colour at Metaplastic epithelium (MS) and increased Stroma (S) with RBCS, x 100.

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CAPTIONS TO FIGURES

Plate No. 3, Figs. 1 to 8

Fig. 1: Prostate of chronic protatitis disorder showing hyperplastic acini (HA) with intraluminal Papillae (P). H-E staining. x 200.

Fig. 2: Prostate of chronic protatitis disorder showing Degenerating acini (DA) cells with hypertrophy and hypertrophid nuclei (arrow), increased Stroma (S) and Muscles (M). H-E staining. x 200.

Fig. 3: Prostate of chronic protatitis disorder stained with PAS. Note moderate staining at Basement membrane (BM), weak to moderate staining at epithelium of Hyperplastic acini (HA) and Stroma (S). 200.

Fig. 4: Prostate same as above (Fig. 3), stained with PAS, showing dilated and Hyperplastic acini (HA) with one of the acini having intensely stained Secretion (SE) and moderate staining at Stroma (S). x 100.

Fig. 5: Prostate same as above (Figs. 3 & 4) stained with AB pH-2.5. Note negative alcianophilia at epithelium of hyperplastic acini (HA) and intra-acinar Papillae (P) and weak alcianophilia at basement membrane and stroma (S). x 200.

Fig 6 Prostate same as above stained with AB pH 1-PAS. Note moderate pink colour at Basement membrane (BM), Stroma (S) and intense staining at Secretion (SE). Also note faint blue staining at apocrine region (arrow) of Hyperplastic acini (HA). x 200.

Fig. 7: Prostate of chronic prostatitis disorder showing stromal region stained with PAS, showing Stroma (S) and Smooth muscles (M) only. Note moderate PAS activity in these regions. x 200.

Fig. 8: Prostate same as above (Fig. 7), stained with AB pH 2.5. Note moderate alcianophilia at Stroma (S). x 200.

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CAPTIONS TO FIGURES

Plate No. 4, Figs. 1 to 7

Fig. 1: Prostate of chronic prostatitis disorder showing increased Stroma (S) stained with AB pH 2.5-PAS. x 200.

Fig. 2: Prostate of chronic prostatitis disorder showing Stroma (S) and blood vessels. Note normal blood vessels in the middle of degenerating blood vessels (RBV) and Degenerating Duct (D). x 200.

Fig. 3: Prostate same as above (Fig. 2) stained with PAS. Note moderate PAS reactivity at the wall of normal bloos vessel (BV), moderate to intense staining at wall of degenerating blood vessels (RBV) and weak staining at Stroma (S) and Duct (D). x 200.

Fig. 4: Prostate same as above (Figs. 2 and 3) showing moderate alcianophilia at wall and intense alcianophilia at endothelium of normal blood vessels (BV), Duct (D) and Stroma (S). x 200.

Fig. 5: Prostate same as above (Fig. 4), stained with AB pH 2.5-PAS. Note moderate to intense AB & PAS reactions at different layers of wall of Blood vessels (BV), moderate bluish-purple staining in the Stroma (S) and only weak magenta at the Duct epithelium (D). x 200.

Fig. 6: Prostate of chronic prostatitis stained with AB pH 1-PAS showing only moderate magenta colour at wall and intense magenta colour at endothelium of obliterating Blood vessel (BV), also note moderate purple colour at Stroma (S). x 100.

Fig. 7: Prostate same as above (Figs. 5 and 6), stained with AB Ph-2.5-PAS showing growth of stroma over the wall of normal blood vessel obliterating it (arrow). Note moderate to intense PAS reaction and weak to trace blue colour in the wall of blood vessel, degenerating blood vessels having only weak magenta colour, bluish-purple staining at Stroma (S) and Duct (D). x 200.

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CAPTIONS TO FIGURES

Plate No. 5, Figs. 1 to 8

Fig. 1: Prostate of acute nonspecific prostatitis with abscesses, showing destroyed Necrotic acini (NA), Stroma (S) and smooth muscles (M). H-E staining. x 200.

Fig. 2: Prostate same as above (Fig. 1) showing destroyed Duct (D) and acini, inflammatory cells infiltrating the Stroma (IC) and Blood cells ( ) in the destroyed acinar region. H-E staining. x 200.

Fig. 3: Prostate of acute nonspecific prostatitis with abscesses, showing well formed microabscesses (A), Inflammatory cells (IC) infiltrating the stroma and increased smooth muscles (M). H-E staining. x 200.

Fig. 4: Prostate same as above (Fig. 3), stained with PAS technique. Note poor PAS activity at microabscesses (A), Duct wall (D) and Stroma (S). x 200.

Fig. 5: Prostate same as above (Figs. 3 & 4) stained with AB pH 2.5. Note negative alcianophilia at Necrotic acini (NA), and microabscesses (A), weak at Duct (D) and moderate alcianophilia at Stroma (S). x 200.

Fig. 6: Prostate same as above (Figs. 4 & 5) stained with AB pH 1-PAS. Note only por magenta colour at necrotic acini (NA) and secretion, Stroma (S) and muscle fibres (S). x 100.

Fig. 7: Prostate same as above (Figs. 5 &6) stained with AB pH 2.5-PAS. Note moderate staining at secretion, weak staining at Necrotic acini (NA) and Duct (D) and purple-magenta colour at Stroma (S). x 200.

Fig. 8: Prostate of acute nonspecific prostatitis with abscesses, stained with AB pH - 2.5-PAS, showing moderate magenta colour at Ascess (A), muscle fibres (M) and purple-magenta at stroma (S). Duct wall showing weak purple magenta colour (D). x 150.

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CAPTIONS TO FIGURES

Plate No. 6, Figs. 1 to 9

Fig. 1: Prostate of Adenocarcinoma showing hyperplastic acini with intra-luminar Papillae (P) which cut across and appear as island of epithelial cell lying free in the acinar lumen (arrow), corpora and amylacea (CA) and Duct (D). H-E staining. x 200.

Fig. 2: Prostate same as above showing dilated acini with corpora amylacea in the lumen (CA) and secretion. Other region shows hyperplastic acini HA, Duct (D) and fibrous Stroma (S). H-E staining. x 100.

Fig. 3: Prostate of Adenocarcinoma showing hyperplastic acini near the tumor region stained with PAS. Note weak PAS reactivity in Hyperplastic acini (HA), Duct (D), Stroma (S) and Muscles (M). x 200.

Fig. 4: Prostate of Adenocarcinoma , showing dilated hyperplastic acini, stained with PAS. Note weak staining at the epithelial lining (HA), moderate PAS reactivity at Secretion (SE) and corpora Amylacea (CA). x 200.

Fig. 5: Prostate same as above, stained with AB pH 1-PAS. Note weak magenta colour at epithelium of hyperplastic Acini (CA), intra-acinar Papillae (P), Stroma (S) and Duct (D), also note moderate bluish-purple staining at Corpora amylaces (CA). x 100.

Fig. 6: Prostate same as Fig. No. 4, stained with AB pH 2.5-PAS, showing moderate magenta colour at the Seretion (SE), moderate bluish-purple colour at brush border (arrow) of hyperplastic acini(HA) and poor purple colour at Stroma (S). x 200.

Fig. 7: Prostate of Adenocarcinoma showing malignant (Tumor) region with glandular tumor cells with hyperchromatic chromatin (GT) and thin stroma. H-E staining. x 200.

Fig. 8: Prostate same as above (Fig. 7) stained with PAS. Note weak PAS staining at the glandular tumor cells (GT), and at stromal region. x 200.

Fig. 9: Prostate same as above (Fig. 7 & 8), stained with AB pH 2.5-PAS, showing weak magenta colour at Glandular tumor cells (GT) at some region weak purple colour is also seen. x 200.

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CAPTIONS TO FIGURES

Plate No. 7, Figs. 1 to 8

Fig. 1: Prostate of anaplastic carcinoma, showing only tumor region. The tumor cells with hyperchromatic nuclei (TC), inflammatory cells (arrow) and smooth muscle fibre strands. H-E staining x 200.

Fig. 2: Prostate same as above (Fig. 1) but magnified to show tumor cells with increased amount of nuclear material, hyperchromatic in nature (TC) and mitotic figure (arrow). H-E staining. x 600.

Fig. 3: Prostate of anaplastic carcinoma showing infiltration of tumor cells forming strands and streaks (TC) in between the smooth muscles (M). The stroma also shows infiltration of inflammatory cells (arrow) H-E staining. x 300.

Fig. 4: Prostate of anaplastic carcinoma disorder stained with PAS. Note moderate to weak staining at Tumor cells (TC), weak PAS reactivity at Duct wall (D) and Muscles (M). x 200.

Fig. 5: Prostate of anaplastic carcinoma disorder stained with PAS. Note weak PAS staining at the Tumor region (TC) as well as at the affected acini which are reduced in size (RA) because of growth of tumor cells. The acini lack the basement membrane. x 200.

Fig. 6: Prostate same as above (Fig. 5) stained with AB pH-2.5. Note negative alcianophilia at Reduced acini (RA) and weak alcianophilia at the Muscle fibres) (M). x 200.

Fig. 7: Prostate of anaplastic carcinoma disorder stained with AB pH 1-PAS showing moderate magenta colour at Tumor cells (TC) weak magenta at inflammatory cells (arrow) and muscle fibres. Stroma at some places shows purple-blue colour. x 200.

Fig. 8: Prostate of anaplastic carcinoma disorder stained with AB pH 2.5-PAS, showing moderate magenta colour at Tumor cells (TC), weak PAS reactivity at epithelium of affected acini (RA) and weak bluish-purple colour at the base of acinar cells (arrow) probably muscle fibres and stroma. x 200.

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CAPTIONS TO FIGURES

Plate No. 8, Figs. 1 to 8

Fig. 1: Prostate of anaplastic carcinoma showing ivaginated prostatic urethral Transitional epithelium (TE) which surrounds tumor. The section also shows Stroma (S) at the base of transitional epithelium. H-E staining. x 150.

Fig. 2: Prostate of anaplastic carcinoma stained with AB pH 2.5-PAS showing moderate PAS staining at apocrine region (arrow) of epithelium of affected Acini (A), weak PAS reaction at Muscle fibres (M) and Stroma (S) showing weak bluish-purple colour. x 200.

Fig. 3: Prostate of anoplastic carcinoma showing ivaginated transitional epithelium stained with PAS. Note weak and uniform PAS staining at basal region of Transitional epithelium (TE), Stroma (S) and epithelium of affected Acini (A). x 150.

Fig. 4: Prostate of anaplastic carcinoma stained with PAS, showing moderate PAS staining at Tumor cells (TC) and weak to moderate PAS staining at wall of Blood vessels (BV) and Stroma (S), x 200.

Fig. 5: Prostate same as above (Fig. 1) stained with colloidal iron showing weak to moderate blue colour at majority of Transitional epithelial cells (TE), but strong basophilia at few cells (arrow). Stroma (S) shows moderate blue colour. x 150.

Fig. 6: Prostate same as above (Fig. 4), stained with AB pH 2.5-PAS, showing moderate magenta colour at Tumor cells (TC) and weak magenta at inflammatory cells (arrow). Note moderate PAS reactivity in the wall of Blood vessel (BV) with traces of bluish-purple staining. x 200.

Fig. 7: Prostate same as Figs. 1 and 5, stained with AB pH 2.5-PAS. Note weak PAS reaction at Transitional Epithelium (EP), but some cells showing moderate bluish-purple colour (arrow). Also note weak magenta staining at affected Acini (A), Duct (D) and Stroma (S). x 150.

Fig. 8: Prostate same as Figs. 4 & 6 stained with AB pH 1-PAS showing moderate magenta colour at wall of Blood vessel (BV) and reduced PAS reaction at wall of blood vessel , where growth of tumor cells is seen (arrow). x 300.

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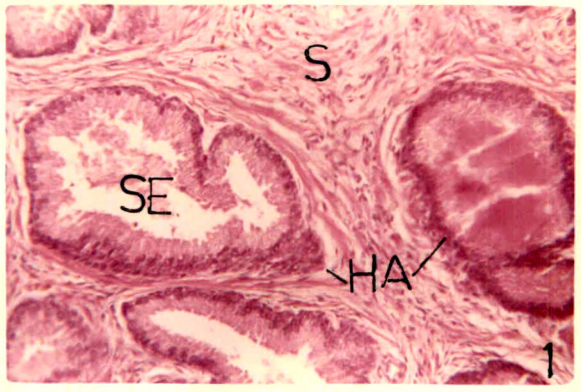
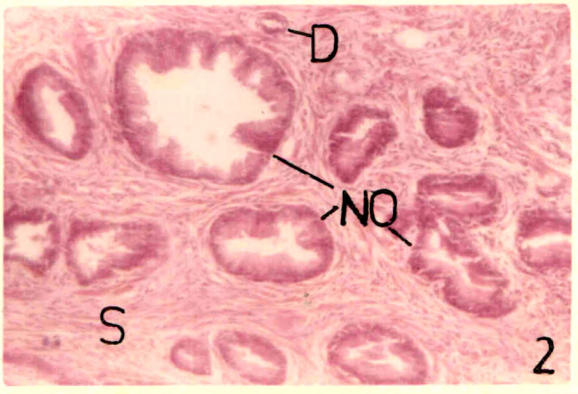
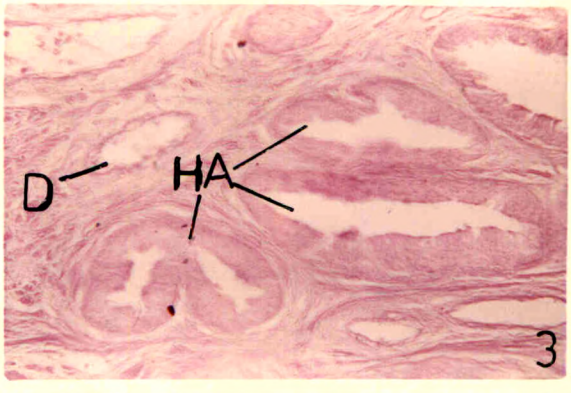
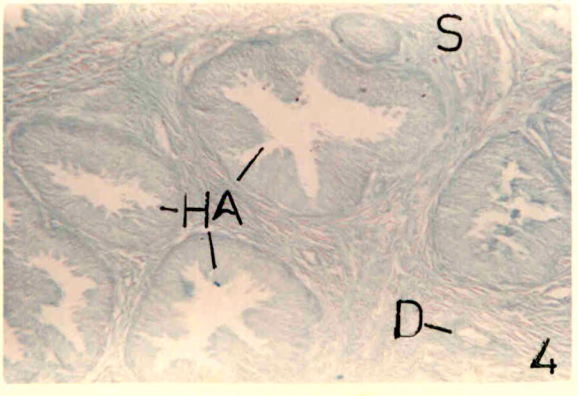
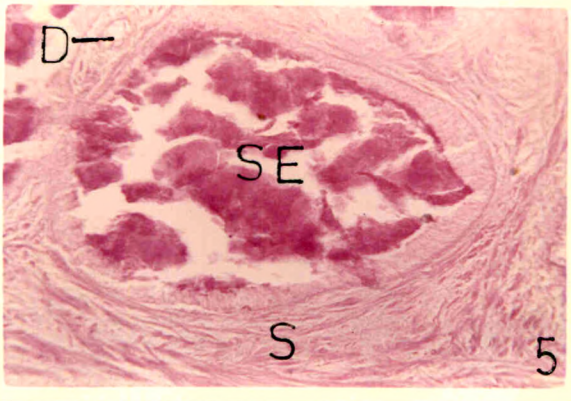
PLATE NO-1

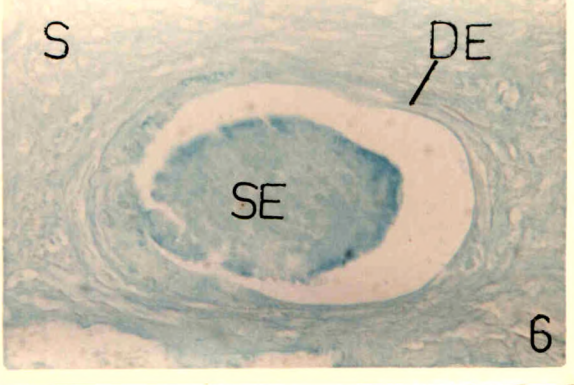


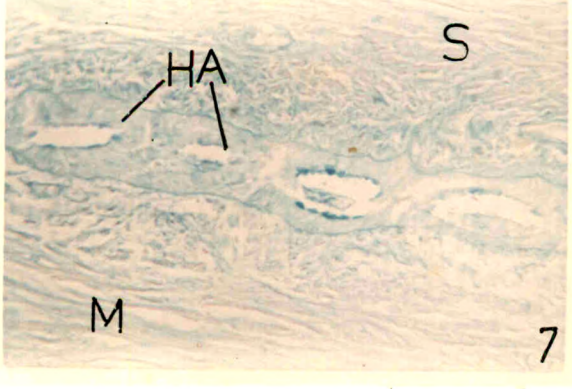


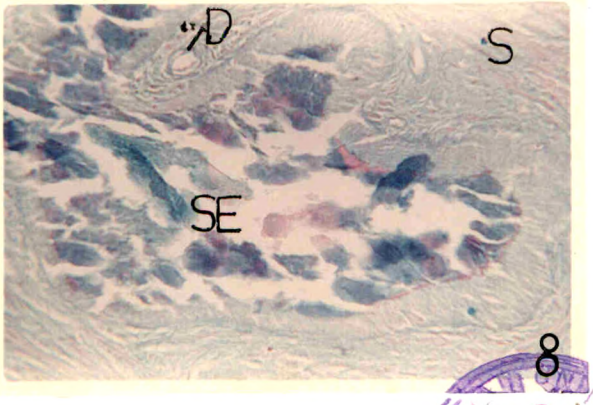












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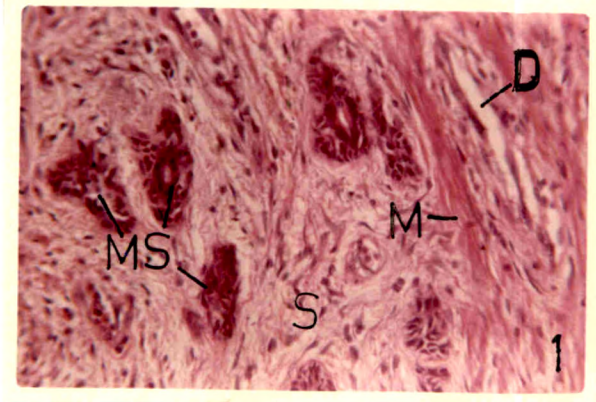
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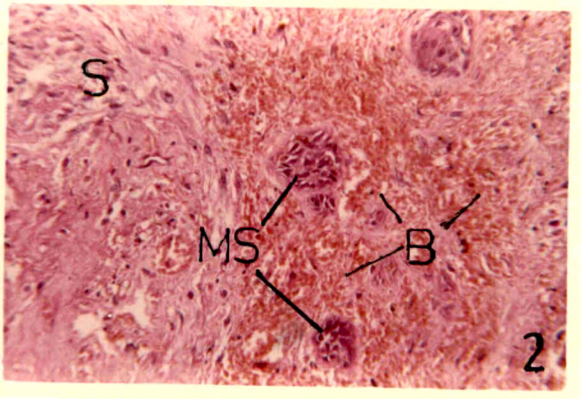
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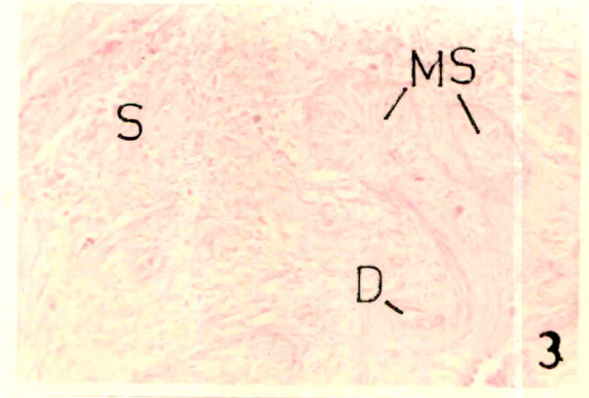
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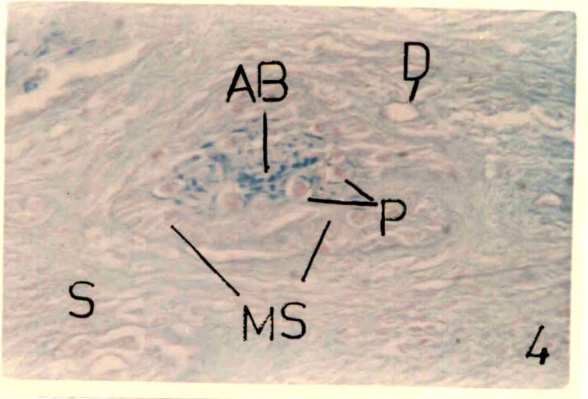
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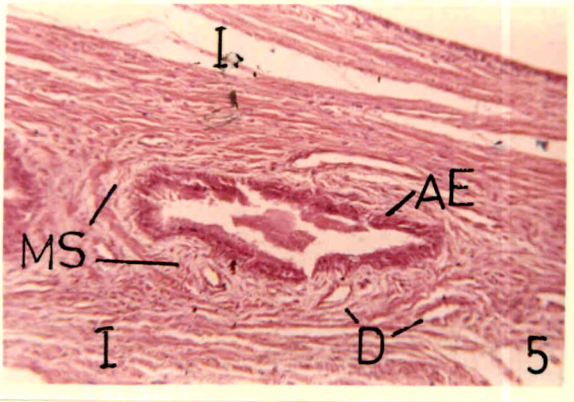
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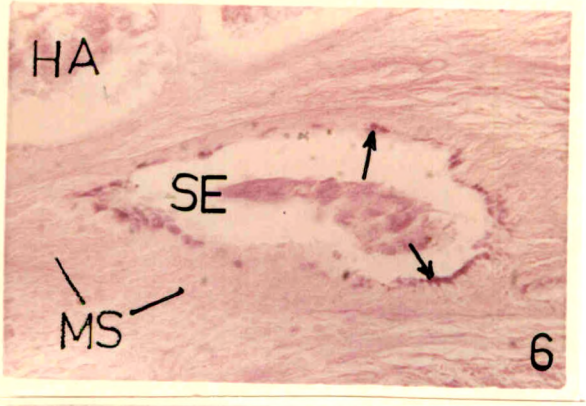
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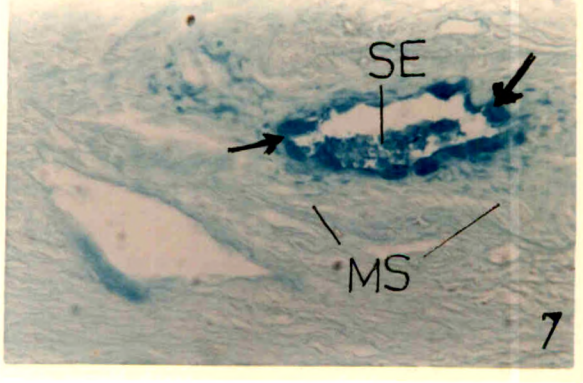
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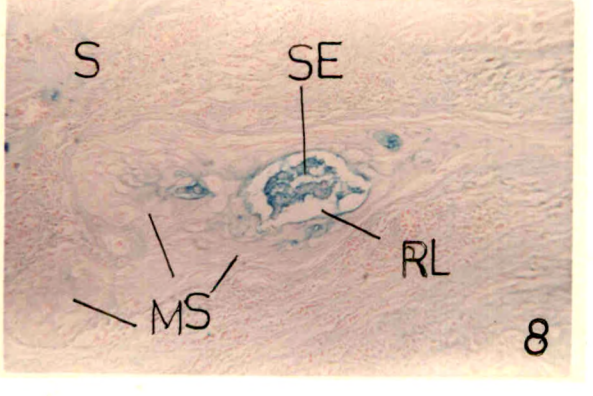
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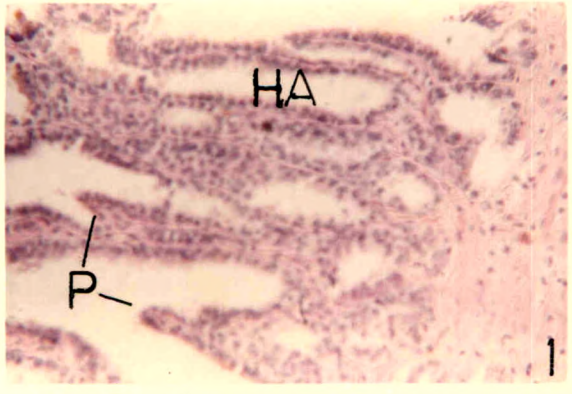

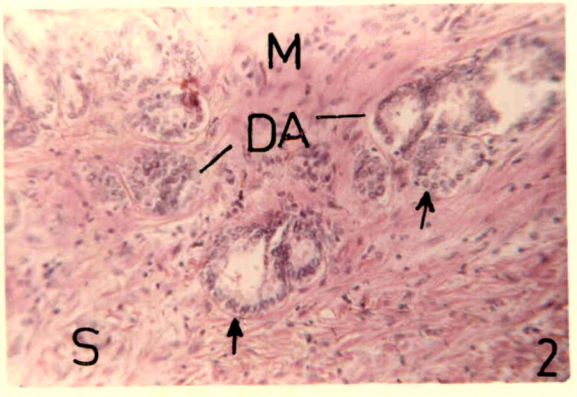

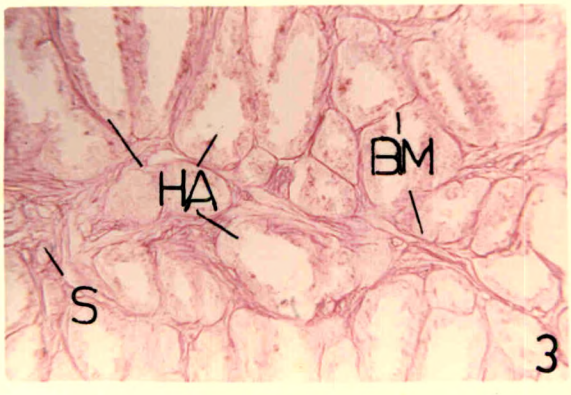

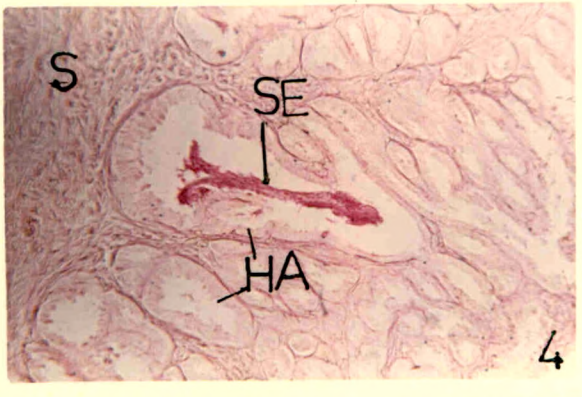

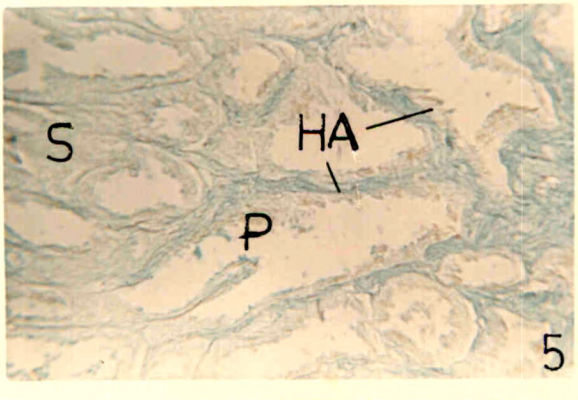

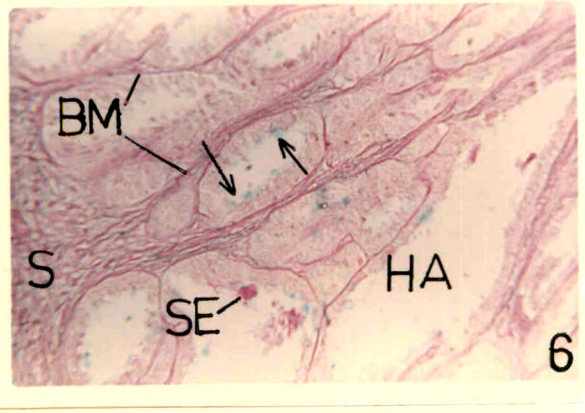

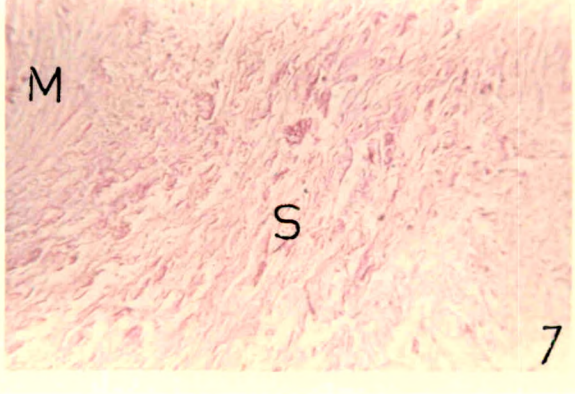

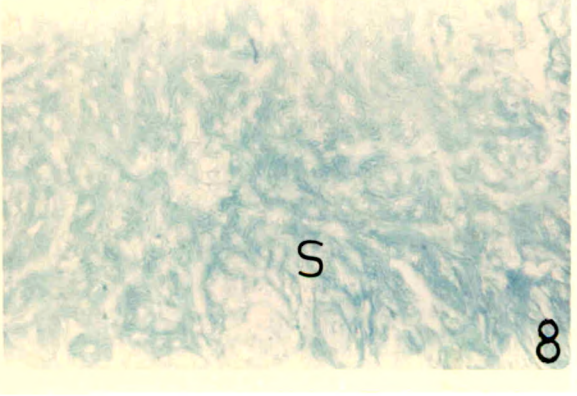

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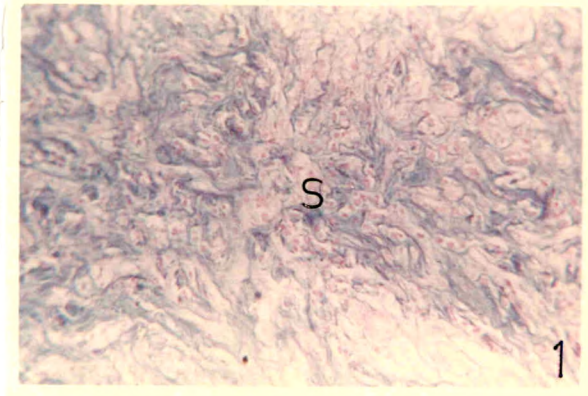

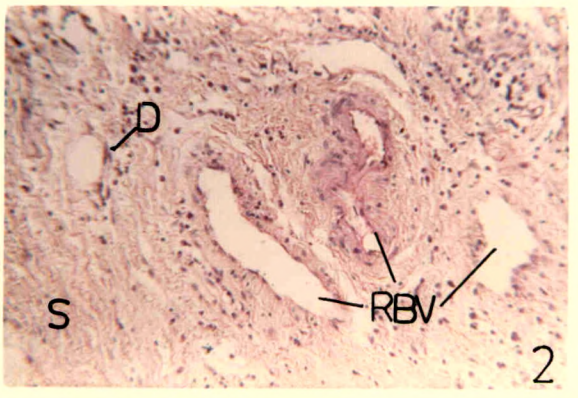

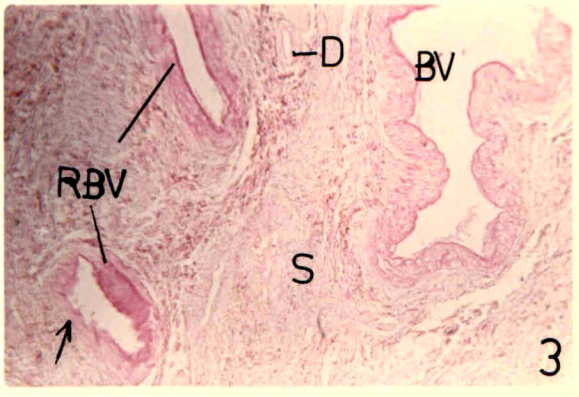

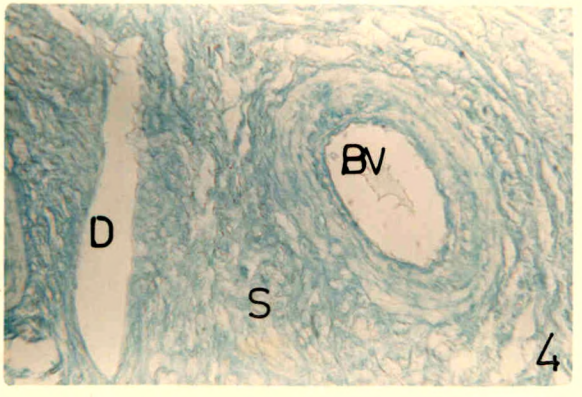

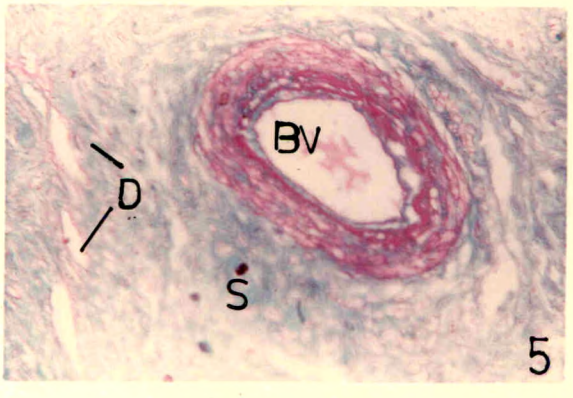

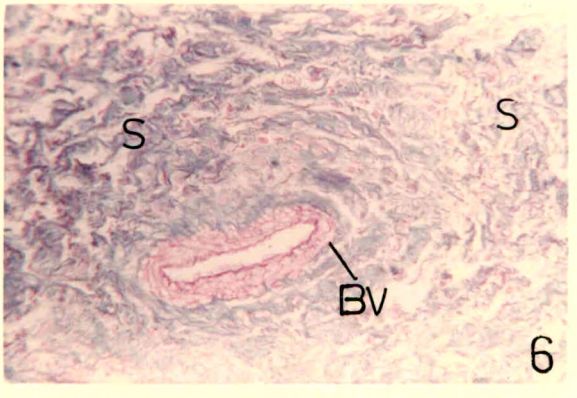

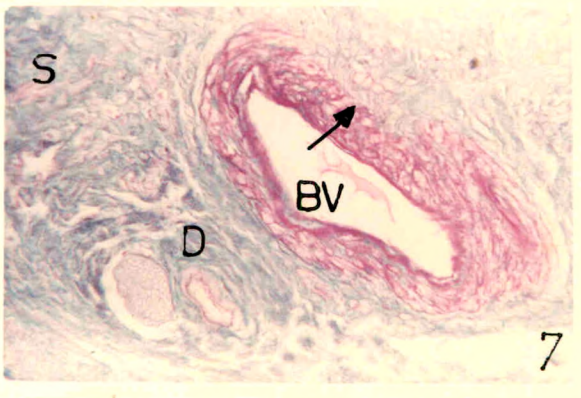

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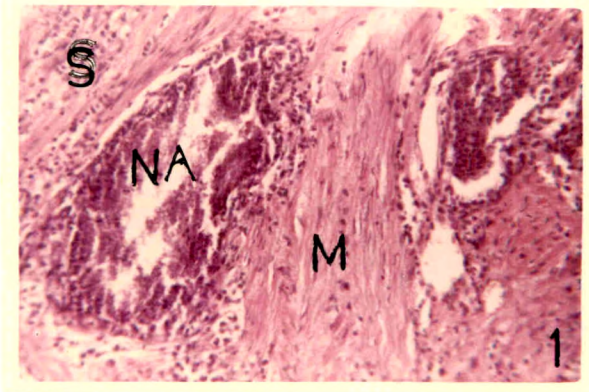

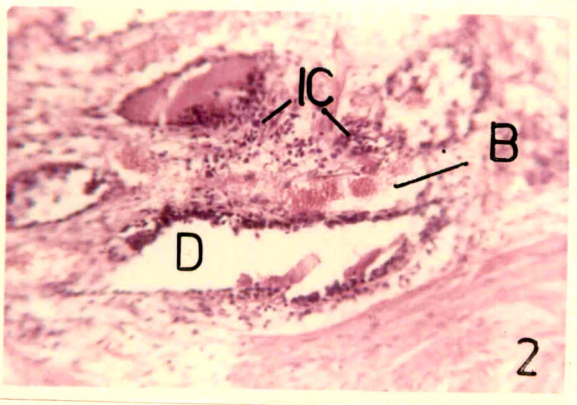

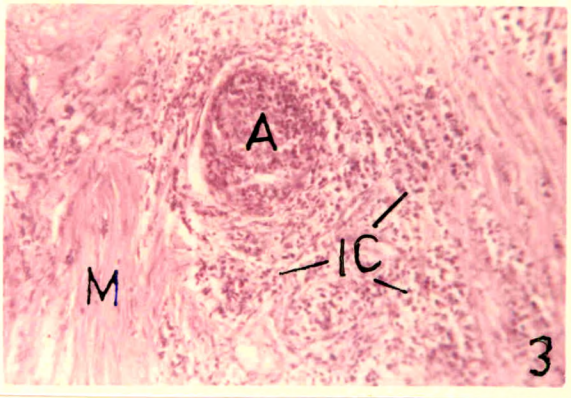

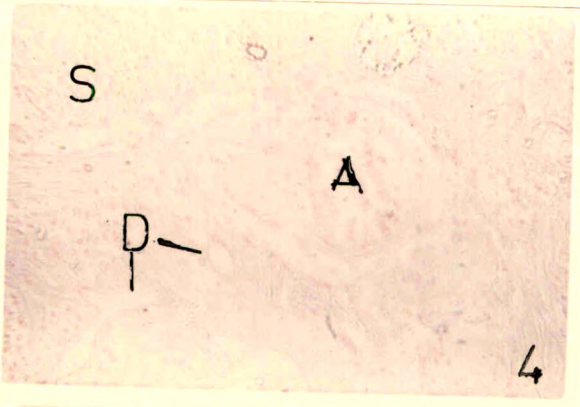

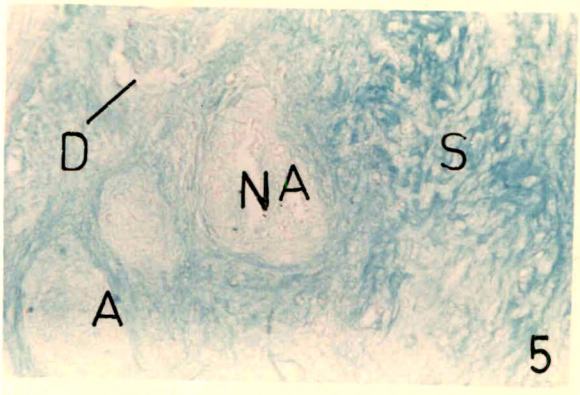

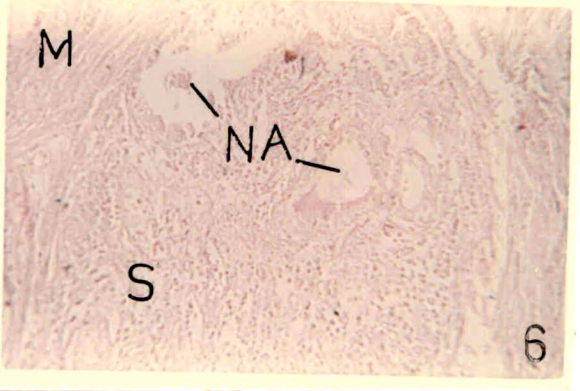

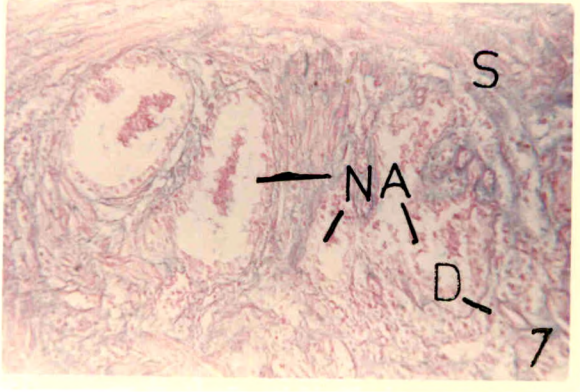

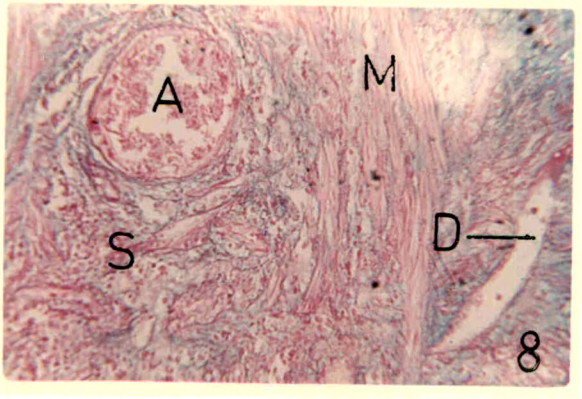

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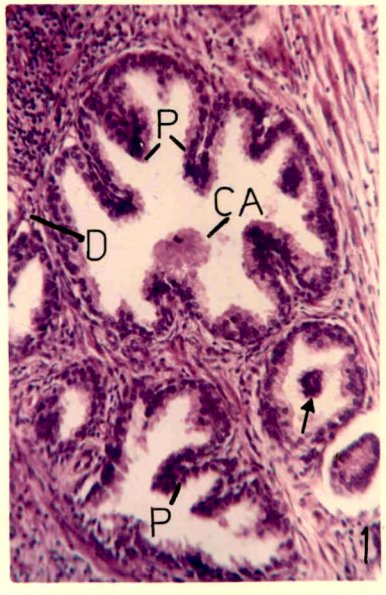
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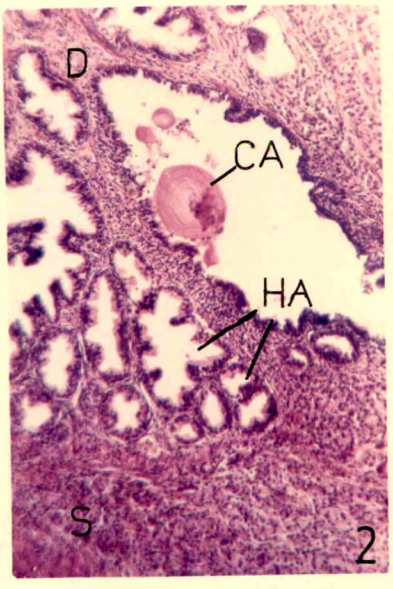
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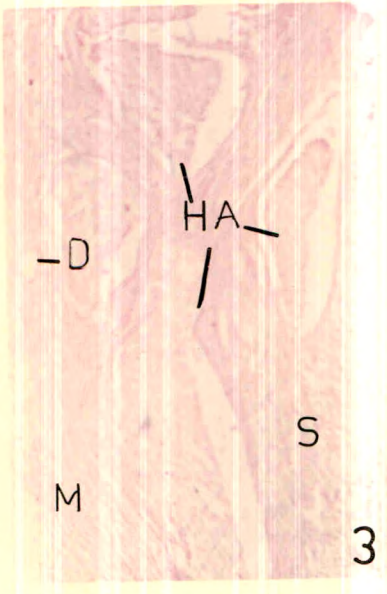
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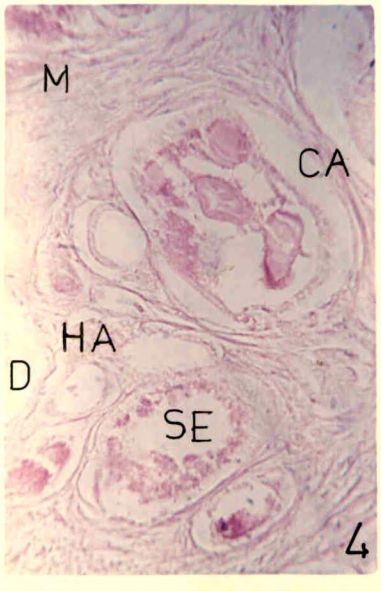
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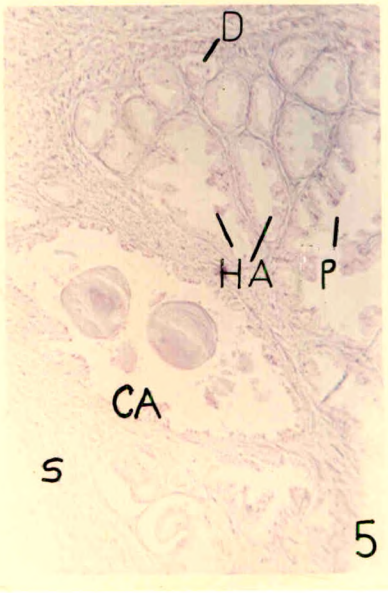
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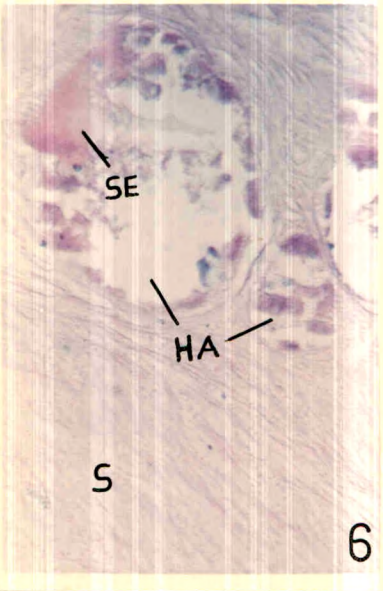
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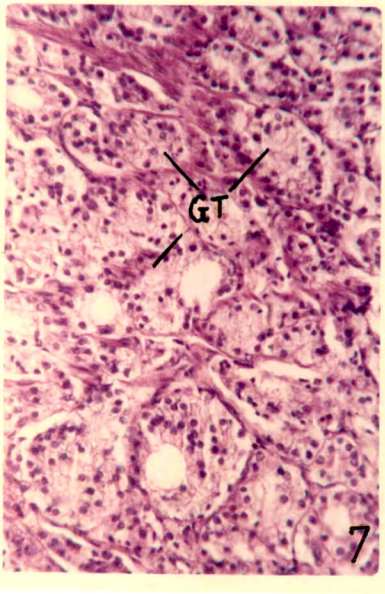
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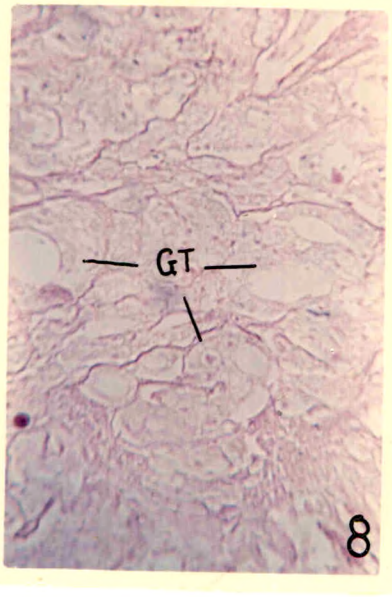
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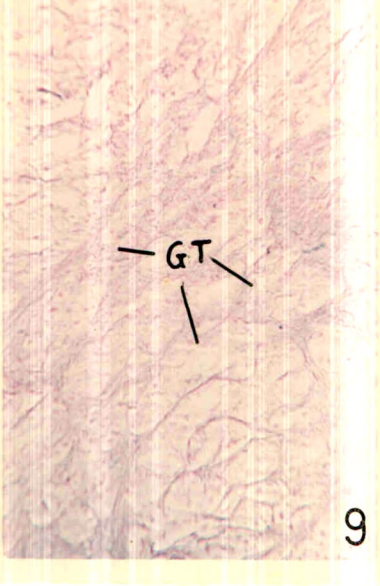
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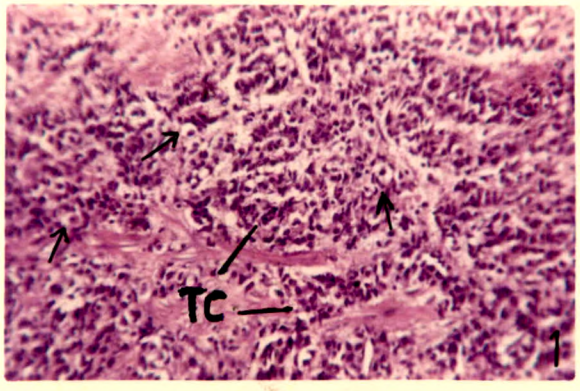
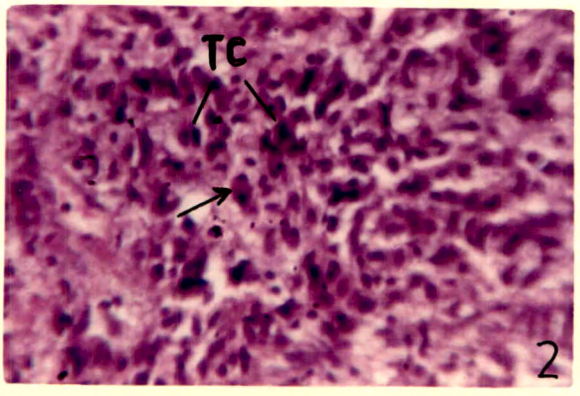
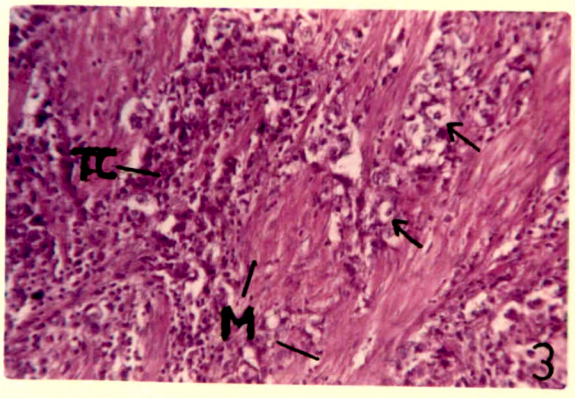
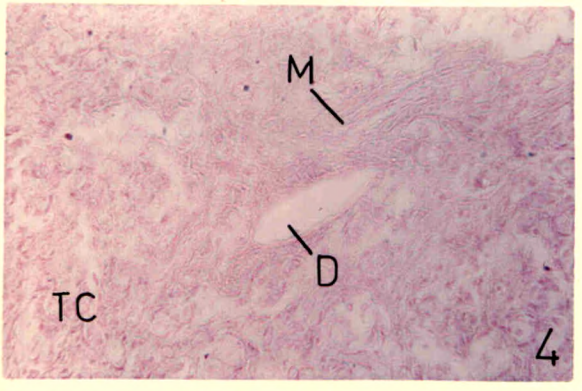
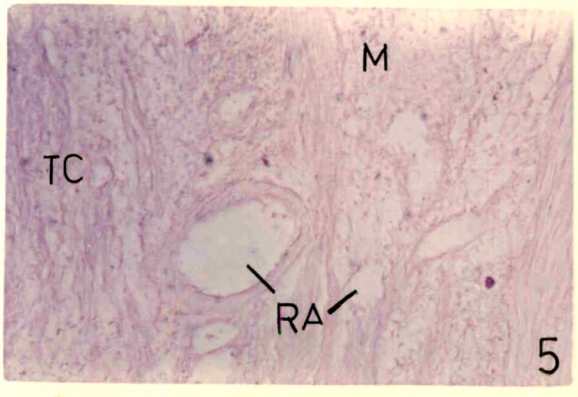
PLATE NO-7

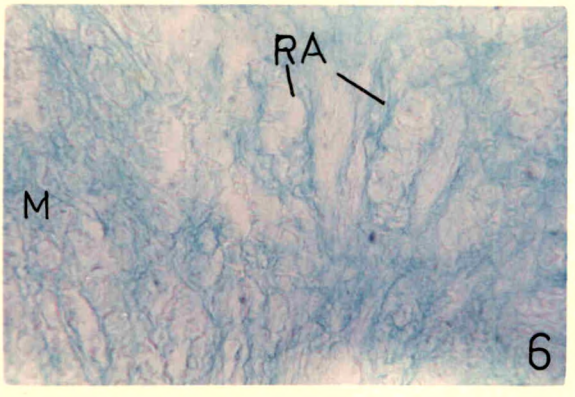


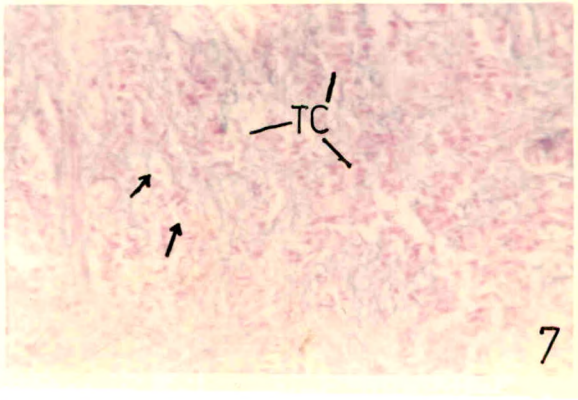












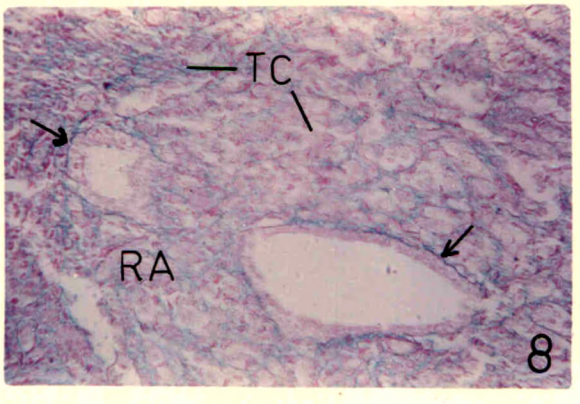
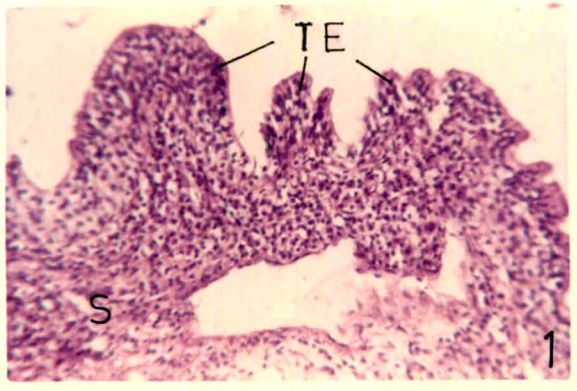
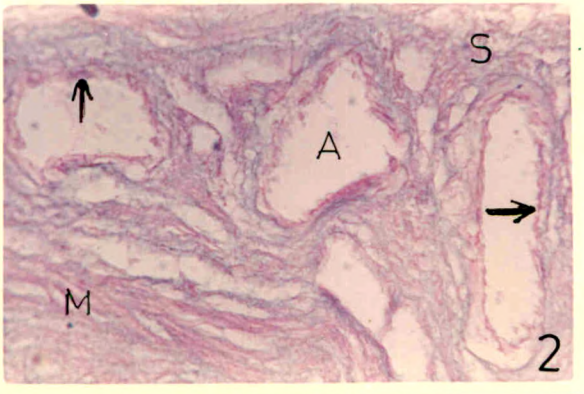
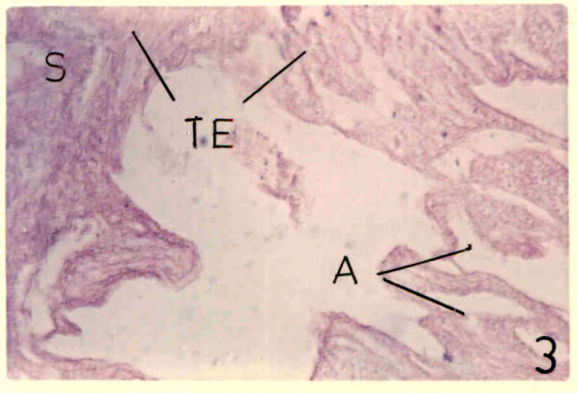
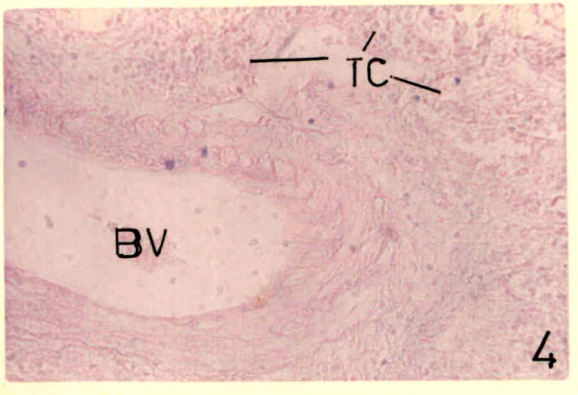


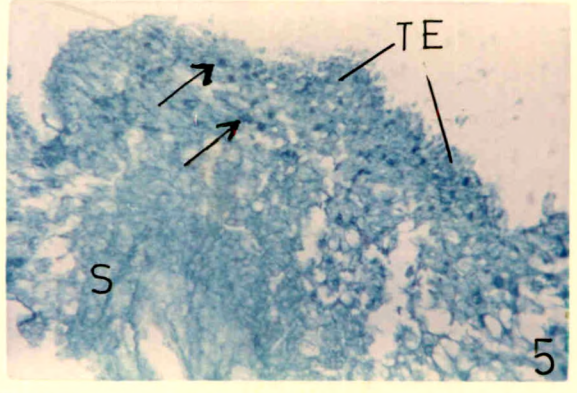
PLATE NO-8

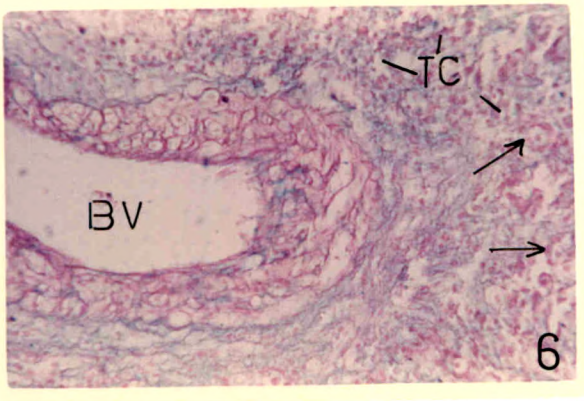


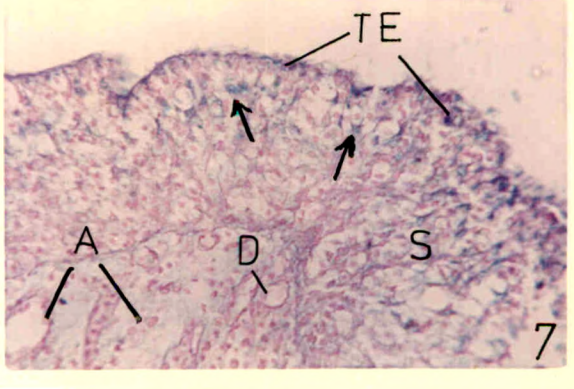


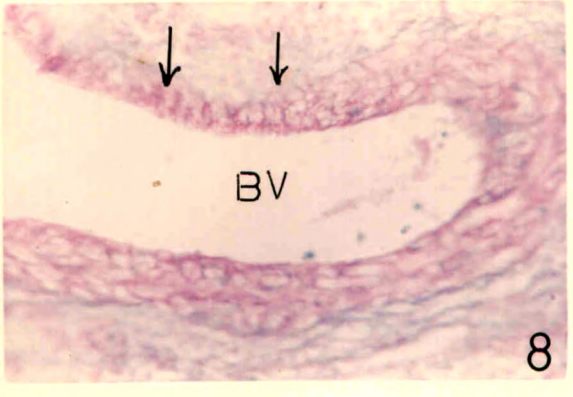












was totally abolished after acid hydrolysis in the basement membrane indicating the presence of weak acidic mucosubstances or sialomucins. With the AB (pH-2.5)-PAS staining procedure (Plate No. 3 Fig. 6) the basement membrane showed bluish pink colour indicating presence of both neutral and sialomucins, whereas epithelium reacted only with PAS indicating only presence of neutral mucins. The AB (pH-1.0)-PAS staining technique at both the structures of hyperplastic acini showed magenta colour indicating the presence of neutral mucins, however, some of the hyperplastic acinar cell brush border was only AB positive indicating presence of weak sulfated mucins (Plate No. 3, Fig. 6). Mild methylation showed loss of alcianophilia at the basement membrane and after saponification the basophilia was restored indicating the presence of carboxyl groups. Similarly, mild methylation and saponification showed no reaction in the epithelium. AF technique showed no reaction at basement membrane and the epithelium indicating absence of sulfomucins, but some of the acinar cell brush border showed weak purple colour. Thus the hyperplastic acinar epithelium and the basement membrane showed presence of neutral mucins (moderate), carboxy mucins (traces, but some hyperplastic cells showed presence of sulfated mucins in the apocrine region).

ii) Secretion:

The secretion found in the lumen of some of the normal

looking and hyperplastic acini exhibited strong PAS reactivity (Plate No. 3, Fig. 4) which was resistant to diastase or α-amylase digestion and phenylhydrazine-PAS reaction, indicating the presence of neutral and acidic mucins and absence of glycogen. The secretion did not exhibit any alcianophilia at AB (pH-1.0), whereas with AB (pH-2.5) and CI it revealed weak blue colour which was lost after acid hydrolysis confirming the presence of sialic acid containing carboxyl mucins. In a combined AB (pH-1.0)-PAS staining procedure the secretion revealed magenta colour only while with AB (pH 2.5)-PAS the secretion showed purple blue colour. This reaction supported the presence of neutral as well as acidic mucins. With the AF staining technique the secretion showed no reaction, indicating absence of sulfate group mucins. Alcianophilia after mild methylation was totally abolished which was restored after saponification. Basophilia after active methylation and de-methylation did not exhibit any reaction. Thus the results of above histochemical techniques indicated the presence of neutral mucins (high concentration), acidic mucins (traces) in the secretion of normal looking and hyperplastic acini.

iii) Ducts:

Histological structure of prostate showed the presence of large and small ducts distributed among the acini and stroma. Both the types of ducts consist of outer wall and inner flat epithelium.

The epithelial layer revealed moderate PAS reactivity which

was retained after diastase digestion but totally lost after the prior treatment of phenylhydrazine. Reaction with AB (pH – 2.5) showed weak to moderate alcianophilia (Plate No. 4, Fig. 4) and AB (pH-1.0) revealed no reaction. Results obtained with AB (pH-1.0 and 2.5)-PAS indicated more or less purple magenta colour (Plate No. 4, Fig. 5), thus showing the presence of neutral and acidic mucins. Alcianophilia after acid-hydrolysis was totally lost in the duct epithelium. Similarly, in the reaction with AF the epithelium did not exhibit any colour. Basophilia after mild methylation was totally lost and restored after demethylation. Alcianophilia after active methylation and demethylation did not exhibit any reaction. Thus the results obtained indicated the presence of neutral mucins (moderate) and small amount of acidic mucins.

iv) Stroma:

The stroma of the hyperplastic part of prostate was moderately stained with PAS (Plate No. 3, Fig. 7). This PAS reactivity remained unchanged after diastase digestion or α-amylase digestion, whereas it was partially lost with prior phenylhydrazine treatment. The reactions with AB (pH-1.0) and (pH – 2.5) and CI showed moderate reactivity (Plate No. 3, Fig. 8 and Plate No. 4, Fig. 4) which was partially retained after acid hydrolysis. In a combined sequential staining with AB (pH-1.0 and 2.5)-PAS technique the stroma revealed blue purple colour (Plate No. 4, Figs, 1, 6 and 7) showing the presence of mixture of neutral, acidic and sulfated mucins. Loss

of alcianophilia was observed after mild and active methylation confirming the presence of both acidic and sulfated mucins in the affected stroma. Similarly, AF technique showed light purple colour in this region. Thus the aforementioned histochemical reactions indicated the presence of neutral mucins (weak to moderate), acidic mucins (weak to moderate) and sulfated mucins (traces).

v) Muscle Fibres:

The prostate tissue stroma is also supported by smooth muscle fibres. The histochemical reactions were carried out to detect the mucopolysaccharide composition. The various reactions showed the presence of PAS positive substane in the muscle fibres (Neutral mucins) along with glycogen and sialomucins in traces (Plate No. 4, Figs. 1 and 7).

H) Blood Vessels:

The histochemical reactions employed for the detection of mucopolysaccharides for this prostatic tissue also gave differential staining for the blood vessels (normal as well as obliterated). The wall of the blood vessel showed moderate to intense PAS reactivity which was not altered after diastase or α-amylase digestion but partially abolished with prior phenylhydrazine treatment, indicating presence of neutral and acidic mucines (Plate No. 4, Fig. 3). Moderate alcianophilia was observed in the well developed blood vessels with the AB (pH-2.5) staining (Plate No. 4, Fig. 4), whereas weak alcianophilia was noted

in the obliterating blood vessel. Alcianophilia at AB (pH-1.0) was weak showing presence of acidic mucins containing carboxyl group and sulfated group. The wall of the blood vessels with combined sequential AB (pH-1.0 and 2.5)-PAS technique showed differential staining at different layers (Plate No. 4, Figs. 5 & 7). The innermost layer showed purple blue colour while the remaining wall showed magenta colour showing weak alcianophilic reactivity at certain places, thus indicating the presence of neutral mucins, sialomucins and sulfated mucins in the wall of the blood vessel. On the other hand, the wall of the obliterating blood vessel showed only moderate magenta colour (Plate No. 4, Fig. 6). The presence of acidic mucin in the normal blood vessel was further confirmed by the loss of alcianophilia after acid hydrolysis and presence of sulfomucins was inferred by weak purple staining with AF. Similarly, with methylation and demethylation technique (at low and high temperature) the presence of acidic mucins (sialomucins) and sulfated mucins was confirmed. Thus the above histochemical reactions showed presence of neutral mucins (moderate to intense), sialomucins (weak), sulfomucins (traces) in the normal blood vessel, whereas only neutral mucins (moderate) in the blood vessel showing obliteration because of increased stroma and smooth muscle fibres.

(IV) Acute Nonspecific Prostatitis with Abscesses

A) Gross Description of the Tissue:

Three pieces of prostate received were irregular in shape,

yellowish in colour and soft to firm in nature measuring about 3 x 2 x 1 $cm.^3$ This prostatic tissue was clinically diagnosed as prostatic abscess.

B) Histopathological Observations:

The sections stained with HE-technique showed abnormal histological structure when compared with the histology of normal prostate tissue. This prostatic tissue was surrounded by fibromuscular tissue. In some of the regions the prostatic acini were normal and lined with single columnar epithelial layer, while in the rest of the area the epithelium of acini was destorted and was necrotic. The lumen of these acini was filld with necrotic substance. The stroma showed diffused, dense and acute inflammtory cell infiltration, predominantly composed of polymorphs, forming many microabscesses (Plate No. 5, Figs. 2 & 3). At certain places stroma also showed blood-spaces (Plate No. 5, Fig. 2). Such a pathological condition of prostate is termed as acute nonspecific prostatitis with abscesses.

C) Histochemical Observations:

The results obtained with various histochemical techniques are illustrated in Plate No. 5 (Figs. 1 to 8) and are recorded in histochemical Table No. 4.

TABLE No. 4

Histochemical observations on the mucosubstances found at various cellular sites of the Acute Non-specific Prostatitis with Abscesses.

Sr. No.	Histochemical Reactions	Normal appearing acini	Necrotic Acini	Secretion	Necrotic secretion	Inflammatory cells	Stroma	Muscles	Ducts
1	PAS	+P	+P	++P	++P	+P	++P	++P	+P
2	D-PAS	+P	+P	++P	++P	+P	+P	+P	+P
3	P-PAS	-	-	-	-	-	-	-	-
4	AB pH-1	-	-	-	-	±B(some)	+B	-	-
5	AB pH-2.5	-	-	±B	±B	+B	+B	±B	±B
6	AB pH-1.0-PAS	+P	+P	++P	++P	+P	++PB	++P	+P
7	AB pH-2.5-PAS	+P	+P	++P	++P	++PB	++PB	++PB	+PB
8	CI	-	-	±B	±B	+B	+B	±B	±B
9	AF	-	-	-	-	±P(some)	-	-	-
10	M 37°c-AB pH-2.5	-	-	-	-	-	-	-	-
11	DM 37°c-AB pH-2.5	-	-	-	-	±	+	-	-
12	M 60°c AB pH-2.5	-	-	-	-	-	-	-	-
13	DM 60°c AB pH-2.5	-	-	-	-	-	-	-	-
14	Acid hydrolysis AB pH-2.5	-	-	-	-	-	-	-	-

i) Necrotic Acini and Microabscesses:

The necrotic acini exhibited weak to moderate PAS reactivity which was resistant to diastase or α-amylase digestion (Plate No. 5, Fig. 3). PAS reactivity was almost abolished by prior phenylhydrazine treatment. These reactions revealed presence of neutral mucins and absence of glycogen. The necrotic acini and microabscesses reacted negatively at AB (pH-1.0) and AB (pH-2.5), and CI showed faint blue colour in this region (Plate No. 5, Fig. 5). In the combined sequential staining with AB (pH-1.0 & 2.5)-PAS, the necrotic acini and microabscesses revealed only pink colour. These regions showed negative reactions with aldehyde fuschin. Mild methylation and active methylation showed no reaction. The above histochemical data clearly indicated presence of only neutral mucins (weak to moderate) in necrotic acini, and microabscesses.

ii) Necrotic Secretion:

The necrotic secretion exhibited weak PAS reactivity when compared with the moderate PAS reactivity in the secretion of the normal acini [Plate No. 5, Figs. 5 & 6). This weak PAS reactivity was resistant to diastase or α-amylase digestion and sensitive to prior phenylhydrazine treatment. Reaction with AB (pH-1.0) and AB (pH-2.5) showed absence of alcianophilia. Reactions with a combined AB (pH-1.0 & 2.5)-PAS technique showed only weak to moderate magenta colour in the secretion (Plate No. 5, Figs. 6 & 7). Thus

the secretion in the necrotic acini showed presence of neutral mucins (less concentration). When compared with secretion of normal prostate, the secretion of necrotic acini showed loss of mucopolysaccharides, this loss was more evident in the case of acidic mucins than in the neutral mucins.

iii) Inflammatory Cells:

The inflammatory cells observed in the affected stroma showed moderate to weak PAS staining reactivity which was partially resistant to diastase or α-amylase digestion and lost with prior phenylhydrazine reaction, indicating the presence of neutral mucosubstances and glycogen. These cells revealed weak alcianophilia with both AB (pH-2.5) and AB (pH-1.0). The alcianophilia was lost after acid hydrolysis. In the combined sequential AB (pH-1.0 and 2.5)-PAS staining procedure the cells displayed only magenta colour but some of the cells showed purple colour with AB (pH-2.5). These cells showed faint purple colour with AF technique. The mild methylation abolished the alcianophilia and was partially restored after saponification. All the above histochemical reactions indicated that inflammatory cells showed the presence of neutral mucins, glycogen and acidic mucins, while some of the inflammatory cells also showed traces of sulfomucins.

iv) Stroma:

The stroma and connective tissue showed moderate to weak staining

reaction when treated with PAS-technique (Plate No. 5, Fig. 4). This PAS reaction was partially blocked with prior diastase or α-amylase digestion and with prior phenylhydrazine treatment. These reactions indicated presence of neutral mucins and glycogen. The stromal cells and connective tissue fibres were stained moderately with AB (pH-2.5) (Plate No. 5, Fig. 5) and weakly with AB (pH-1.0). The stroma displayed weak alcianophilia after acid hydrolysis. With combined AB (pH 1.0 and 2.5)-PAS staining reactions the stroma showed bluish purple colour (Plate No. 5, Figs. 7 & 8). These reactions indicated presence of acidic mucins as well as sulfomucins. AF technique showed weakly positive reaction in the stromal cells. Mild and active methylation abolished alcianophilia and it was partially restored after saponification in the mild methylation technique. Thus the stroma and connective tissue revealed presence of neutral mucins, glycogen, acidic mucins (sialomucins) and traces of sulfomucins.

v) Ducts:

The prostatic tissue studied in this pathological disorder showed very few ducts distributed in the acinar region and stroma. The duct system responded weakly with the PAS staining technique, this weak reactivity was retained after diastase or α-amylase digestion (Plate No. 5, Fig. 4) and abolished with prior phenylhydrazine-PAS technique. Weak alcianophilic reaction was observed at AB (pH-2.5) but no reaction with AB (pH-1.0). In a combined sequential AB (pH-1.0 and 2.5)-PAS technique magenta and purple blue colour (Plate No.

5, Fig. 7 & 8) was observed respectively. With the remaining histochemical techniques employed, the duct system showed regative reaction, thus showing presence of neutral mucins and traces of acidic mucins and absence of sulfated mucins.

vi) Muscle Fibres:

The muscle fibres surrounding the prostatic tissue and those present in the stroma showed more or less similar histochemical reactions. These reactions revealed presence of only PAS positive substance in the muscle fibres (Plate No. 5, Fig. 8). The PAS-positive substance was shown to contain neutral mucins and glycogen.

(V) Adenocarcinoma of Prostate

A) Gross Description of the Tissue:

Two lobes of prostatic glands were selected. The larger lobe measured about 3 x 2 x 1 cm^3 and smallere 2 x 2 x 1 $cm.^3$ The external surface of both the lobes was nodular and cut surface showed greyish white colour with small cystic area.

B) Histopathological Observations:

(Plate No. 6, Figs. 1 & 2)

The sections studied for histopathology of this tissue mainly consisted of tumor, reducing the normal glandular appearance of prostate. The tumor composed of glandular cells arranged in adenoid cysts and streaks (Plate No. 6, Fig. 7). The tumor cells showed

round vesicular nuclei. The cytoplasm of these cells appeared to be eosinophilic as well as clear. The occasional focus of normal appearing prostatic acini was also seen (Plate No. 6, Figs. 1 and 2). The stroma and connective tissue became thick due to the increase in tumor glandular tissue. The stroma showed mild chronic inflammatory cell infiltration (Plate No. 6, Figs. 1 & 2). This condition in pathology, is diagnosed as adenocarcinoma of prostate. In this condition some of the acini showed corpora amylacea.

C) Histochemical Observations:

The results obtained with the histochemical techniques are illustrated in Plate No. 6 (Figs. 1 to 9) and are recorded in the histochemical Table No. 5.

i) Affected Acini:

The affected acini occasionally seen in this tissue, when stained with PAS, revealed weak reactivity (Plate No. 6, Figs. 3 & 4). This PAS reactivity remained unaltered after diastase digestion or α-amylase digestion but abolished with prior phenylhydrazine-PAS technique. With AB (pH-1.0) and AB (pH-2.5) the normal acini showed negative reactions. With combined sequential AB (pH-1.0 & 2.5)-PAS staining technique the acini revealed weak magenta colour only. With CI, AF, active methylation and demethylation, mild methylation and demethylation techniques the normal acini showed negative reactions. Thus the affected acini showed the presence of neutral mucins only.

TABLE NO. 5

Histochemical observations on the mucosubstances found at various cellular sites of the Adenocarcinoma of the Prostate.

Sr. No.	Histochemical Reactions	Normal appearing acini	Secretion	Amylacea	Glandular tumor cells	Stroma Normal tissue	Stroma tumor region	Muscles	Duct
1	PAS	+P	++P	++P	+P	++P	+P	+P	+P
2	D-PAS	+P	++P	++P	+P	++P	+P	±P	+P
3	P-PAS	-	±P	+P	-	±P	±P	-	+P
4	AB pH-1	-	±	+B	-	±B	-	-	-
5	AB pH-2.5	±B	+	+B	±B	+B	±B	±B	±B
6	AB pH-1.0-PAS	+P	+PB	+PB	+P	+P	+P	+P	+P
7	AB pH-2.5-PAS	+PB	++PB ++P	++PB	+P	+PB	+PB	+PB	+PB
8	CI	±B	+B	+B	-	+B	±B	±B	±B
9	AF	-	-	±P	-	-	-	-	-
10	M 37°c-AB pH-2.5	-	-	-	-	-	-	-	-
11	DM 37°c-AB pH-2.5	±B	±B	±B	-	±B	-	-	-
12	N 60°c AB pH-2.5	-	-	-	-	-	-	-	-
13	DM 60°c AB pH-2.5	-	-	-	-	-	-	-	-
14	Acid hydrolysis AB pH-2.5	-	±B	±B	-	-	-	-	-

ii) Secretion:

The secretion of affected acini showed weak to moderate pink colour with PAS technique (Plate No. 6, Figs. 3 & 4). The PAS reactivity was not affected by diastase digestion, while it was partially abolished with prior phenylhydrazine treatment. These reactions clearly indicated presence of PAS-positive material and absence of the glycogen. The secretion exhibited weak alcianophilia with AB (pH-2.5) and no alcianophilia at AB (pH-1.0). Acid hydrolysis technique showed partial loss of blue colour. With combined AB (pH-1.0 and 2.5)-PAS procedure the secretion showed moderate magenta colour with AB (pH-1.0) and purple blue colour with AB (pH-2.5) (Plate No. 6, Fig. 6). With CI and AF techniques the secretion showed weak reactions. The mild methylation abolished the alcianophilia, while it was partially restored after saponification. Thus the above histochemical reactions showed that the secretion of affected acini contained neutral mucins in moderate concentration and carboxyl mucins (sialomucins) in traces.

iii) Corpora Amylacea:

(Plate No. 6, Figs, 2,4 & 5)

The amylacea which was observed in some of the prostatic acini showed moderate PAS reactivity which was resistant to diastase digestion and sensitive to the prior phenylhydrazine treatment indicating presence of neutral mucins. Alcianophilia with AB (pH-2.5) was

moderate, whereas with AB (pH-1.0) it was weak. Acid hydrolysis procedure showed partial loss of alcianophilia. With a combined AB (pH-1.0 & 2.5)-PAS staining procedure amylacea showed purple blue colour (Plate No. 6, Fig. 5).

The AF technique showed light purple colouration. The mild methylation abolished the total basophilia which was partially restored after saponification. Thus amylacea showed the presence of neutral mucins in moderate concentration and carboxy mucins (sialomucins) in small amount and sulfomucins (traces).

iv) Tumor Cells:
(Plate No. 6, Figs. 7 to 9)

The tumor cells which represent glandular appearance, exhibited weak PAS reactivity which was resistant to diastase digestion PAS reactivity (Plate No. 6, Fig. 8) and lost with prior phenylhydrazine treatment. Alcianophilia with AB (pH-1.0) and AB (pH-2.5) was negative. With sequential combined AB (pH-1.0 and 2.5)-PAS technique these cells showed only weak magenta colour. These results indicated that the tumor cells showed presence of only neutral mucins and absence of AB reactive mucins. Other methods such as acid hydrolysis, AF, methylation at different temperatures and saponification showed negative results. Thus the glandular tumor cells showed the presence of only neutral mucins in weak concentration.

v) Ducts

Ducts of large and small size present in the normal and tumor region showed weak PAS reactivity. This PAS reactivity was sensitive to phenylhydrazine treatment. Diastase digestion - PAS reaction showed no effect on the PAS-reactivity. Alcianophilia at AB (pH-2.5) showed poor reaction, whereas at AB (pH-1.0) it was negative. The combined AB-PAS reactions at both pH revealed only pink colour. Colloidal iron reaction showed very weak reaction, whereas AF showed doubtful reaction. Thus the duct system in the glandular adenocarcinoma of the prostate showed only the presence of neutral mucins in less concentration with traces of acidic mucins.

vi) Stroma:

The stroma from normal region as well as tumor region showed similar intensity of reactions for mucopolysaccharides studied. The stroma exhibited weak PAS reactivity which was resistant to diastase digestion (Plate No. 6, Fig. 4) and was almost abolished with prior phenylhydrazine treatment. The stroma reacted poorly at AB (pH-1.0) and (pH-2.5). Acid hydrolysis showed no effect on alcianophilia. With sequential combined technique at AB (pH-1.0 and 2.5)-PAS, purple blue coloured stroma was observed (Plate No. 6, Figs. 5 & 6). With AF the stroma showed no reaction. Alcianophilia with mild methylation was totally lost and partially restored after demethylation. Active methylation showed negative alcianophilia. Thus the above reactions showed the presence of neutral (weak)

as well as carboxy mucins (traces) in the stroma of glandular adenocarcinoma.

vii) Muscle Fibres:
(Plate No. 6, Figs. 3 & 4)

The smooth muscle fibres present in the stroma of tumor region and normal part of prostate showed similar pattern of mucins as found in other pathological conditions, that is muscle fibres showed presence of neutral mucins with small amount of glycogen.

(VI) Anaplastic Carcinoma

A) Gross Description of the Tissue:

The tissue removed after operation consisted of two pieces. The large piece was soft, irregular, flat and grey white in colour measuring about 1.0 x 0.3 x 2 $cm.^3$ The second piece was very tiny, greyish white and soft in nature measuring about 0.1 x 0.1 $cm.^2$

B) Histopathological Observations:

Sections studied from both the pieces showed strip of transitional eplithelium, beneath which a well differentiated tumor was seen. Such transitional epithelium proliferates from the wall of the prostatic urethra and invades the prostate gland. The tumor was composed of cells arranged in sheets and clusters (Plate No. 7, Figs. 1 & 3). The cytological structure of these cells showed hyperchromatic nuclei

and vacuolated to eosinophilic cytoplasm. Inflammatory cells infiltration was also seen. The infiltration of tumor cells in muscle fibres was also observed. The original structure of prostate gland was lost. Occasionally some reduced but functional acini were observed. Major part of the tissue consisted of tumor cells invading acini and also grow over the wall of the blood vessels. Such a histopathological condition in the pathological studies is diagnosed as anaplastic carcinoma.

C) Histochemical Observations:

The results obtained with various histochemical techniques are illustrated in the Plate No. 7 (Figs. 1 to 8) and Plate No. 8 (Figs. 1 to 8) and are recorded in histochemical Table No. 6.

i) Affected Acini:

The acini in the region of tumor cell infiltration showed weak to moderate PAS reactivity which was resistant to diastase or α-amylase digestion (Plate No. 7, Fig. 5) and labile to prior phenylhydrazine treatment showing presence of neutral and acid mucins and absence of glycogen. These acini reacted very weakly with AB (pH-2.5) (Plate No. 7, Fig. 6) and negatively with AB (pH-1.0) indicating the presence of acidic mucins containing sialic acid. The presence of sialic acid containing mucins were further confirmed, as acid hydrolysis completely abolished the alcianophilia. In the combined sequential staining with AB (pH-1.0 and 2.5)-PAS

TABLE NO. 6

Histochemical observations on the mucosubstances found at various cellular sites of the Anaplastic Carcinoma.

Sr. No.	Histochemical Reactions	Normal appearing acini	Transitional epithelium	Secretion	Tumor cells	Stroma	Muscles	Duct	Blood Vessels
1	PAS	++P	+P	+P	++to+++P	++P	+P	+P	++P
2	D-PAS	++P	+P	+P	++P	+P	+P	+P	++P
3	P-PAS	+P	±P	-	+P	±P	-	-	+P
4	AB pH-1	-	+B(some cells)	-	-	±B	-	-	-
5	AB pH-2.5	±B	++B	±B	+B	+B	-	±B	±B
6	AB pH-1.0-PAS	++P	++PB	+P	++PB	+PB	+P	+P	++P
7	AB pH-2.5-PAS	++PB	++PB	+P	++BP	++PB	+P	+P	++P
8	CI	±B	+B	±B	+B	+B	-	±B	±B
9	AF	-	+B	-	±P	-	-	-	-
10	M 37°c-AB pH-2.5	-	-	-	-	-	-	-	-
11	DM 37°c-AB pH-2.5	±B	+B(some cells)	-	±B	±B	-	-	-
12	M 60°c AB pH-2.5	-	-	-	-	-	-	-	-
13	DM 60°c AB pH-2.5	-	±B(some cells)	-	-	-	-	-	-
14	Acid hydrolysis AB pH-2.5	-	±B(some cells)	-	-	-	-	-	-

(Plate No. 8, Fig. 2), the affected acini revealed only moderate magenta colour and weak purple colour showing the presence of neutral and acidic mucins. The remaining histochemical techniques showed negative results. Thus the above results revealed that the affected acini in anaplastic carcinoma showed the presence of neutral mucins (moderate to weak) and acidic mucins (traces), thus showing partial loss of both the mucins when compared with normal acinar mucins.

ii) Secretion:

The secretion found in affected acini showed poor PAS reactivity which remained unaffected after distase or α-amylase digestion. PAS reactivity was sensitive to prior phenylhydrazine treatment. The secretion reacted positively with AB (pH-2.5) and negatively with AB (pH-1.0). The combined AB-PAS reaction at both pH indicated poor magenta colour. However in some acini the secretion revealed weak purple blue colour at AB (pH-2.5)-PAS reaction. CI technique also showed weak blue colour. For the remaining histochemical techniques the secretion showed negative results. Thus the secretion of affected acini in this pathological condition showed the presence of neutral mucins and at some places mixture of neutral and acidic musosubstances. The secretion was found to be decreased in the affected acini as compared to the secretion of normal acini.

iii) Transitional Epithelium:
(Plate No. 8, Figs. 1,3,5 & 7)

The transitional epithelium present in the form of strips around the tumor exhibited weak PAS reactivity (Plate No. 8, Fig.2). This PAS reactivity was resistant to diastase or α-amylase digestion and labile to prior phenylhydrazine treatment. These reactions revealed the presence of neutral and acidic mucins. Moderate alcianophilia was observed at AB (pH-2.5) (Plate No. 8, Fig. 5) and weak at AB (pH-1.0) showing the presence of acidic and sulfated mucins. With the sequential combined AB (pH-1.0 & 2.5)-PAS staining procedure there was a purple blue colour at pH 2.5 and only pink colour at pH 1.0. Alcianophilia was partially lost after acid hydrolysis confirming the presence of sialic acid containing mucins, while the AF technique showed light purple colour confirming the weak sulfated mucins. Partial restoration of the alcianophilia was noted after saponification of previously methylated slides confirming the presence of acidic and sulfated mucins. Few of these cells in sequential combined staining with AB (pH-1.0 & 2.5)-PAS showed bluish purple colour (Plate No. 8, Fig. 7) thus showing elaboration of sulfated mucins, whereas the remaining cells showed elaboration of neutral and acidic mucins. The above histochemical reactions suggested that the distribution of these mucopolysaccharides is not uniform in all the transitional epithelial cells. As it was clear from the sections stained with sequential staining with AB (pH-1.0 & 2.5)-PAS some cells stained

magenta colour and showed the presence of neutral mucins (moderate) and other cells stained blue purple colour showing the presence of mixture of neutral and acidic mucins (The acidic mucins containing sialic acid with traces of sulfated mucins).

iv) Inflammatory Cells:
(Plate No. 7, Figs. 3 & 7)

The inflammatory cells infiltrated in the stromal region revealed weak PAS reactivity. This PAS reactivity was labile to prior treatment of phenylhydrazine PAS and resistant to diastase digestion PAS reaction. Alcianophilia with AB (pH-2.5) was weak and with AB (pH-1.0) it was negative. In a combined sequential staining technique the inflammatory cells showed purple blue colour with AB (ph-2.5)-PAS, whereas only weak magenta colour with AB (pH-1.0)-PAS. With AF technique these cells showed light purple colour. Methylation at 37°c showed complete loss of alcianophilia which was partially restored after saponification. CI technique also showed light blue colour with these cells. Thus the inflammatory cells showed the presence of neutral mucins and sialomucins (traces).

v) Tumor Cells:
(Plate No. 7, Figs. 4,7 & 8; Plate No. 8, Figs. 6 & 8)

Tumor cells found in various regions of prostate gland showed more or less similar pattern of histochemical results. The tumor cells when stained with PAS technique revealed moderate activity.

The PAS reactivity was labile partially to diastase or α-amylase digestion and totally to prior phenylhydrazine treatment. Alcianophilia at AB (pH-2.5) was weak and negative at AB (pH-1.0), whereas CI technique gave light blue colour in these cells. Acid hydrolysis abolished the alcianophilia. In a combined sequential AB (pH-1.0 & 2.5)-PAS technique the tumor cells showed moderate purple blue colour with AB (pH-2.5), whereas moderate magenta colour with AB (pH-1.0). Active methylation and mild methylation abolished the basophilia, which was restored only after saponification at 37°c. The above histochemical techniques showed that the tumor cells contained neutral mucins (moderate), acid mucins (traces) and glycogen (traces). The tumor cells showed more increased PAS reactivity than the cells of other region of prostate; this increase may be apparently due to the presence of glycogen.

vi) Duct:

(Plate No. 7, Fig. 4)

The ducts found in the normal region as well as in the tumor region showed similar pattern of histochemical results. The ducts reacted moderately with PAS staining. This PAS reactivity was completely abolished with prior phenylhydrazine-PAS reaction and was resistant to distase or α-amylase digestion. These reactions revealed presence of neutral mucins. Alcianophilia at both pH was negative showing absence of carboxylic and sulfated mucins. The duct wall and epithelium showed moderate magenta colour in sequential

combined AB (pH-1.0 &2.5)-PAS reaction. The other histochemical reactions employed revealed negative reactions in the above regions. Thus the duct wall and epithelium showed presence of only neutral mucins in moderate concentration.

vii) Stroma:

The connective tissue and stroma present in the acinar and tumor region revealed weak to moderate PAS reactivity which was resistant to diastase or α-amylase digestion, while it was slightly abolished with prior phenylhydrazine PAS treatment. Reactions at AB (pH-2.5) and AB (pH-1.0) showed moderate and weak alcianophilia respectively. CI technique also showed moderate blue colour. In a combined sequential AB (pH-2.5)-PAS staining technique the stroma showed moderate bluish purple colour (Plate No. 7, Fig. 8) and with AB (pH-1.0)-PAS moderate magenta and weak purple colour was noted. All these reactions revealed the presence of neutral, carboxy and sulfated mucins. The AF technique revealed light purple colour with the stroma confirming the presence of sulfated mucins. The basophilia was totally abolished after active methylation, while partially abolished after mild methylation; further basophilia was restored after saponification at low temperature only. Partial loss of alcianophilia after acid hydrolysis confirmed the presence of carboxy mucins (sialic acid). Thus the above histochemical technique revealed that the stromal cells contained neutral mucins (moderate), acidic mucins that to sialic acid (weak concentration) and traces

of sulfomucins.

viii) Muscle Fibres:

The smooth muscle fibres showed positive results with PAS staining technique. This PAS activity was partially abolished after diastase or α-amylase digestion and resistant to prior phenylhydrazine treatment. With the combined sequential AB (pH-1.0 & 2.5)-PAS staining technique the muscles showed poor magenta colour. The other histochemical techniques employed gave negative results. Thus the muscle fibres showed the presence of neutral mucins (weak) and glycogen.

ix) Blood Vessels:

The wall of the blood vessels showed moderate to intense PAS reactivity (Plate No. 8, Fig. 4) which was not altered after diastase or α-amylase digestion but partially abolished with prior phenylhydrazine treatment, indicating the presence of neutral and acidic mucins. Weak alcianophilia was observed with AB (pH-1.0) and AB (pH-2.5), while the combined sequential staining with AB (pH-1.0 and 2.5)-PAS showed magenta colour in which traces of purple coloured staining was distributed, showing the presence of neutral mucins and sialomucins (Plate No. 8, Fig. 6). The presence of acidic mucins was confirmed by loss of alcianophilia after acid hydrolysis. The methylation and demethylation and also the AF techniques

gave negative reactions indicating absence of sulfomucins. The above histochemical rections showed that the wall of blood vessel contained neutral mucins (moderate amount), and sialomucins (weak to traces).