HISTOLOGICAL AND CERTAIN HISTOCHEMICAL STUDIES ON THE PAROTID SALIVARY GLANDS OF GOAT (*Capra hircus*)

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR)

BIHAR

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

By

PRABHAWATI KUMARI

In partial fulfilment of the requirement

For the degree of

MASTER OF VETERINARY SCIENCE

(VETERINARY ANATOMY)

AUGUST, 1997

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DEDICATION

This Thesis dedicated

to my father

(Late Ram Sharan Prasad)

Department of Veterinary Anatomy & Histology
Rajendra Agricultural University,
Bihar.

CERTIFICATE - I

This is to certify that the thesis entitled " HISTOLOGICAL AND CERTAIN HISTOCHEMICAL STUDIES ON THE PAROTID SALIVARY GLANDS OF GOAT (Capra hircus)" submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science with major in Veterinary Anatomy, Rajendra Agricultural University, Bihar, is a bonafide research work carried out by Dr. Prabhawati Kumari under my supervision and guidance and that it incorporates the results of her independent and original study.

6/8/97

(Dr. M. K. Roy)
Major Advisor
Associate Professor

Endorsed :

6/8/97

(Chairman/Head of Department)

CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of Dr. Prabhawati Kumari, a candidate for the Degree of Master of Veterinary Science with major in Veterinary Anatomy have gone through the manuscript of the thesis and agree that the thesis entitled " HISTOLOGICAL AND CERTAIN HISTOCHEMICAL STUDIES ON THE PAROTID SALIVARY GLANDS OF GOAT (*Capra hircus*)" may be submitted by Dr. Prabhawati Kumari in partial fulfilment of the requirements for the Degree.

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Chairman, Advisory Committee.

Members of the Advisory Committee :

1. Dr. A. K. Barnwal - 6/8/97
2. Dr. R. B. Singh -
3. Dr. S. B. Verma -

CERTIFICATE - III

This is to certify that the thesis entitled " HISTOLOGICAL AND CERTAIN HISTOCHEMICAL STUDIES ON PAROTID SALIVARY GLANDS OF GOAT (Capra hircus)" submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science with major in Veterinary Anatomy of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on 29.1.1998.

29/1/98

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Dean, Post-Graduate Studies,

A C K N O W L E D G E M E N T

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Prabhawati Kumari

(PRABHAWATI KUMARI)

Place: B.V.C.Patna,

Dated :

I N D E X

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INTRODUCTION

1. INTRODUCTION

The productive role of goat keeping is now well established in India's rural economy. According to F A O (1995), India ranks on the top in goat population (118.30 million) in the World (609.43 million). It thus becomes the most important livestock for poor and marginal farmers.

Inspite of such multifaceted importance of goat in our country, very limitted informations are available on the histological and histochemical features of various organs including major salivary glands.

The parotid salivary gland is one of the major salivary glands of mammals that secretes the major quantum of total saliva in animal's body. In addition to generalised mechanical and chemical functions of saliva (Trautmann and Feibiger, 1957), the parotid salivary gland in ruminant exhibits additional functions through its secretion. The saliva becomes very essential for microbial digestion in non-glandular forestomach by maintaining the optimum fluid enviroment and normal pH in the rumen (Phillipson, 1992). The immunological functions are also thought to be carried out through the plasma cells distributed in the parotid salivary gland (Banks, 1981). Considering the above facts and figures the present investigation on histological and histochemical features on the goat parotid salivary gland

has been undertaken to provide basic informations which can be well utilised by the research workers in the various fields of biological sciences in general and Veterinary Sciences in particular.

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REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 HISTOLOGY :

2.1.1 Capsule and Supporting tissue :

Trautmann and Fiebiger (1957) stated that the salivary glands of domestic animals were enveloped by a connective tissue capsule which contained elastic fibres and some smooth muscle fibres and conducted vessels and nerves. It gave off strands which ramify extensively in the gland. Further, they also described that the interstitial and perivascular connective tissue of ruminant parotid contained abundance of mast cells that increased in number with age.

Carleton and Short (1965) mentioned the occurrence of lymphnodules in the interstitial connective tissue of the parotid salivary gland of mammals.

Ham (1969) described that the parotid salivary gland was enclosed in a well defined fibrous connective tissue capsule and also mentioned the accumulation of fat cells in the connective tissue septa of human parotid salivary gland.

Venkatakrishnan and Mariappa (1969) observed that the parotid stroma in buffalo resembled to that of the Ox, but smooth muscle in the form of isolated and discrete bundles were seen in the connective tissue stroma.

Sengar and Singh (1970) noted that in buffaloes, a well defined fibrous connective tissue capsule was present around the parotid. The septae of connective tissue divided and subdivided the parenchyma into lobes, lobules and alveoli or acini. They were traversed by blood vessels, nerves and ducts.

Pal, Chandra and Bharadwaj (1972) found that the capsule of parotid gland of young buffaloes frequently contained adipose tissue and nerve bundles. The capsule, interlobular and intralobular connective tissues consisted of fine collagenous boundle and few elastic and reticular fibers. In adult animals the capsule contained densely packed collagenous bundle with a few elastic fibers. The interlobular tissue reduced to a thin partition. The elastic fibers surrounding the acini and ducts became more pronounced.

Copenhaver, Kelly and Wood, (1978) noted that in the parotid gland, there was definite connective tissue capsule which enclosed the gland and blended with the connective tissue of surrounding structures. Connective tissue septae divided each gland into lobes and lobules. The larger ducts, the blood vessels and occasional ganglion cells were found in these septae. Fat cells were frequent in the connective tissue of the parotid and they tended to increase in number with age in mammals including man.

Banks (1981) described that the juxta-alveolar connective tissue of the salivary glands contained numerous plasma cells and small lymphocytes. Immunoglobulin-A was produced by plasma cell in domestic animals.

Stinson and Calhoun (1987) described that the stroma of compound glands included the capsule and the internal supportive framework. The capsule, composed of collagen, elastic and reticular fibers, completely surrounded the gland which gave rise to connective tissue sheets (septae) or strands (trabeculae) that extended well into the parenchyma. These septae clearly defined the lobes and lobules and provided support for the various interlobar and interlobular ducts. Fine reticular fibers encircled the individual secretary units.

2.1.2 Parenchyma :

2.1.2.1 Secretory end pieces :

Junqueira, Sessot and Nahas (1951) recorded that the secretory end pieces of none of the 10 different mammals contained a purely serous type.

Trautmann and Fiebiger (1957) mentioned that the parotid gland as serous gland but young carnivores and lambs contained mucous end pieces. They also described the presence of myoepithelial cells on the inner surface of basement mambrane of secretory cells in domestic animals which joined each other by their cytoplasmic processes to form basket like cell around the secretory cells.

VenLennep (1957) reported that the parotid gland of adult camel was a serous gland. It's glandular cells contained a variable amount of mucoids.

Scott and Pease (1959) observed the presence of myoepithelial cells were not a common feature of the rat parotid although they were commonly present in other major salivary glands.

Rausch and Emodi (1961) noted that the parotid gland was not exclusively serous and it always kept the potential capacity of a mixed gland.

Duancie and Posinovec (1962) recorded the human parotid gland as a mixed gland.

Shackleford and Klapper (1962) noted that the acini of bovine and ovine parotid glands were very long and tubular and the lining cells tended to be cuboidal rather than usual pyramidal shape.

Munger (1964) reported that the typical seromucous cells were found in the parotid gland of human. The seromucous cells secreted a watery product, but the secretory granules contained a variable amount of mucopolysaccharide also in the secretory granules.

Carleton and Short (1965) noted that the parotid glands of all mammals were of serous type, but in man a few mucous alveoli were found around the main duct. The adipose tissue was often distributed in the glands of man and monkey.

Blairwest, Coghlan and Denton (1969) reported that the alveoli of sheep parotid gland were surrounded by a mesh of muscular process i.e. myoepithelial cells. There were no myoepithelial cells in relation to the ducts. It was proposed that the saliva which appeared due to sympathetic stimulation was expelled from the alveoli by contraction of the myoepithelial network.

Ham (1969) described that the human parotid salivary gland was compound tubulo-alveolar gland and of serous type. The cytoplasm contained eosinophilic zymogen granules. Myoepithelial cells lied between the bases of the secretory cells and the basement membrane.

Leeson (1969) noted that parotid salivary gland was purely serous type in spider monkey. Myoepithelial cells were present around the acini and intercalated duct.

Shackleford and Wilborn (1969) reported that the bovine parotid gland had some differential structures than of non-ruminant mammals. Acinar cell contained more mitochondria and less granular endoplasmic than parotid of non-ruminant animals. The acini were tortuous, branched and lined with cells of different heights. Two types of secretory materials were found in the acinar cell. Myoepithelial cells were mostly found at the junctions of acini and intercalated ducts, where they attached to both acinar and ductal epithelium.

Sengar and Singh (1970) observed that the parotid gland of buffaloes was purely serous type with elongated acini that empty into the intercalated ducts.

Leeson and Leeson (1971) observed that the myoepithelial cells were found in relation to both secretory units and intercalated ducts. They lied within the basal lamina of secretory acini and intercalated ducts and did not protrude from the external surface. They contained a central nucleus and surrounding cytoplasm with several cytoplasmic arms in rat parotid salivary gland.

Pal, Chandra and Bharadwaj (1972) reported that the parotid gland of buffalo was tubulo-alveolar-acinar type. The alveoli were widely separated and had a duct like appearance. The spherical nucleus of alveolar cells were situated towards the centre of the cell and the supra nuclear zone stained densely. PAS reactive colloid like mass was found in the venule surrounding the secretory end pieces.

Tandler and Erlandson (1976) reported that the parotid gland of baboon was of serous type. The acini consisted of pyramidal cells with abundant secretory granules, varying in size. Myoepithelial cells were present at the acinar perimeter.

Lennep, Kennerson and Compton (1977) mentioned the sheep parotid gland as compound tubular gland, secreting large amounts of fluid with very little protein.

Barnwal (1978) reported three distinct cell-types in the parotid salivary gland of buffalo.

Copenhaver, Kelly and Wood (1978) described that the parotid salivary gland was purely serous type in man, dog, cat and rabbit. Myoepithelial cells lied in close contact with the secreting cells and they formed a sort of basket work around the alveolus.

Banks (1981) described the parotid salivary gland as serous type in all domestic animals, man and rodents. A few mucous cells or adenomeres might be present in carnivores. It might be mixed gland in young puppies and lambs. Myoepithelial cells were juxtaposed intimately to the secretory epithelial cells of the alveoli. The contraction of myoepithelial cell assisted the movement of secretory products from the alveoli.

Barnwal and Sinha (1985) mentioned that the secretory end pieces of parotid salivary gland of buffalo were comprised of acini, alveoli and tubules. The parotid gland represented seromucus character in buffalo.

Dellmann and Brown (1987) described that the argyrophillic character of reticular fibre was due to the presence of glycoprotein layer around it.

Stinson and Calhoun (1987) described that parotid salivary gland was predominantly serous type in domestic animals although occasional isolated mucous secretory units might occur in the dog and cat. Structurally it was compound

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gland. Stellate-shaped myoepithelial cells were located between the secretory cells and the basement membrane.

Kishore and Rao (1992) studied that the parotid salivary gland of goat as compound tubulo alveolar type of gland. They further classified the gland as a purely serous type.

2.1.2.2 Duct System :

Trautmann and Feibiger (1957) described the duct system of parotid salivary gland of domestic animals was composed of intercalated, striated, interlobular and the main ducts. The intercalated ducts were lined by simple cuboidal and rarely squamous cells. The thin basement membrane of the striated ducts bore simple columnar epithelium. The cells were high and strongly eosinophilic. The interlobular excretory ducts had a simple columnar epithelium in the begining and the larger ducts were lined with two layered epithelium. The terminal portion was lined with high stratified columnar with a few goblet cells in some glands, and near the opening the epithelium of main duct changed to stratified squamous. In ruminant, considerable number of mast cells were found around the striated duct and within their actively secreting two-layered epithelium.

Munger (1964) reported the absence of goblet cells in the duct system of human parotid gland.

Shackleford and Wilborn (1969) noted that the intercalated ducts in the bovine parotid gland connected acini with the striated ducts which inturn empty into the collecting ducts located between gland lobules. They also pointed out that the striated ducts of the calf parotid were not well developed as regards the number of mitochondria as compared to those of other salivary glands.

Shear (1969) mentioned the intercalated ducts of rat parotid gland consisted of a number of proximal segments, which united to form a distal segment. Proximal segments were part of the secretory acini from which they arised. They contained secretory granules, and the basement membrane was seen between them and the parent secretory acinus. Therefore, they had been called juxta acinar cells. Fine cytoplasmic processes extended from acinar cells. The cells of the distal segments contained secretory granules. These cells lied on the basement membrane and were surrounded by myoepithelial cells. The distal segments of the intercalated ducts being surrounded by myoepithelial cells were responsible for expelling the secretion into the striated ducts.

Pal, Chandra and Bharadwaj (1972) reported that the intralobular ducts in the parotid of calf and adult buffaloes were lined with simple columnar epithelium. The infranuclear zone was vacuolated in all age groups. The interlobular ducts were lined by high cuboidal or columnar cells. The mucosa of the main parotid duct was highly folded and was lined with

startified columnar epithelium with predominance of goblet cells. They had not observed striated tubules in the gland of any age group. Goblet cells appeared in the interlobular ducts and increased in number towards the oral cavity. Increased infranuclear vacuolations due to fatty acid concentration (negative for acid mucopolysaccharides) distinguished duct cells from gland cells. Intraepithelial cysts frequently appeared in the main parotid duct.

Tandler and Erlandson (1976) studied the baboon parotid gland and noted that the intercalated duct cells contained granules. The striated ducts consisted of tallcells interlocked in a complex fashion. The duct walls were composed primarly of tall columnar epithelial cells with a few small pyramidal cells. Vertically oriented mitochondria were responsible for the striated appearance of these ducts in the light microscope. Excretory ducts consisted of simple to pseudostratified columnar epithelium and lacked basal striation or apical blebs.

Copenhaver, Kelly and Wood (1978) mentioned that the intercalated ducts were lined by low cuboidal epithelium. Striated ducts were lined by single layer of columnar cells, and main duct was lined with pseudo-striatified or stratified cells were found in intercalated and striated duct.

Jerry, Boshell and Wilborn (1978) studied the pig parotid gland and mentioned that the intercalated duct cells

near acini contained secretory granules. Striated ducts were lined with two types of columnar epithelial cells, "light" cells and "dark" cells.

Banks (1981) described that the intercalated ducts were lined with a low cuboidal epithelium. The striated ducts were lined with a columnar epithelium. Intralobular duct was lined with simple columnar epithelium. Bistratified cuboidal or bistratified columnar epithelium might be present at point of transition between intralobular ducts and lobular ducts in feline salivary gland. Intralobar ducts, lobar duct might be lined by pseudostratified columnar epithelium. Main excretory duct was lined by stratified squmous epithelium.

Suzuki, Nishinakagawa and Otsuka (1981) mentioned the cells of the intercalated duct had a few smooth spherical granule. Epithelial cells of the striated duct did not contain granules in parotid gland of goat.

Barnwal and Sinha (1984) studied the parotid salivary gland of buffalo and noted that the duct system of parotid salivary gland was comprised of intercalated, striated, interlobular and the main duct. The intercalated ducts were lined with flattened to simple cuboidal epithelium, the striated ducts with simple columnar in adults and cuboidal to columnar epithelium in calves. The basal striations were not well marked in the striated ducts. The interlobular duct epithelium varied from simple columnar to stratified columnar.

The parotid duct possessed a stratified columnar epithelium with preponderance of goblet cells and a number of intra-epithelial cysts and glands.

Stinson and Calhoun (1987) described that the intercalated duct was lined by low cuboidal epithelium and striated duct by cuboidal or columnar epithelium. Interlobular ducts were located in the connective tissue septa between lobules. These ducts were lined by simple columnar epithelium, which changed to stratified columnar epithelium as ducts became larger. The interlobular ducts fused to form main parotid duct, where it opened into the oral cavity, as the epithelium changed from striatified columnar to stratified squamous epithelium.

Yasear and Ibrahim (1992) noted that the parotid salivary gland of sheep was characterized by the presence of a large number of goblet cells. Serial sectioning showed that the number of goblet cells increased as the luminal diameter increased. The goblet cells represented a constant feature of the stratified cuboidal and squmous epithelium of the ductal system.

2.2 HISTOCHEMISTRY :

Hill and Brown (1954) mentioned that in the parotid and other salivary glands of rat, cat and mouse, the acinar cells were intensely PAS positive. The intercalated and interlobular duct cells were PAS negative in these glands. They found that some of the acinar cells of the parotid salivary gland were in demilune shape in rat, cat and mouse and were slightly positive to alkaline phosphatase but most intense reaction was given by the capillaries surrounding the base of the acini and ducts.

Cantatore (1959) studied the parotid salivary gland and found the serous adenomeres were very rich in mucopolysaccharide contents.

Kawakatsue et al. (1959) observed that the acinar cells of the mouse and dog parotid were non-reactive to alkaline phosphatase. There was slight activity in the rat, moderate to diffusely distributed activity in guinea pig and rabbit. Moderate or high phosphatase activity had been observed in the capillaries.

Ito and Asano (1961) noted that a parotin like substance parotin was found in bovine parotid gland. £-parotin was considered to be glycoprotein.

Duancie and Posinovec (1962) studied human parotid salivary gland and classified as mixed type. They also mentioned that the mucoid elements were mostly in the glands

from male persons.

Morelli and Perucci (1963) studied the structure of parotid glands in men and oxen and reported the distribution of acid and neutral mucopolysaccharides in the acini and in the epithelium of the excretory system.

Snkurko (1963) observed that the secretory granules in the cat parotid gland were PAS positive. Zymogenic granules were positive for glycoprotein and mucoproteins, while staining with toludine blue the periphery of the granules showed B metachromasia.

Munger (1964) reported that the serous cell secreted proteinaceous material without significant polysaccharides in the secretory granules such as the pancreatic acinar cell and gastric cheif cell. He further considered the seromucus cells of human parotid salivary gland as serous cells containing intensely PAS positive demonstrable polysaccharide in their secretory granules. However these granules were not distinctly positive for colloidal-iron and Alcian blue. Similar reactions were also reported in the apical cytoplasm of secretory duct.

Fava de moraes, Giuffrida and Jungueira (1967) found that the glycogen positive striated duct of the salivary glands acted as an energy store in mammals. When the glands were stimulated there was marked decrease in the glycogen content of the striated duct.

Carpenter (1968) mentioned that the intralobular fat cells were usually present in the human parotid salivary gland.

Ham (1969) described that the presence of fat cells in the connective tissue of parotid salivary gland of man.

Shear (1969) noted that the intercalated duct of rat was PAS positive and also noted presence of Glycoprotein.

Pal, Chandra and Bharadwaj (1972) found that the cytoplasm of the acinar cells in buffalo was moderate to mild PAS reactive and occasionally intense PAS reactive and acinar lumen contained highly PAS positive secretory mass. The lining cells of acini and alveoli of the parotid were mildly metachromatic. In calves and adult animals acid mucopolysaccharide was present in different part of the cell cytoplasm. The supranuclear cytoplasm of interlobular ducts stained mildly with PAS. Only the apical border of duct cells showed positive reaction for acid mucopolysaccharides. PAS reactive acinar cells did not reveal the presence of glycogen after treatment with saliva. They further showed the absence of fat in the secretory cell cytoplasm of the buffalo parotid but treatment with nile blue sulphate showed the presence of fatty acids. There was presence of fatty acids in the duct cells but were negative for neutral fat. The simple columnar to pyramidal glandular cells contained fatty acids in the infranuclear cytoplasm. Other parts of cells cytoplasm were positive for PAS, acid mucopolysaccharide and alkaline phosphatase. The activity of the alkaline phosphatase at the apical border and basement membrane was moderate, mild in

supranuclear cytoplasm in buffalo parotid gland.

Copenhever, Kelly and Wood (1978) noted that the presence of fat cells in the connective tissue of parotid salivary gland of mammals.

Jeery, Boshell and Wilborn (1978) studied the adult pig parotid gland and reported that the acini had little affinity for PAS and were alcian blue-negative at pH 2.6 or 0.5. These results indicated a paucity of neutral mucins and absence of sialo and sulfomucins. The acinar cells differed histochemically from a typical serous cells and were classified as special serous cells with lipid in the secretory granules.

Vignoli and Noguira (1981) noted that glycogen was demonstrated in secretory tubules and ducts of zebu parotid gland. Neutral mucosubstances were detected in the secretory tubules and rarely in some duct cells, reaction for sialomucins were weak in the secretory tubules. Parenchyma was positive to protein, with reduction in intensity from younger to older zebu.

Barnwal and Sinha (1985) studied the parotid salivary gland of buffalo and mentioned that the secretory end-pieces showed weak to moderate PAS positivity. They further noticed that the cells of secretory end pieces were mildly reactive for acid mucopolysaccharides, neutral fat and fatty acids, NH_2 bound protein and tyrosin. However, the cells were moderately positive for neutral mucin, glycogen and alkaline phosphatase.

Dellmann and Brown (1987) described that the reactivity of connective tissue fibres with Periodic Acid Schiff were due to presence of thin layer of glycoprotein around the argyrophilic reticular fibres.

Yasear and Ibrahim (1992) studied the sheep parotid salivary gland and noted the goblet cells of duct were PAS positive. Alcian blue negative and suggested the presence of neutral polysaccharides.

Singh, Pawar and Roy (1995) studied the parotid salivary gland of castrated donkey and found the acinar cells and ductular epithelium were also positive for sudanophilic lipid. The mucous and serous acinar cells were positive for alkaline phosphatase. Myoepithelial cells had better domonstrable oxidoreductases and A.T. Pase.

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MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigations were conducted on the parotid salivary glands from twelve healthy adult crossbred Bengal goats. They were 1½ to 3½ years of age. Out of twelve goats six were male and rest were female.

The tissue samples were collected from freshly killed animals for proper fixation. The tissue samples measuring 3 to 5 mm in thickness were secured from 3 different parts of each gland. Similarly, pieces of main parotid duct were also collected from each side at three different sites. The following fixative solutions were used for preservation of tissues (Luna, 1968):

1. 10% Neutral buffered formalin
2. Zenker's solution
3. Bouin's solution
4. Regaud's solution
5. Chilled acetone
6. Absolute alcohol.

After proper fixation, the tissue samples were processed through standard schedules viz., Washing, Dehydration, Clearing, Paraffin infilteration and Block preparation to procure the paraffin sections (Humason, 1967 and Luna, 1968). The paraffin sections were cut at five to seven microns with the help of rotary microtome. The frozen sections were cut at ten to fifteen micron from formalin fixed tissue for lipid staining.

The following histological and histochemical staining methods were employed.

A. Histological staining methods :

i. Haematoxylin and Eosin stain (Luna, 1968) for routine study.

ii. Mod. Mallory's trichrome stain (Crossman, 1937) for connective tissue fibres.

iii. Gomori's reticulin stain (Humason, 1967) for reticular fibres.

iv. Van Gieson's stain (Luna, 1968) for collagen and muscle fibres.

v. Gomori's Aldehyde Fuchsin stain (Pearse, 1968) for elastic fibres and mucins.

vi. Weigert's Resorcin-fuchsin method (Culling, 1974) for elastic fibres.

vii. Verhoeff's elastin stain (Humason, 1967) for elastic fibres.

viii. Unna's methods (Luna, 1968) for mast cells.

ix. Luna's method (Luna, 1968) for mast cells.

x. Diazo method (Luna, 1968) for argentaffin granules.

xi. Altmann's method (Humason 1967) for mitochondria.

xii. Cain's method (Luna, 1968) for mitochondria.

xiii. Mallory Heidenhain's Azan stain (Humason, 1967) for connective tissue, muscle etc.

B. Histochemical staining method :

i. Periodic Acid Schiff stain with or without saliva digestion (Pearse, 1968) for glycogen and neutral mucosubstances.

ii. Aldehyde Fuchsin method (Luna, 1968) for mucosubstances.

iii. Modification of Mowry's colloidal iron stain stain (Luna, 1968) for acid mucopolysaccharides.

iv. Best's carmine method (Luna, 1968) for glycogen.

v. Mercury-Bromophenol blue method (Pearse, 1968) for proteins.

vi. Feulgen reaction (Pearse, 1968) for D N A .

vii. Oil red O in propylene glycol method (Luna, 1968) for fat.

viii. Gomori's stain (Davenport, 1969) for alkaline phosphatase.

ix. Mayer's mucihaematein stain (Culling, 1974) for mucin.

Micrometry :

Different measurments viz., epithelial height, nuclear size and diameters of secretory end pieces and various segments of the ducts upto interlobular regions were made with the help of caliberated occular micrometer. For the ducts with irregular outline, only epithelial heights were measured.

The above measurement were done on randomly selected sections from the different regions of gland proper and main excretory ducts of each animal.

Statistical analysis :

The mean, standrad Error (S.E.) Analysis of variance (ANOVA) and critical difference test (C.D.test) were calculated on different data of male and female animals for statistical interpretations (Snedecor and Cochran, 1967).

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RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 Histology :

4.1.1 Capsule and Supporting Tissue :

The parotid salivary gland of goat was invested with dense irregular connective tissue containing collagen fibres. It was substanciated with fine reticular fibres and a little amount of elastic fibres. The connective tissue cells mainly comprised of fibrocyte and well marked by heterochromatic flattened nucleus which were distributed between the connective tissue fibres. The blood vessels, nerves, lymphatic spaces and clusters of unilocular adipocytes were also observed in the capsule. The present finding was in agreement with the findings of Trautmann and Fiebiger (1957) in domestic animals, Ham (1969) in man, Venkatkrishnan and Mariapa (1969), Senger and Singh (1970) in buffalo, Pal, Chandra and Bharadwaj (1972) in buffalo, Copenhever, Kelly and Wood (1978) in mammals and Stinson and Calhoun (1987) in domestic animals, who reported predominance of collagen fibres alongwith little amount of reticular and elastic fibres in the capsule of parotid salivary gland. The smooth muscle fibres were not recorded in the capsular tissue of goat parotid salivary gland although Trautmann and Fiebiger (1957) reported some smooth muscle fibres in the capsule of domestic animals. The isolated distribution of the cluster of adipocyte in the capsule could be compared with the

findings of Pal, Chandra and Bharadwaj (1972) who frequently recorded adipose tissue in the capsule of young buffaloes.

The connective tissue septae arising from the capsule divided the parotid salivary gland into various lobes and lobules. The interlobular connective tissue was marked by the presence of fine collagen bundles alongwith few elastic and reticular fibres (Figs. 1, 2, 3). Through these septae, the interlobular ducts, blood vessels, nerves lymphatics traversed (Fig. 7). Occasionally some isolated patches of smooth muscle fibres were recorded with Van-Gieson stain which were suppose to be transitional section through tunica media of passing blood vessels (Fig. 9). Distributions of adipocyte clusters were not uncommon in the larger connective tissue septae. The perivascular connective tissue did not exhibite mast cells, however occasional distribution of plasma cell with heterochromatic nucleus was noticed in the connective tissue (Fig. 18). The histological features of interlobular septae recorded in the present investigations were in agreement with the findings of Ham (1969) in human, Senger and Singh (1970) in buffaloes, Pal, Chandra and Bharadwaj (1972) in buffaloes, Copenhever, Kelly and Wood (1978) in mammals and Stinson and Calhoun (1987) in domestic animals. The smooth muscle fibres observed in the connective tissue septae of goat parotid salivary gland could be compared with the findings

of Venkatkrishnan and Mariapa (1969) who recorded isolated and discreate bundles of smooth muscles in the stroma of buffalo parotid salivary glands. The occurence of plasma cells in connective tissue could be compared with report of Banks (1981) who described the presence of plasma cells at the juxta-alveolar connective tissue of the gland. The intralobular connective tissue was drastically reduced and was mainly distributed around the intralobular ducts (Figs. 6, 11, 12, 13). Around the secretory part of parenchyma the fine network of reticular fibre was recorded (Figs.4, 5). With Azan stain, the similar network of fine collagen fibres were observed around the glandular tubule and acini (Fig.6). The elastic fibres were altogether absent in the intralobular connective tissue except for the area around the intralobular duct (Fig. 2). The present finding was contrast to the findings of Pal, Chandra and Bharadwaj (1972) who recorded elastic fibres around the acini in buffalo parotid salivary gland. However fine reticular fibrous network surrounding the indivisual secretory unit had been described in the parotid salivary glands of domestic animal by Stinson and Calhoun (1987).

The stromal tissue of parotid salivary gland in goat presented sporadic distribution of diffused lymphoid tissue. No lymph nodules were, however, observed. Carleton and Short (1965) described occurence of lymph nodules in the interstitial connective tissue of parotid salivary gland of mammals.

4.1.2 Parenchyma :

The parenchyma of the parotid salivary gland was made up of tubulo-acinar type of secretory units associated with various subdivision of excretory ducts (Figs. 1, 6, 10, 11, 12, 13, 14, 17, 18). Histomorphologically, it was thus typed as compound tubulo-acinar gland. Pal, Chandra and Bharadwaj (1972) and Barnwal and Sinha (1985) reported buffalo parotid salivary gland as compound tubulo-alveolar-acinar type. Lennep, Kennerson and Compton (1977) described sheep parotid salivary gland as compound tubular gland. However, Kishore and Roy (1992) opined that the parotid salivary gland of goat was compound tubuloalveolar type.

4.1.2.1 Secretory end Pieces :

The secretory end pieces of parotid salivary gland in goat comprised of glandular tubules terminating into acini (Figs. 11, 12, 13, 14). The glandular cells were mostly pyramidal in shape having spherical to oval nucleus. Shackleford and Klapper (1962) recorded cuboidal cells in glandular tubules and acini of bovine and ovine parotid salivary glands. The cytoplasm contained variable amount of acidophilic granules which were more concentrated at supuranuclear position. These granules were distinctly stained with azocarmine with Azan stain (Fig. 6). These cells exhibited the microscopic profile of serous cell as

distinct basophilia of the cytoplasm was lacking. This was incontrast to the findings of Junquira, Sessot and Nahas (1951), Roush et al. and Emodi (1961), Duancie and Posinovec (1962) and Munger (1964) who reported the parotid salivary glands in different mammals as mixed gland. However, Trautmann and Fiebiger (1957) in domestic animals, VenLennep (1957) in adult camel, Carleton and Short (1965) in mammals except man, Ham (1969) in man, Leeson (1969) in spider monkey, Senger and Singh (1970) in buffalo, Tandler and Erlandson (1976) in baboon, Banks (1981) in domestic animals, Stinson and Calhoun (1987) in domestic animals and Kishore and Roy (1992) in goat classified the parotid salivary gland as purely serous type. Banks (1981) in young puppies and lamb and Stinson and Calhoun (1987) in dog and cat described the presence of few mucus cells in the parotid salivary glands.

The variability in the distribution of eosinophilic zymogen granules alongwith change in the nuclear profile resulted in the distribution of three definite cell types viz. secretory, exhausted and resting cells. The secretory cells contained variable amount of zymogen granules with vesicular nucleus. The exhausted cells appeared oval in outline having clear cytoplasm with highly vesicular nucleus (Fig. 13). The resting cells were filled with densely packed zymogen granules having a hetrochromatic oval nucleus placed basally (Fig. 14). Barnwal (1978) also reported

3 distinct types of cells in the parotid salivary gland of buffalo. The acinar and tubular secretory end pieces were surrounded by thin network of connective tissue fibres mainly made up of fine reticular and collagen fibres. The fibrous network around the end pieces were associated with the presence of few fibroblasts. With reticulin stain the cytoplasm of the glandular cells presented variable distribution of argyrophlic granules (Figs. 4, 5). These granules were faintly stained with Diazo method for argentaffin granules. The presence of these granules might be denoting glycoprotein in the cells as described by Dellmann and Brown (1987).

With the available histological techniques the distribution of myoepithelial cells around the acini or tubules could not be ascertained, however a few flattend nuclei were observed between the glandular cells and the surrounding connective tissue fibres. These nuclei might be denoting the nuclei of myoepithelial cells placed between the glandular cell and the basement mambrane. Scott and Pease (1959) reported that the presence of myoepithelial cells were not a common feature of rat parotid gland. However, many authors described the distribution of myoepithelial cells around the secretory cells of parotid salivary gland in various mammals (Trautmann and Fiebiger, 1957; Blairwest, Coghlan and Denton, 1969; Ham, 1969; Leeson, 1969; Sahckleford and Wilborn, 1969; Leeson and Leeson, 1971; Tandler and

Erlandson, 1976; Copenhaver, Kelly and Wood, 1978; Banks, 1981; and Stinson and Calhoun, 1987).

The average diameter of glandular end pieces measured 20.50 ± 0.45 µm in acinar and 14.17 ± 0.23 µm in tubular region of adult male. Similarly 20.68 ± 0.45 µm and 14.23 ± 0.26 µm in acinar and tubular region of adult female respectively. The mean, standard error and coefficient of variation percentage of diameter in secretory units of crossbred Bengal goats have been depicted in Table 1. Analysis of variance (Table 2) of the diameter of different secretory units revealed no significant differences between sex and interaction between sex and secretory units. It was observed that the mean diameter (µm) of acinar secretory unit pooled over sexes was significantly ($P < 0.01$) 6.39 µm more than the tubular secretory unit.

The average height of glandular cells in acinar and tubular segments were 6.85 ± 0.19 µm and 6.99 ± 0.14 µm in adult male respectively. In female, the average epithelial height of acinar was 6.82 ± 0.19 µm and of tubular segment was 7.06 ± 0.15 µm. The mean ± S.E. alongwith C.V.% of epithelial height (µm) of secretory units in crossed Bengal goat have been presented in Table 3. Analysis of variance of the cell height (Table 4) showed no significant differences between sex, secretory units and interaction between sex and secretory units.

The average diameter of spherical nucleus was 4.18 ± 0.11 /um in adult male and 4.30 ± 0.11 /um in adult female . The average length of oval nucleus in the acini was 7.03 ± 0.20 /um in female having its average width 2.58 ± 0.11 /um in male 2.73 ± 0.10 /um in female . The average diameter of spherical nucleus of tubular segment measured 3.19 ± 0.05 /um in adult male and 3.22 ± 0.05 /um in adult female. The average length of oval nucleus was 4.88 ± 0.05 /um in male and 4.88 ± 0.05 /um in male and 4.99 ± 0.04 /um in adult female with average width of 2.58 ± 0.11 /um in male and 2.70 ± 0.10 /um in female.

4.1.2.2 Duct System :

The excretory duct system of parotid salivary gland of goat consisted of intercalated ducts, the intralobular ducts, the interlobular ducts and main parotid ducts. Trautmann and Fiebiger (1957), Banks (1981) and Stinson and Calhoun (1987) also described similar duct system in domestic animals. The intercalated duct was narrowest terminal segment of the duct system. It connected the glandular end pieces with intralobular duct (Figs. 14, 15). These terminal ducts were long and were lined with simple squamous to low cuboidal epithelium. The cytoplasm of the ductular epithelium appeared pale with comparatively hyperchromatic nucleus. Occasionally several intercalated ducts appeared to join

with each other before joining the intralobular duct (Fig. 16). The non-secretory epithelium of intercalated duct did not show the presence of myoepithelial cells. Trautmann and Fiebiger (1957) described that the intercalated ducts in the parotid salivary gland of domestic animals were lined by simple cuboidal epithelium with rare presence of squamous cells. The non secretory characters of the epithelial cells of intercalated duct were incontrast to the findings of Shear (1969), Tandler and Erlandson (1976), Suzuki, Nishinakagawa and Otuska (1981) reported presence of cytoplasmic granules in the epithelial cells of intercalated duct of rat, baboon and goat parotid salivary gland respectively.

The average diameter of intercalated duct measured 18.35 ± 0.45 /um in adult male and 18.22 ± 0.41 /um in adult female goat. The average height of the epithelium was 5.28 ± 0.14 /um in adult male and 5.25 ± 0.12 /um in female. The average diameter of spherical nucleus 3.65 ± 0.09 /um and 3.59 ± 0.08 /um in male and female respectively. The average size of oval nucleus was 5.93 ± 0.16 /um in length and 3.04 ± 0.08 /um in width of male goat. In female the average length of oval nucleus measured 5.65 ± 0.14 /um with 2.98 ± 0.09 /um in width.

The intralobular ducts appeared very tortous having lined with simple columnar epithelium. The intercalated ducts

opened in the intralobular ducts, thus establishing the connection between the intralobular duct with the secretory end pieces (Figs. 6, 9, 10, 11, 12, 13, 17). Trautmann and Fiebiger (1957) in domestic animals, Schakleford and Wilborn (1969) in bovine, Shear (1969) in rat, Tandler and Erlandson (1969) in baboon, Copenhaver, Kelly and Wood (1978) in man, Jerry, Boshell and Wilborn (1978) in pig, Banks (1981) in domestic animals, Barnwal and Sinha (1984) in buffalo and Stinson and Calhoun (1987) in domestic animals designated the intralobular duct as striated duct and was lined with simple cuboidal to simple columnar epithelium.

The average diameter of intralobular duct measured 47.18 ± 1.59 /um in male and 45.98 ± 1.55 /um in female. The average epithelial height of intralobular duct was measured 14.54 ± 0.32 /um in male and 13.95 ± 0.26 /um in female. The average diameter of spherical nucleus was 4.36 ± 0.10 /um in male and 4.33 ± 0.11 /um in female. The mean, S.E. and C.V.% of diameter (/um) of intercalated and intralobular duct in crossbred Bengal goats have been shown in Table 5. Analysis of variance (Table 6) of diameter of duct system revealed no significant difference between sex and interaction between sex and duct. However, there was significant difference ($P < 0.01$) between the diameter of two duct system in crossbred Bengal goats. It was observed that (Table 5) the mean diameter of intralobular duct pooled over sexes was 28.27 /um more than the intercalated duct.

The cytoplasm of the epithelium of intralobular duct was slightly acidophilic with basophilic dark, spherical or oval nucleus. The nucleus was usually placed paracentrally towards the base of the epithelial cells. The basal striations were not clearly visible. However, with Altmann methods, fine granular refractile mitochondria were discerned in these cells. The present findings were in agreement with the findings of Pal, Chandra and Bharadwaj (1972) and Barnwal and Sinha (1984) who failed to record basal striations in the epithelial cells of the intralobular duct of buffalo parotid salivary glands. Although Tandler and Erlandson (1976) in baboon and Stinson and Calhoun (1987) in domestic animals clearly recorded the basal striations in the epithelial cells of intralobular striated duct due to vertical orientation of mitochondria.

The interlobular ducts of parotid salivary gland in goat were found in the interlobular spaces surrounded by interlobular connective tissue, blood vessels, lymphatic and nerves. Several intralobular ducts were seen to open in the interlobular duct, which was suggestive for the formation of interlobular duct by the union of several intralobular ducts in the interlobular spaces (Fig. 9). The epithelium of interlobular duct was simple cuboidal to simple columnar type (Fig. 17). The nucleus of the epithelial cell was faintly basophilic and vesicular. The

cytoplasm of the cell was faintly eosinophilic or paler in colour. The intial portion of duct presented regular spherical lumen which became irregular and wide towards the terminal ends before joining the other interlobular ducts to form main excretory duct (Fig. 7). The duct epithelium at initial segment did not reveal the presence of goblet cells. However, towards the terminal end the epithelium contained goblet cells as well (Fig. 18). The average epithelial height of interlobular duct measured 16.04 ± 0.52 /um in male and 16.01 ± 0.48 /um in female.

Trautmann and Fiebiger (1957) described that the interlobular excretory duct of parotid salivary gland in domestic animals were lined by simple columnar epithelium in the begining and the larger ducts were lined with two layered columnar epithelium. Pal, Chandra and Bharadwaj (1972) recorded that the interlobular duct epithelium in parotid salivary gland of buffalo was of high cuboidal or columnar type containing goblet cells which increased in number towards the oral cavity. The present findings were in agreement with the findings of Pal, Chandra and Bharadwaj (1972), Barnwal and Sinha (1984) in buffalo and Stinson and Calhoun (1987) in domestic animals however, reported that the interlobular ducts of parotid salivary gland were lined by simple columnar epithelium to stratified columnar epithelium. The occurrence of the goblet cells

in the epithelial lining of interlobular duct could be well compared with the findings of Pal, Chandra and Bharadwaj (1972) in buffalo, Barnwal and Sinha (1984) in buffalo and Yasear and Ibrahim (1992) in sheep who recorded the presence of goblet cells in the interlobular ducts. However, goblet cells were lacking in duct system of human parotid gland (Munger, 1964).

The main excretory duct was formed by the union of various wide and irregular lumen interlobular ducts (Figs. 7, 19). The intial and middle portion of the main excretory duct was lined by pseudostratified to stratified columnar epithelium surrounded by perieptheliał connective tissue sheath predominantly made up of collagen fibres. The terminal part of the duct near the opening in the oral cavity was lined with nonkeratinized type of stratified squmous epithelium (Fig. 20). The pseudostratified to stratified columnar epithelial lining of the main excretory duct frequently revealed the distribution of intra epithelial glands formed by the clusters of mucous cells and a few isolated goblet cells (Fig. 19). The cytoplasm of the cell appeared vacuolated with peripherally placed hyperchromatic nucleus. The average epithelial height of main duct, lined by pesudostratified to stratified columnar epithelium with intraepithelial gland was 30.0 $\pm$ 0.24 μm in male 29.35 $\pm$ 0.33 μm in female. The epithelial height of stratified squmous

epithelium of main duct measured 46.38 ± 0.29 /um in male 46.51 ± 0.28 /um in female. Pal, Chandra and Bharadwaj (1972) and Barnwal and Sinha (1984) in buffalo reported intra-epithelial glands and cyst in the main parotid duct. The present findings on the distribution of entraepithelial glands and a few goblet cells were in agreement with the findings of Pal, Chandra and Bharadwaj (1972) and Barnwal and Sinha (1984). The present observation in goat was incontrast to the findings of yeasear and Ibrahim (1992) who observed the presence of goblet cells as constant feature of stratified cuboidal or squmous epithelium of ductal system in the parotid salivary gland of sheep.

The mean ± S.E. alongwith C.V.% of epithelial height of duct system in crossbred Bengal goat have been shown in Table 7. Analysis of variance (Table 8) of epithelial height of duct system revealed no significant difference between sex and interaction between sex and duct system. However, there was significant difference ($P < 0.01$) between the epithelial height of different duct system.

The epithelial height pooled over sexes was found to be highest (46.44 /um) in main duct system whereas it was lowest (5.25 /um) in intercalated duct system. The epithelial height of main duct was significantly more by 41.19 /um, 32.20 /um and 30.41 /um than intercalated, intralobular and

interlobular ducts respectively. The epithelial height of interlobular duct was observed to be 10.78 /um and 1.79 /um significantly ($P < 0.01$) more than intercalated and intralobular duct. Besides, it was also observed that the overall mean epithelial height of intralobular duct was significantly more by 8.99 /um than the overall mean of intercalated duct (Table 7).

TABLE - 1

Mean ± S.E. alongwith C.V.% of diameter (/um) of secretory units in crossed Bengal goats.

Secretory units	SEX Male Mean ± S.E.	C.V.%	Female Mean ± S.E.	C.V.%	Over all mean
Acinar	20.50±0.45	16.28	20.68±0.45	16.39	20.59^{a}
Tubular	14.17±0.23	12.35	14.23±0.26	13.49	14.20^{b}
Over all mean	17.33^{a}		17.46^{a}		

Means with different superscripts differed significantly ($P < 0.01$) both in row and column separately.

TABLE - 2

Analysis of variance of diameter (/um) of secretory units in crossed Bengal goats.

Source of variation	Degree of freedom	Mean squares	F
Between sex	1	0.817259	0.111^{NS}
Between secretory units	1	2207.746	299.898^{XX}
Sex X Secretory units	1	0.203	0.027^{NS}
Error	212	7.361	

NS : Non Significant

XX : Significant at $P < 0.01$

TABLE - 3

Mean ± S.E. alongwith C.V.% of cell heights (/um) of secretory units in crossed Bengal goats.

Secretory units	SEX				Over all mean
	Male Mean ± S.E.	C.V.%	Female Mean ± S.E.	C.V.%	
Acinar	6.85±0.19	20.43	6.82±0.19	21.40	6.83^{a}
Tubular	6.99±0.14	15.59	7.06±0.15	15.86	7.03^{a}
Over all mean	6.92^{b}		6.94^{b}		

Means with same superscripts both in row and column separately did not differ significantly.

TABLE - 4

Analysis of variance of cell heights (/um) of secretory units in crossed Bengal goat.

Source of variation	Degree of freedom	Mean square	F
Between sex	1	0.018	0.010^{NS}
Between secretory units	1	1.972	1.196^{NS}
Sex X secretory units	1	0.129	0.078^{NS}
Error	212	1.649	

NS : Non Significant

TABLE - 5

Mean ± S.E. alongwith C.V.% of diameter (/um) of duct system (Intercalated and Intralobular) in crossed Bengal goats.

Duct	SEX				Over all mean
	Male Mean ± S.E.	C.V.%	Female Mean ± S.E.	C.V.%	
Intercalated	18.35±0.45	18.20	18.22±0.41	16.79	18.29^{a}
Intralobular	47.18±1.59	24.84	45.98±1.55	24.77	46.58^{b}
Over all mean	32.76^{a}		32.10^{a}		

Means with different superscripts differed significantly (P < 0.01) both in row and column separetly.

TABLE - 6

Analysis of variance of diameter (/um) of duct system (Intercalated and Intralobular) in crossed Bengal goats.

Source of variation	Degree of freedom	Mean squares	F
Between sex	1	22.93	0.318^{NS}
Between duct	1	43237.79	600.64^{XX}
Sex X duct	1	16.57	0.23^{NS}
Error	212	71.98	

NS : Non Significant

XX : Significant at (P < 0.01).

TABLE - 7

Mean ± S.E. alongwith C.V.% of epithelial height (/um) of duct system in crossed Bengal goats.

Ducts	SEX				Over all Mean
	Male Mean±S.E.	C.V.%	Female Mean±S.E.	C.V.%	
Intercalated	5.28± 0.14	20.26	5.25± 0.12	18.09	5.25^{a}
Intralobular	14.54± 0.32	16.23	13.95± 0.26	13.95	14.24^{b}
Interlobular	16.04± 0.52	24.06	16.01± 0.48	22.42	16.03^{c}
Main	46.38± 0.29	4.67	46.51± 0.28	4.55	46.44^{d}
Over all mean	20.56^{a}		20.43^{a}		

Means with different superscript differed significantly ($P < 0.01$) both in row and column separetly.

TABLE - 8

Analysis of variance of epithelial height (/um) of duct system in crossed Bengal goats.

Source of variation	Degree of freedom	Mean squares	F
Between sex	1	1.85	0.30^{NS}
Between duct	3	34715.07	5714.88^{XX}
Sex X duct	3	2.60	0.429^{NS}
Error	424	6.07	

NS : Non Significant

XX : Significant at ($P < 0.01$).

4.2.2 PARENCHYMA :

4.2.2.1 Secretory end Pieces :

The glandular cells of the secretory units were mildly positive for periodic Acid Schiff reaction but negative for Munihaematin stain. These staining reactions were suggestive for absence for mucopolysaccharides in the secretory cells (Table 9). The mild periodic Acid Schiff positivity was probably due to the presence of glycoprotein similar to the findings of Ito and Asano (1961) in the bovine parotid gland. The present findings were, however, not in agreement with the findings of Cantatore (1959), Dauncie and Posinovec (1962), Morelli and Perucci (1963), Snkurko (1963), Pal, Chandra and Bharadwaj (1972) and Barnwal and Sinha (1985) in different mammals including large ruminants reported the variable presence of periodic Acid Schiff positive muco substances in the secretory cells of parotid salivary gland.

The secretory cells were negative for acid mucopolysaccharide when stained with colloidal iron and Aldehyde fuchsin stains. Morelli and Perucci (1963) in men and oxen, Pal, Chandra and Bharadwaj (1972) in buffalo and Barnwal and Sinha (1985) in buffalo, however reported the presence of acid mucopolysaccharide in these cells. The present finding was however inagreement with the findings of Munger (1964) who recorded indistinct positive reaction for colloidal iron

4.2 HISTOCHEMISTRY :

4.2.1 Capsule and Supporting Tissue :

Histochemically the capsule, connective tissue septae between the lobules and connective tissue network around the secretory end pieces and intralobular ducts of the lobule reacted mildly with periodic Acid Schiff reaction, colloidal iron reaction and Mercury Bromophenol blue (Figs. 21, 22). However, the supporting tissues were negative for glycogen and alkaline phosphatase. The nuclei of connective tissue cells showed mild to moderate reaction for D N A. Oil red O positive neutral fat globules were moderately distributed in the capsule and the larger interlobular septae (Fig. 28). Occasionally a few lipid droplets were also distributed in the connective tissue around the intralobular duct (Table 9).

Carpenter (1968) in human, Ham (1969) in human and Copenhever, Kelly and Wood (1978) in mammals also reported the presence of fat cells in the connective tissue of parotid salivary gland. According to Dellmann and Brown (1987) the periodic Acid Schiff reactivity of connective tissue fibres were due to presence of the thin layer of glycoprotein around the argyrophilic reticular fibres.

and Alcian blue in the serous cells of human parotid salivary gland. Similarly Jerry, Boshell and Wilborn (1978) indicated the paucity of neutral mucins and absence of sialo and sulfomucins. The secretory cells of both sexes were negative for glycogen with Best's carmine stain and Periodic Acid Schiff reaction with saliva digestion which was inconsonace to the findings of Pal, Chandra and Bharadwaj (1972) who reported the absence of glycogen in the secretory cells of buffalo parotid salivary gland. Although Vignoli and Noguira (1981) demonstrated glycogen in the secretory tubules of the parotid salivary glands in Zebu cattle.

The Feulgen reaction demonstrated mild to moderate positivity of D N A in the nucleus of glandular cells. The variablity of Feulgen reaction was suggestive for functional status of nucleus of secretory cells (Table 9).

With Mercury Bromophenol blue technique, the secretory cells of parotid salivary gland in male and female goats reacted moderately for protein and peptides (Fig. 27). Vignoli and Noguira (1981) reported the positive reaction for protein in the parenchyma of parotid salivary gland of Zebu cattle. Barnwal and Sinha (1985) also noted the presence of NH_2 bound protein and tyrosin in the secretory cells of buffalo parotid salivary glands.

The secretory cells reacted negatively for oil red O stain suggesting the absence of neutral fat in the cytoplasm although Jerry, Boshell and Wilborn (1978) reported the

presence of lipid in the serous cells of the pig perotid gland. Similarly the present finding in goat was incontrast with the findings of Barnwal and Sinha (1985) in buffalo and Singh, Pawar and Roy (1993) in castrated donkey, who reported the presence of lipid droplets in the acinar cells.

The acinar cells were non reactive for alkaline phosphatase enzyme. Hill and Brown (1954) in rat, cat and mouse, Pal, Chandra and Bharadwaj (1972) in buffalo, Barnwal and Sinha (1985) in buffalo and Singh, Pawar and Roy (1993) in castrated donkey reported variable degree of positivity for alkaline phosphatase in the secretory cells of parotid salivary gland. Singh, Pawar and Roy (1995) further reported strong reaction for alkaline phosphatase in myoepithelial cells, which were not clearly demonstrable in the present investigation.

4.2.2.2 Duct System :

The simple epithelium of the intercalated duct, striated duct/intralobular duct and intial part of the interlobular duct not having the goblet cells reacted variable degree of mild positivity for Periodic Acid Schiff reaction. The mucihaematin stain did not show positive reaction to these epithelial cells, the goblet cells lining the interlobular and mainduct and intra epithelial glands of main excretory duct reacted strongly positively for PAS reaction

and mucihaematin stain (Table 9; Figs. 21, 22, 25). Such staining affinities were suggestive for presence of neutral mucopolysaccharides in the goblet cells and intra epithelial glands with a trace of glycoprotein in other epithelial cell lining of duct system. It was in contrast to the findings of Hill and Brown (1954) reported negative reaction for PAS in the epithelial cells of the duct system in the parotid salivary glands of cat, rat and mouse. The present findings tallied with Shear (1969) who reported PAS positive glyco-progein in the intercalated duct of rat parotid salivary gland. Pal, Chandra and Bharadwaj (1972) also reported mild PAS positivity in the epithelial cells of interlobular duct of the buffalo perotid salivary gland. Yasear and Ibrahim (1992) also recorded the PAS positive goblet cells in the duct of sheep parotid salivary gland.

The ductular epithelium in goat parotid salivary gland were negative for sulfated and acid mucopolysaccharides except for goblet cells and intra epithelial gland of main duct which were intensely positive with colloidal iron reaction and moderately with Aldehyde Fuchsin stain (Figs. 23, 24, 26). This findings could be co-related with the findings of Morelli and Perucci (1963) who reported the distribution of acid and neutral mycopolysaccharide the epithelium of excretory system of parotid salivary gland in men and Oxen. However, the present observations on goat did not tally with the findings of Yasear and Ibrahim (1992) who reported that the goblet

cells of the duct in sheep parotid salivary glands showed the presence of only neutral mucopolysaccharide and were negative for Alcian blue.

The epithelial cells of the duct system did not show the presence of glycogen granules when stained with PAS saliva digestion and Best carmine method. Although Fava de Moraes, Giuffrida and Junquira (1967) found the glycogen positivity in the striated duct of mammalian salivary glands. Similarly Vignoli Noguira (1981) in Zebu cattle also reported the presence of glycogen in the ducts of parotid salivary gland.

Histochemically, the epithelial cells of the duct system in the parotid salivary gland of goat were negative for the presence of alkaline phosphatase. Similar findings were reported by Hill and Brown (1954) in rat, cat and mouse, Kawakatsue et al. (1959) in mouse and dog and Singh, Pawar and Roy (1995) in castrated donkey.

The ductular epithelium, in general, was mildly positive for proteins and peptides (Fig. 27). The nuclei of ductular epithelial cells were mild to moderatly positive for D N A when treated for Feulgen reaction. Stongest D N A positivity was in the nuclei of the epithelial cells lining the intercalated duct (Table 9).

TABLE - 9

HISTOCHEMICAL CHARACTERS OF VARIOUS COMPONENTS IN PAROTID SALIVARY GLAND OF GOAT

Components	P A S	A F	C I	M H	B C	M B B	F R (D N A)	O R O	A K P
(A) Stroma									
* Capsule	+	+ (E F only)	+	-	-	+	+ to ++	++	+ (only at luminal margin of blood vessels)
* Interlobular septa	+	+ (E F only)	+	-	-	+	+ to ++	++	+ (only at luminal margin of blood vessels)
* Intralobular septa	+	+ (E F only)	+	-	-	+	+ to ++	±	+ (only at luminal margin of blood vessels

Components	P A S	A F	C I	M H	B C	M B B	F R (D N A)	O R O	A K P
(B) Parenchyma									
* Glandular epithelium	+	-	-	-	-	++	+ to ++	-	-
* Epithelium of intercalated duct	+	-	-	-	-	+	++ to +++	-	-
* Epithelium of intralobular duct	+	-	-	-	-	+	+ to ++	-	-
* Epithelium of interlobular duct	+	-	-	-	-	+	+ to ++	-	-
* Epithelium of main duct	+	-	-	-	-	+	+ to ++	-	-
* Goblet cells of epithelium	+++	++	+++	+++	-	+	+	-	-
* Intraepithelial glands of main duct	+++	++	+++	+++	-	+	+	-	-

(-) Negative, (+) Mild, (++) Moderate, (+++) Intense, (±) Occasional, (PAS) Periodic Acid Schiff, (AF) Aldehyde Fuchsin, (CI) Colloidal Iron, (MH) Mucihaematin, (BC) Best Carmine, (MBB) Mercury-Bromophenol blue, (FR) Feulgen reaction, (ORO) Oil Red O, (AKP) Alkaline Phosphatase, (EF) Elastic fibres.

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Histological and certain histochemical observations were studied of twelve crossbred Bengal goats of 1½ to 3½ years of age. Out of twelve goats six were male and rest were female.

5.1 Histology :

5.1.1 Capsule and Supportive Tissue :

The parotid salivary gland of goat was invested with dense irregular connective tissue containing more collagen fibres with fine reticular fibres and a little amount of elastic fibres. The connective tissue cells mainly comprised of fibrocytes. The blood vessels, nerves, lymphatic spaces and clusters of unilocular adipocytes were also observed in the capsule. The smooth muscle fibres were not recorded in the capsule.

The connective tissue septae arising from the capsule divided the gland into various lobes and lobules. The interlobular septae traversed with the interlobular ducts, blood vessels, nerves, lymphatics. The perivascular connective tissue did not exhibit mast cells, however occasional distribution of plasma cell was noticed. Fine networks of reticular fibres and collagen fibres surrounded the secretory end pieces. The elastic fibres were altogether absent in the intralobular connective tissue, except for the area around the intralobular

duct. At certain areas of stromal tissue, diffused lymphoid tissue appeared.

5.1.2 Parenchyma :

The parenchyma of the parotid salivary gland was made up of tubuloacinar type of secretory units associated with various subdivision of excretory ducts.

The secretory end pieces of the gland in goat comprised of glandular tubules terminating into acini. The glandular cells were mostly pyramidal in shape having spherical to oval nucleus. The cytoplasm contained variable amount of acidophilic granules which were more concentrated at superanuclear position. The gland in the goat was of purely serous type. The variability in the distribution of eosinophalic zymogen granules alongwith change inthe nuclear profile resulted in the distribution of three definite cell types viz., secretory, exhausted and resting cells. With the available histological technics the distribution of myoepithelial cells around the acini or tubule could not be ascertained. Statistical analysis revealed no significant difference ($P < 0.01$) in the diameter of different secretory units of the gland between sex and interation between sex and secretory units. However, the mean diameter of acinar secretory units pooled over sexes was significantly more than tubular secretory units. Further analysis showed that the cell height of different secretory units did not differ significantly ($P < 0.01$) between sex, secretory units and intraction between sex and secretory units.

The excretory duct system of parotid salivary gland of goat consisted of intercalated ducts, the intralobular duct, the interlobular ducts and main parotid duct. The intercalated duct was narrowest terminal segment of the duct system that connected the glandular end pieces with intralobular duct. These terminal ducts were long and were lined with simple squmous to low cuboidal epithelium. The cytoplasm of the ductular epithelium appeared pale with compartively hyperchromatic nucleus. Occasionally, several intercalated ducts appeared to join with each other before joining the intralobular duct. The epithelium of intercalated duct did not show the presence of myoepithelial cells. The intralobular ducts appeared very tortuous having lined with simple columnar epithelium. The basal striations were not clearly visible, however, with Altmann method fine refractile mitochondria were discerned in these cells. The interlobular ducts of this salivary in goat were surrounded by interlobular connective tissue, blood vessels, lymphatic and nerves. The epithelium of interlobular duct was simple cuboidal to simple columnar type. The nucleus of the epithelial cell was faintly basophilic and vesicular. The cytoplasm of the cell was faintly eosinophilic or paler in colour. The initial segment did not reveal the presence of goblet cells. However, towards the terminal end the epithelium contained goblet cells as well.

The main excretory ducts was formed by the union of interlobular ducts. The intial and middle portion of the main

excretory duct was lined by pseudostratified to stratified columnar epithelium surrounded by peripthelium connective tissue sheath, made up of predominantly collagen fibres. The terminal part of the duct near its opening in the oral cavity was lined with nonkeratinized type of stratified squamous epithelium. The pseudostratified to stratified columnar epithelial lining of the main excretory duct frequently revealed the distribution of intraepithelial glands formed by the clusters of mucous cells and a few isolated goblet cells. The cytoplasm of the such glandular cell appeared vacuolated with peripherally placed hyperchromatic nucleus. Statistically there was no significant difference in the diameter of intercalated duct and intralobular duct between sex and interaction between sex and duct. However, there was significant difference ($P < 0.01$) between the diameter of two duct system in the parotid salivary gland of crossbred Bengal goats. It was further revealed that there was no significant difference in the epithelial height of the intercalated, intralobular, interlobular and main excretory ducts between sex and interaction between sex and duct. However, there was significant difference ($P < 0.01$) between the epithelial height of different ducts.

5.2 Histochemistry :

The capsule and all connective tissue septae of the gland were mildly reactive with Periodic Acid Schiff (PAS), colloidal iron and Mercury Bromophenol blue but negative with mucihaematin. The supporting tissues were negative for glycogen and alkaline phosphatase. The nuclei of connective tissue cells showed mild to moderate reaction for DNA. Fat globules were moderately distributed in the capsule and larger interlobular septae. Occasionally a few lipid droplets were found in the connective tissue around the intralobular duct.

The glandular cells of the secretory units were mildly positive for Periodic Acid Schiff reaction but negative for mucihaematin stain, suggesting the absence of mucopolysaccharides in the secretory cells. The secretory cells were negative for glycogen, when stained with Best's carmine and PAS with saliva digestion. The Feulgen reaction demonstrated mild to moderate positivity of DNA in the nucleus of glandular cells. The variability of Feulgen reaction was suggestive for functional status of nucleus of secretory cells.

With Mercury Bromophenol blue technique, the secretory cells of parotid salivary gland reacted moderately for protein and peptides.

The secretory cells reacted negatively for Oil Red O stain suggesting the absence of neutral fat in the cytoplasm.

The secretory cells did not show alkaline phosphatase enzyme.

The epithelium of the intercalated duct, striated duct/ intralobular duct and intial part of the interlobular duct not having goblet cells reacted positive mildly for Periodic Acid Schiff reaction but negative with mucihaematin stain. The goblet cells of interlobular duct and main duct and intra-epithelial gland of main excretory duct reacted strongly positive for Periodic Acid Schiff reaction and mucihaematin stain. Such affinities were suggestive for presence of neutral mucopolysaccharides in the goblet cells and intraepithelial gland with a trace of glycoprotein in other epithelial cell lining of duct system.

The ductular epithelium of goat parotid salivary gland were negative for sulfated and acid mucopolysaccharide except for goblet cells and intraepithelial gland of main duct which were intensely positive with colloidal iron reaction and moderatly with Aldehyde Fuchsin stain. The epithelial cells of duct system did not show the presence of glycogen granules when stained with PAS with saliva digestion and Best carmine method. Histochemically, the epithelial cells of the duct system in the parotid salivary gland of goat were negative for the presence of Alkaline phosphatase.

The ductular epithelium, in general, was mildly positive for protein and peptides. The nuclei of ductular

epithelium were mild to moderatly positive for D N A when treated for Feulgen reaction. Strongest D N A positivity was in the nuclei of the epithelial cells lining the intercalated duct.

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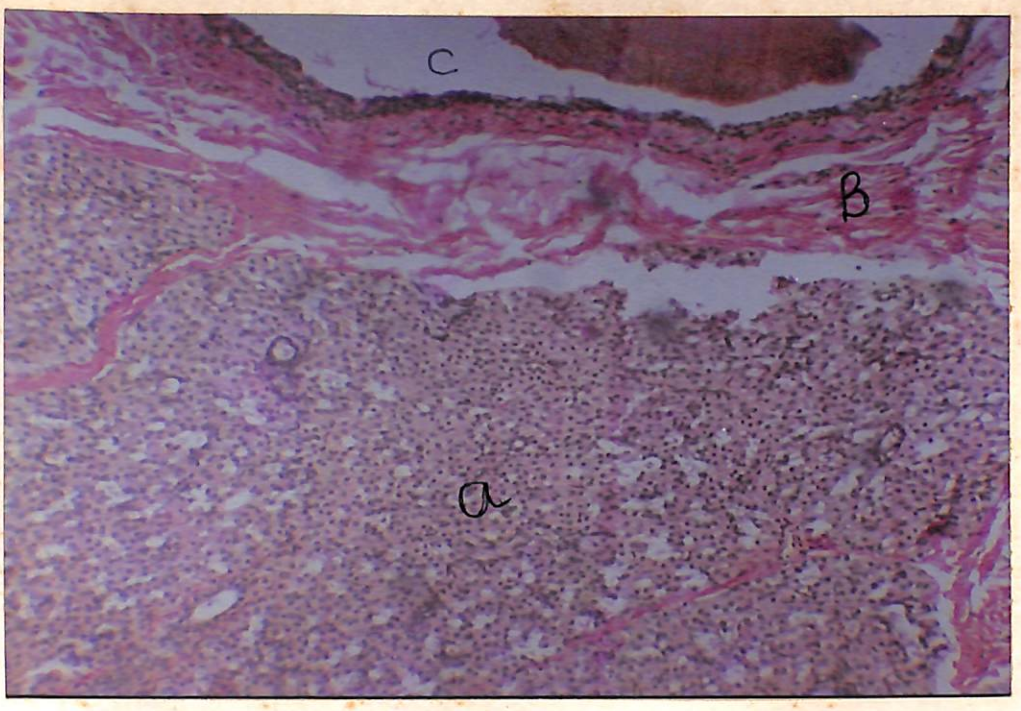
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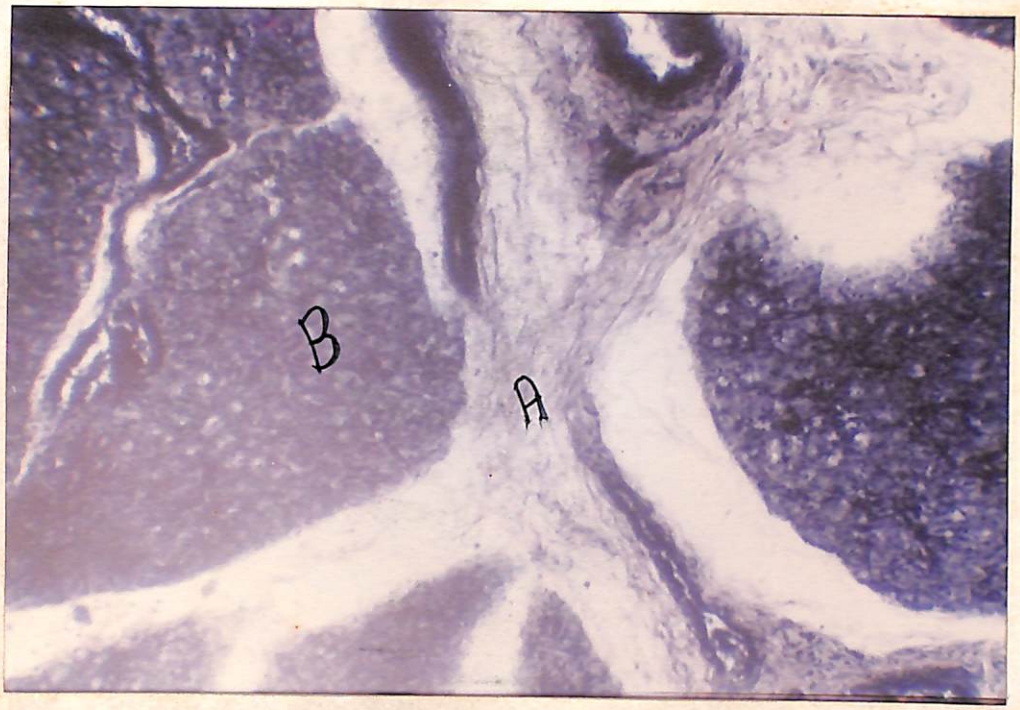
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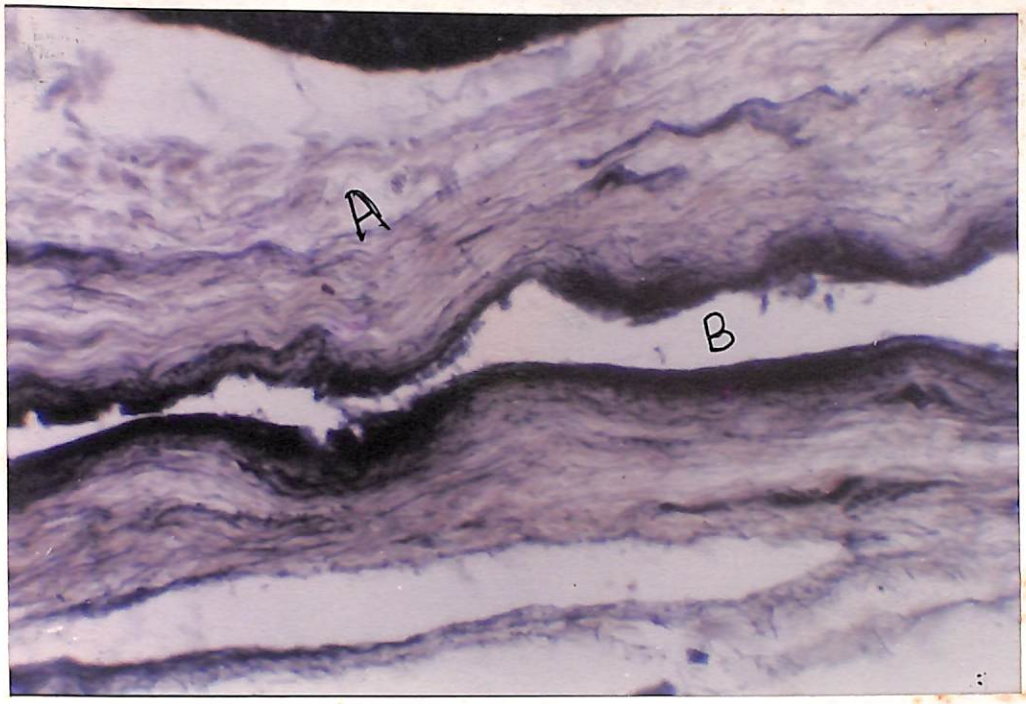
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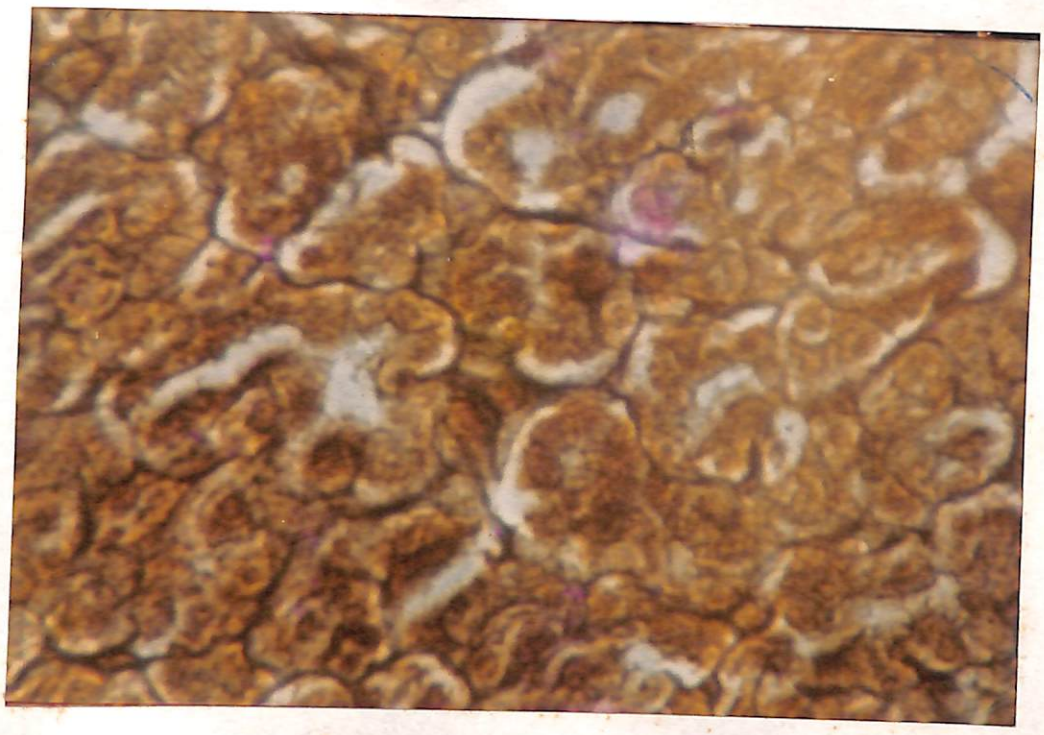

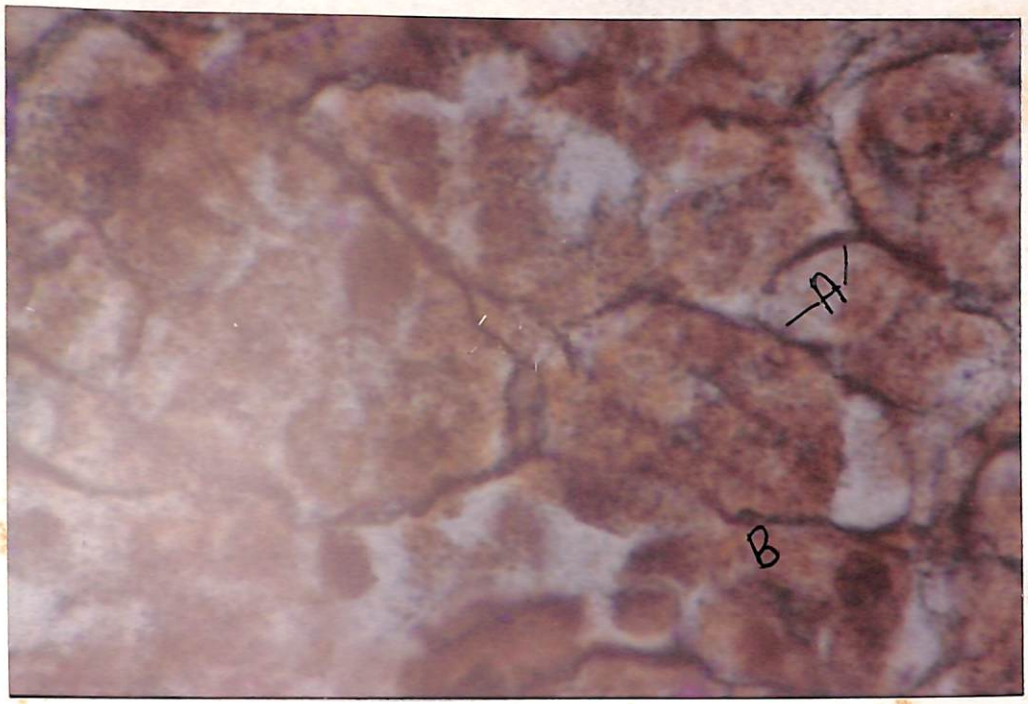
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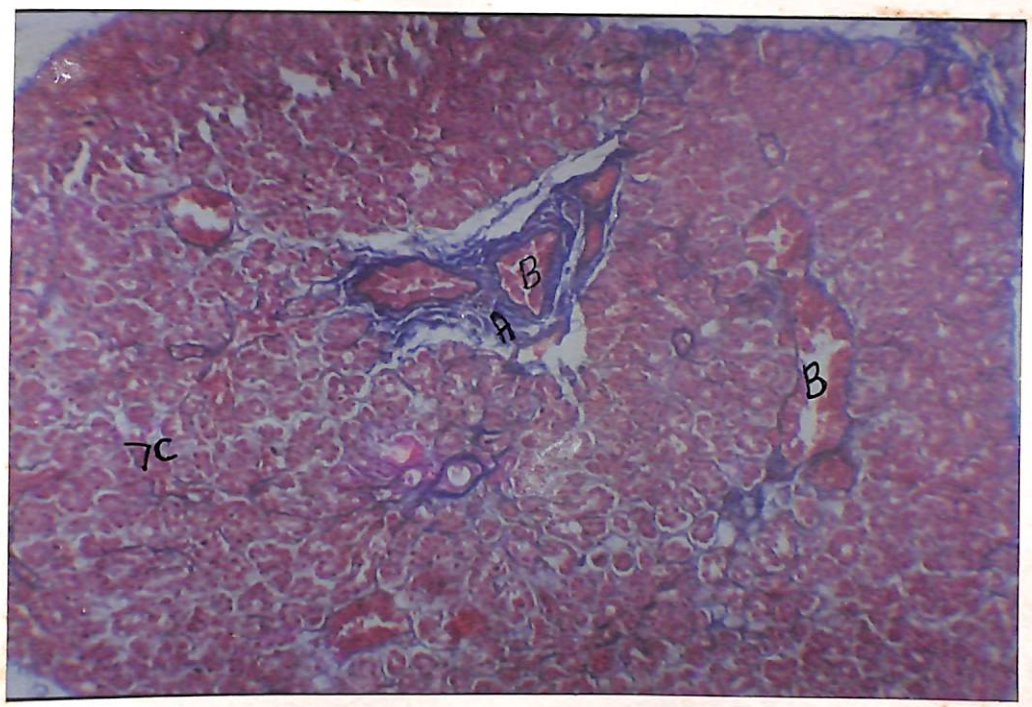
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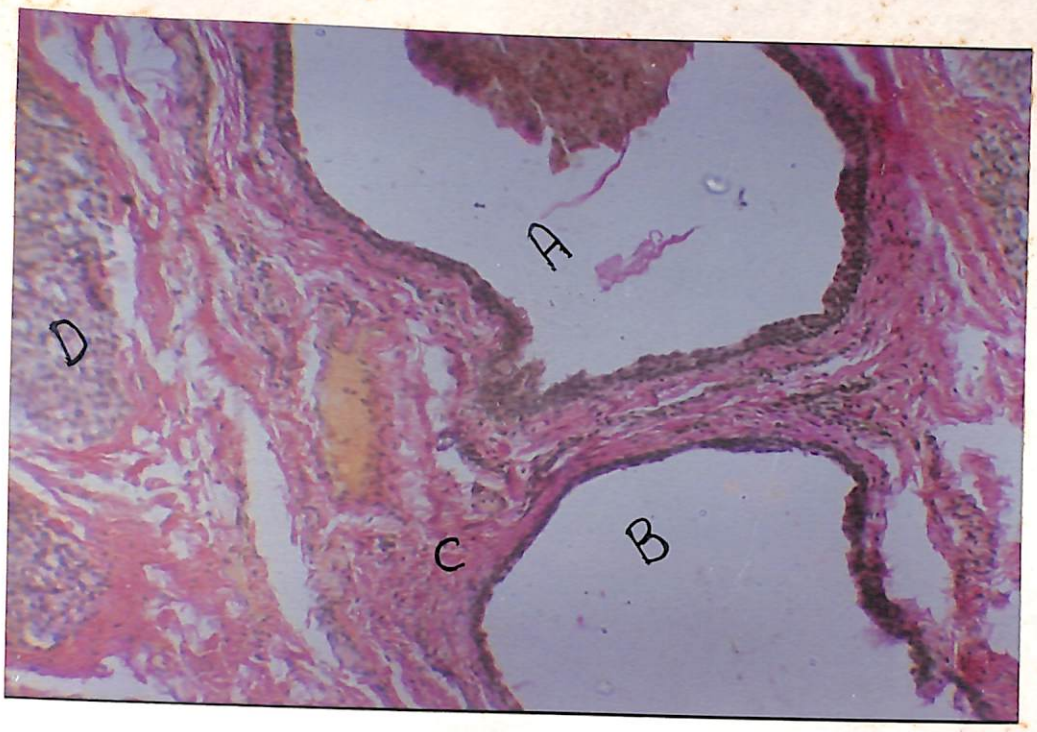
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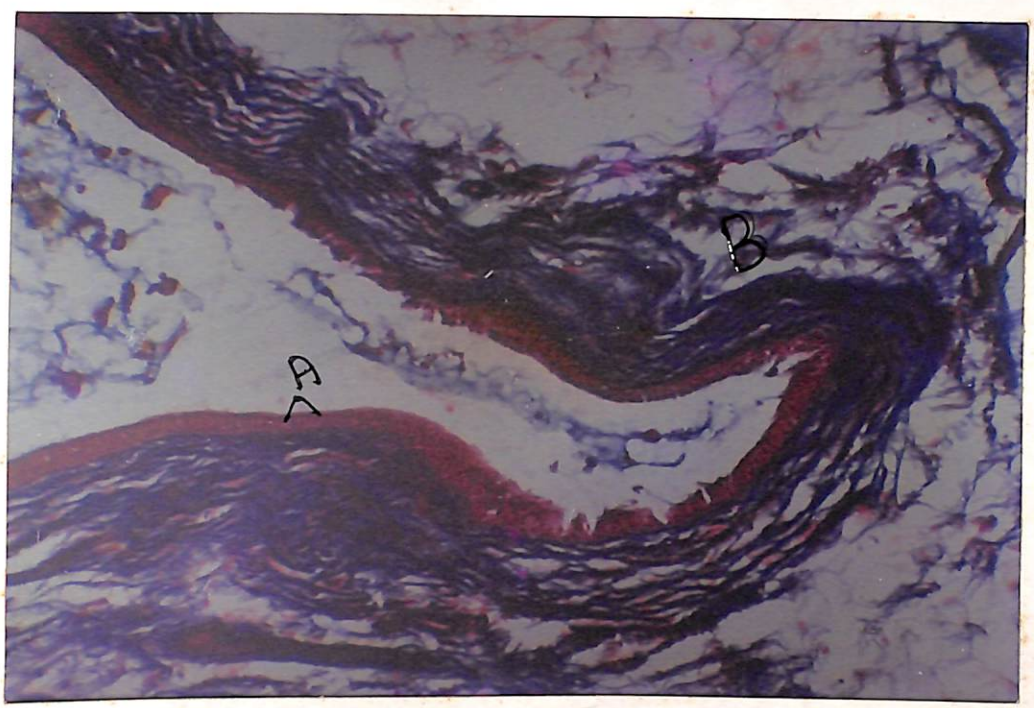
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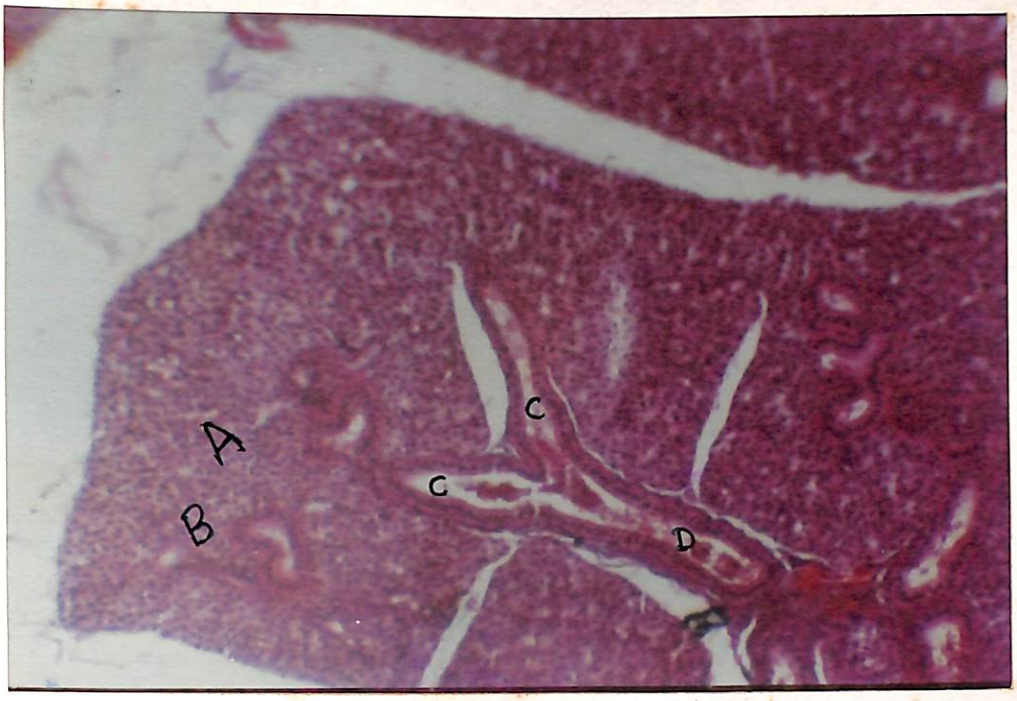
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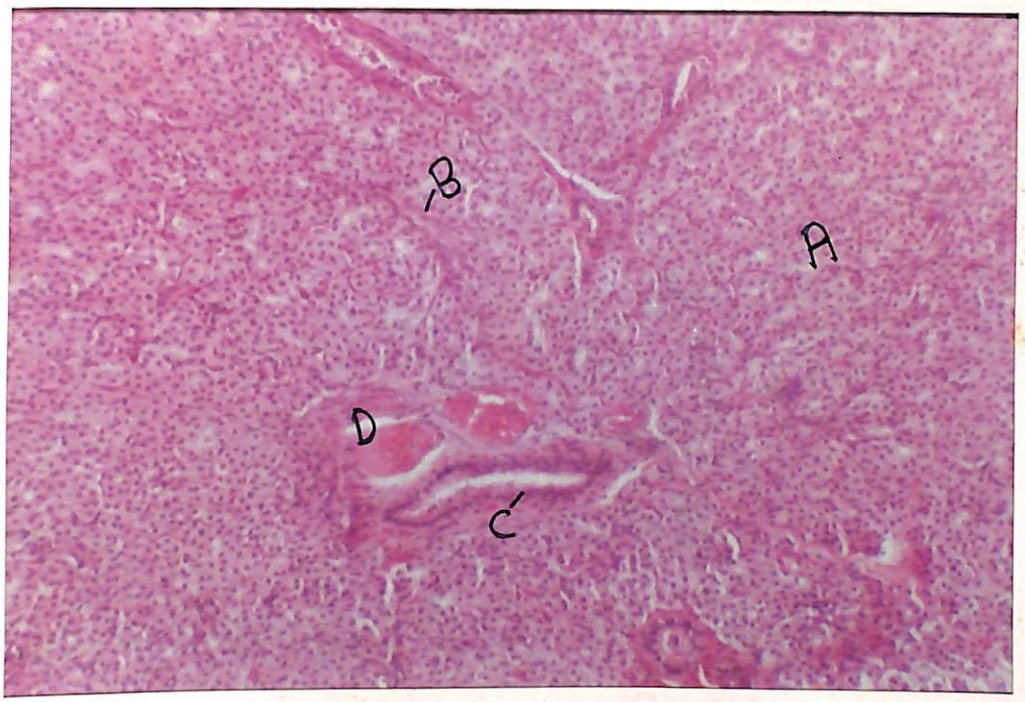
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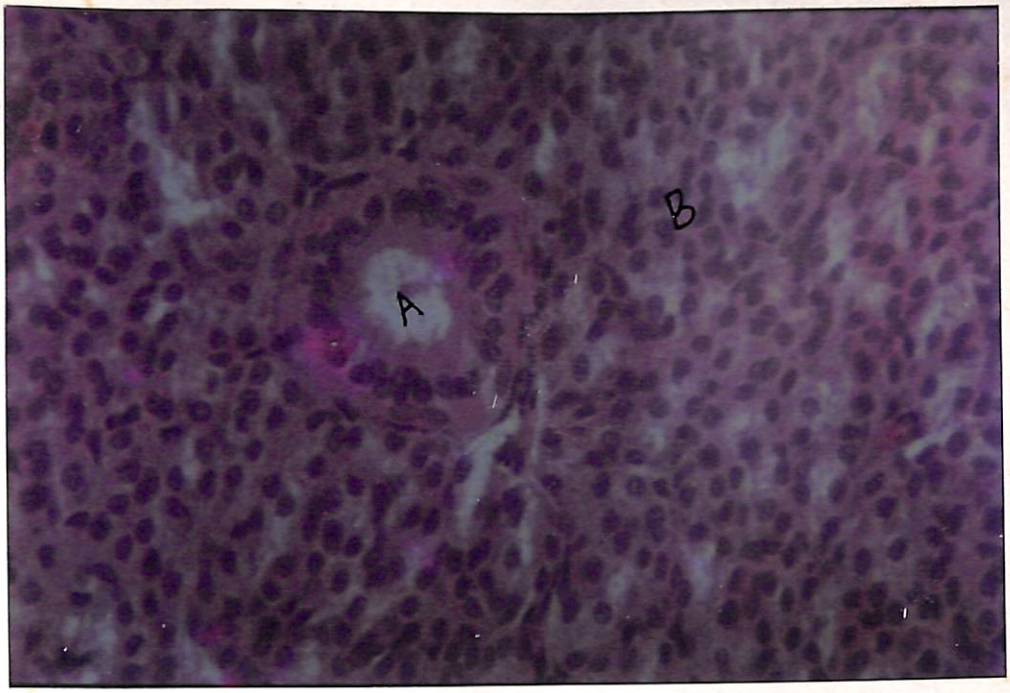
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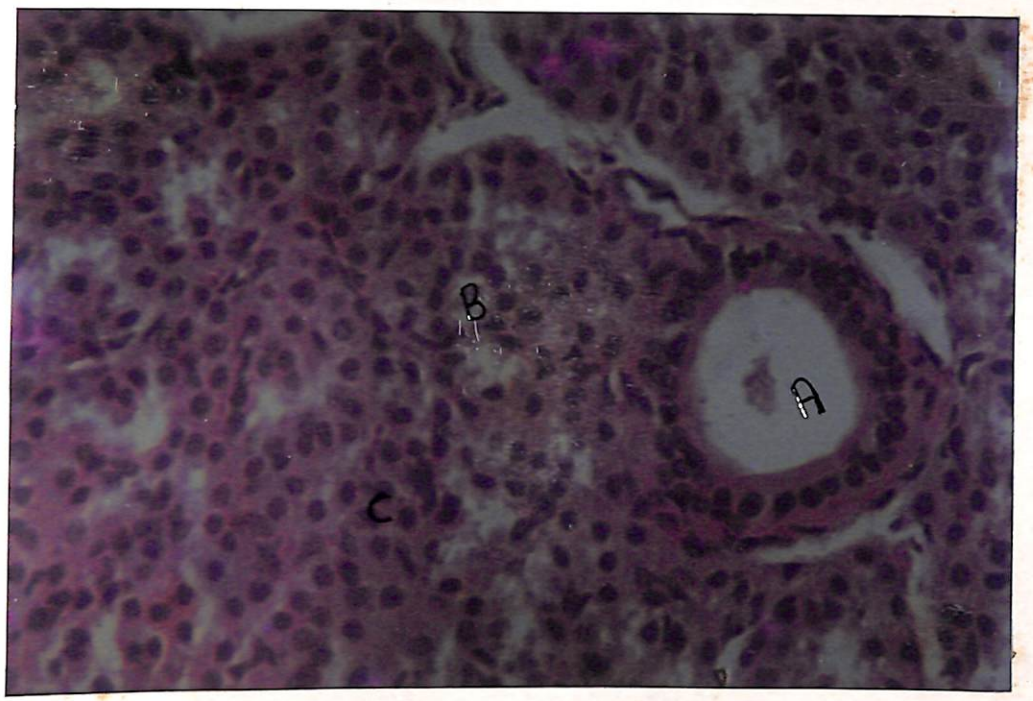
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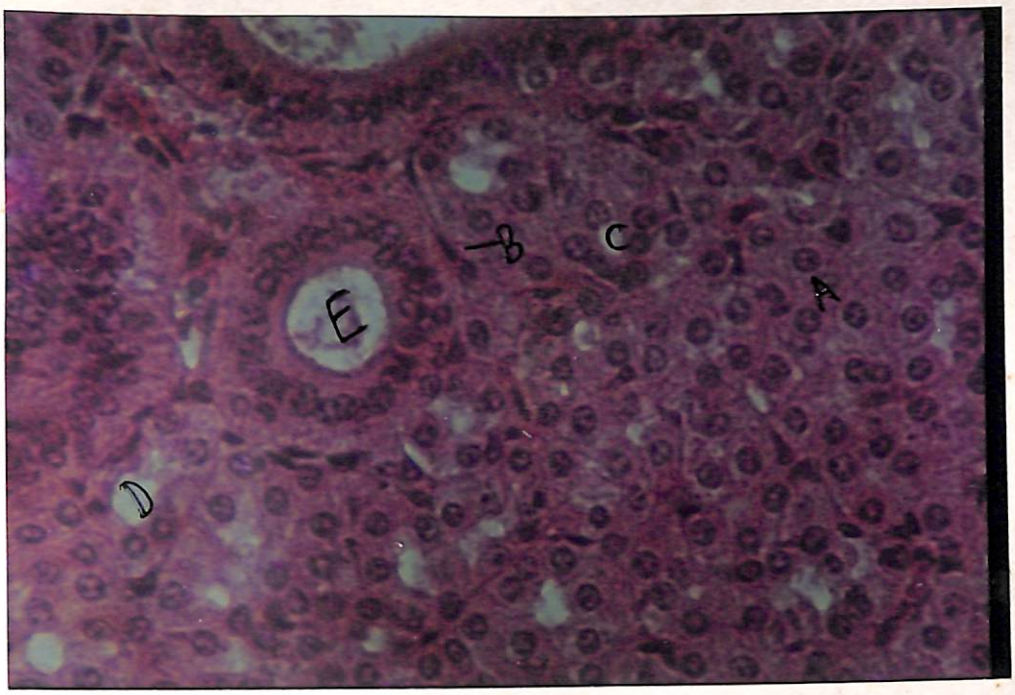
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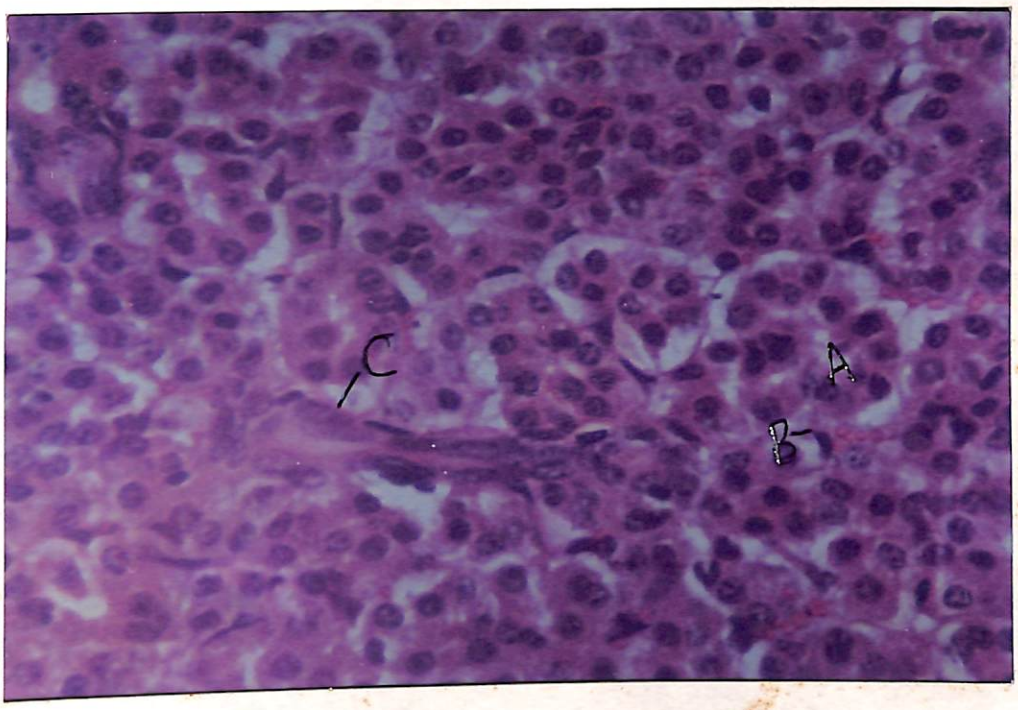
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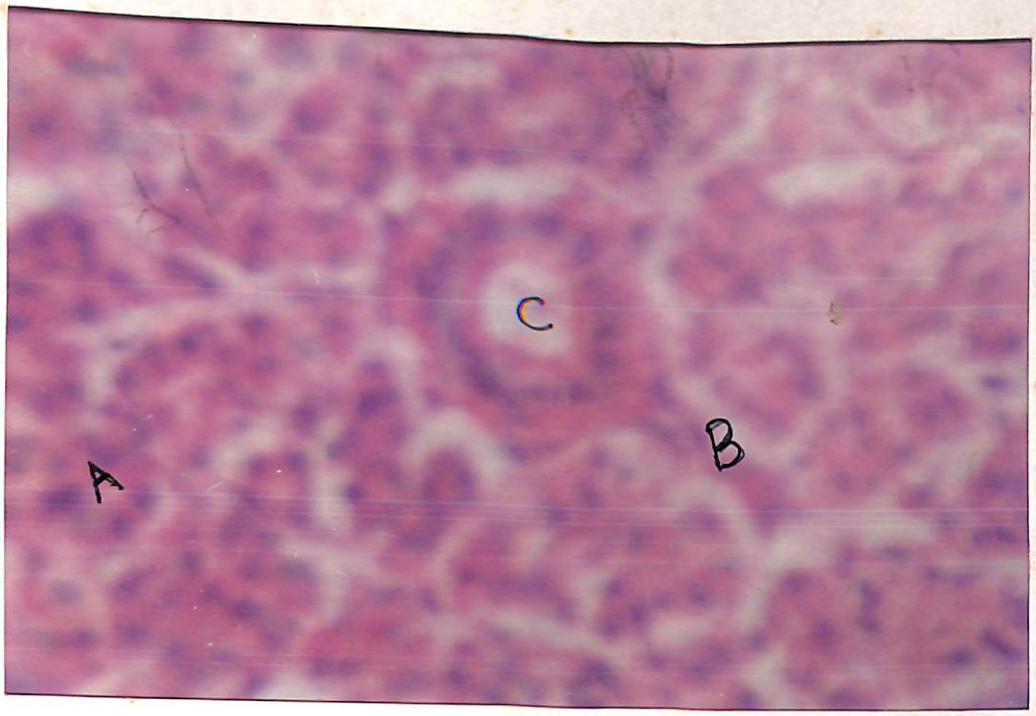
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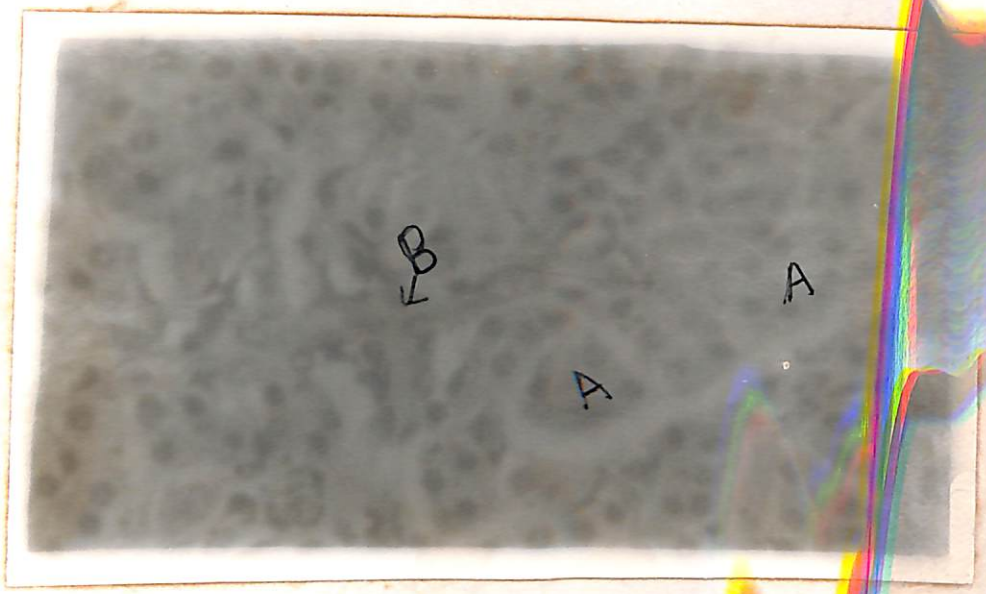
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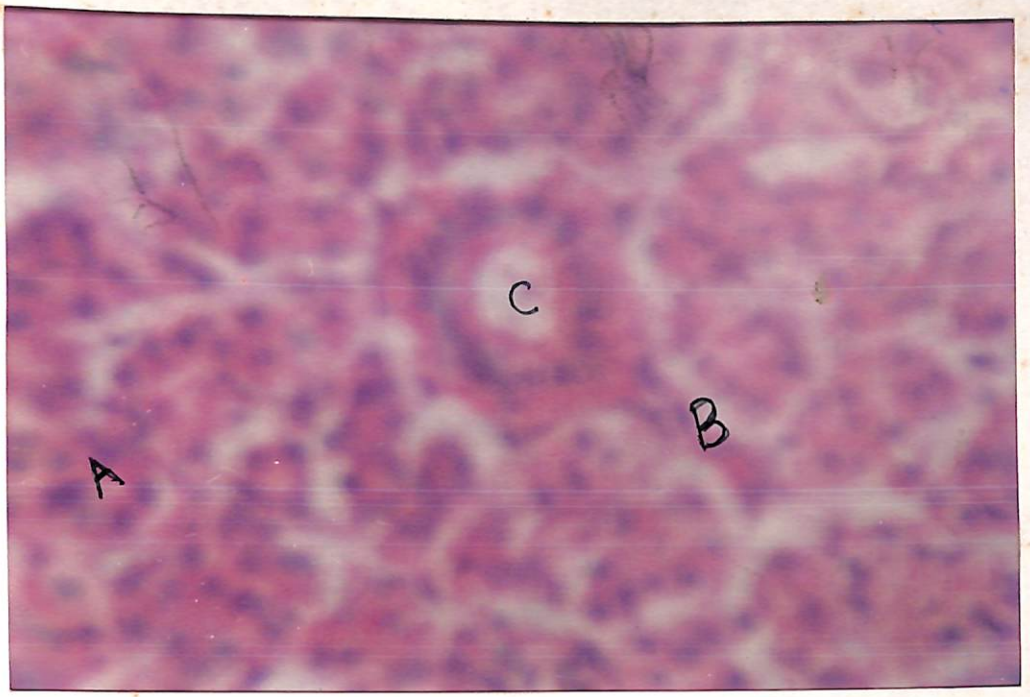
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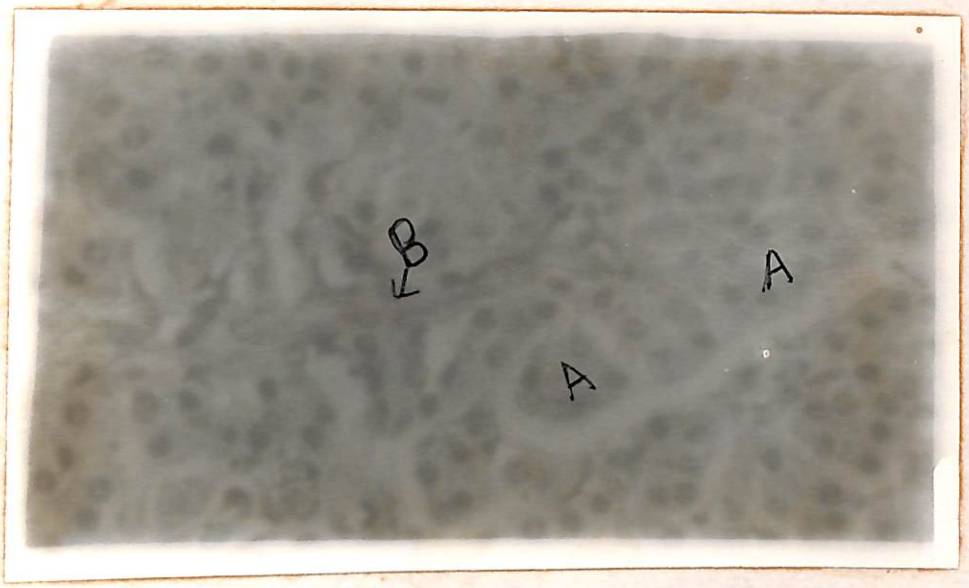
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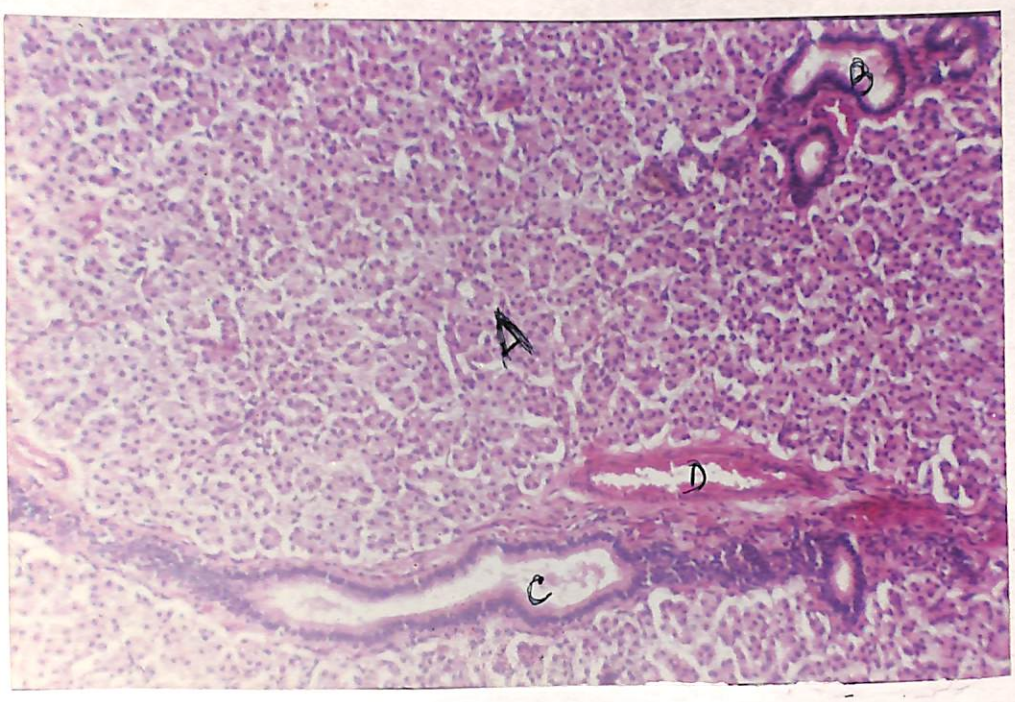
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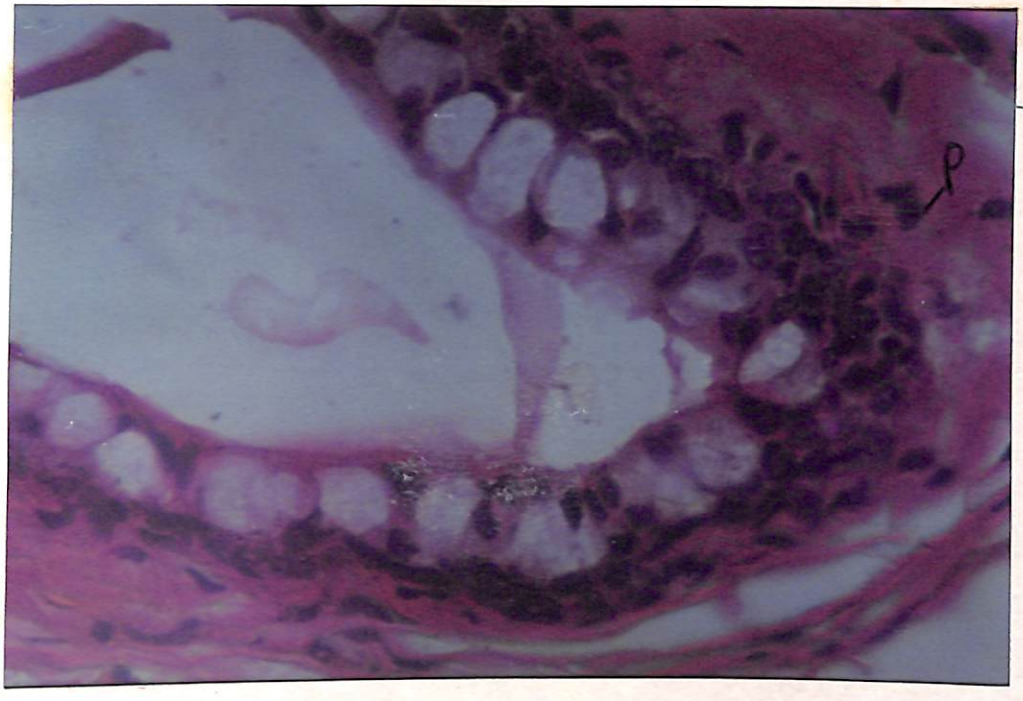
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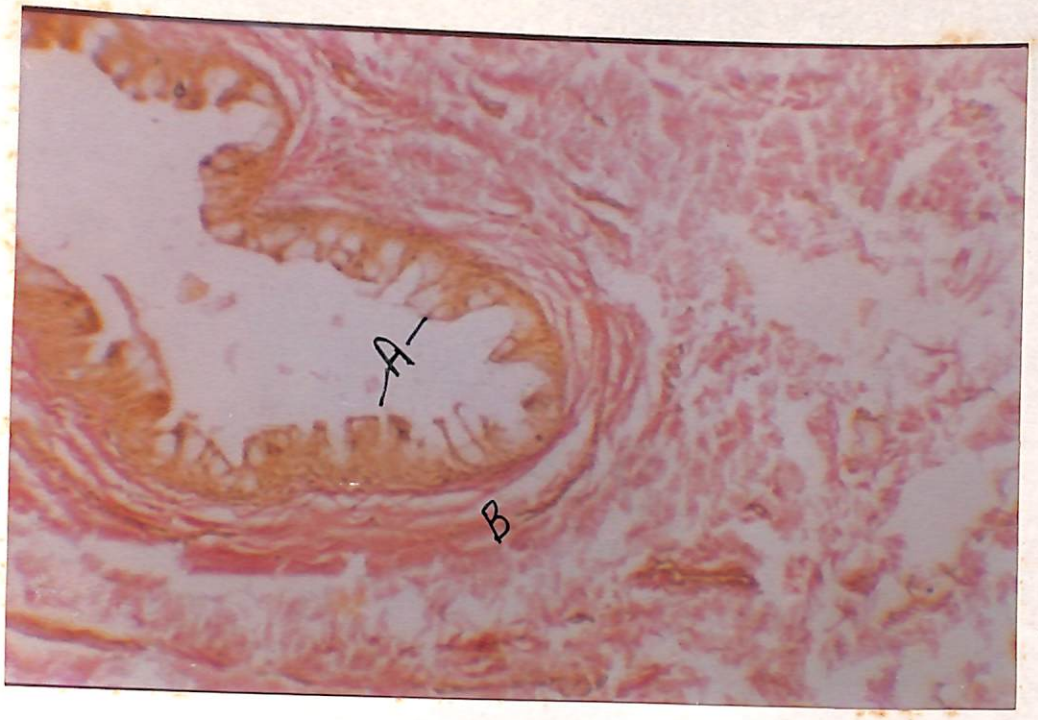
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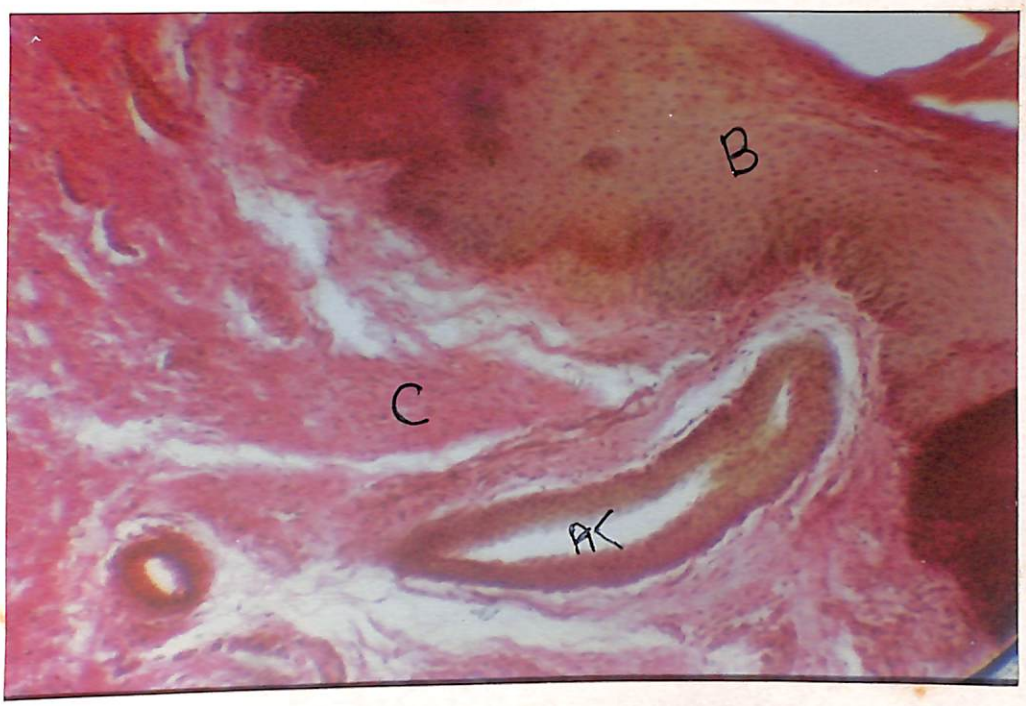
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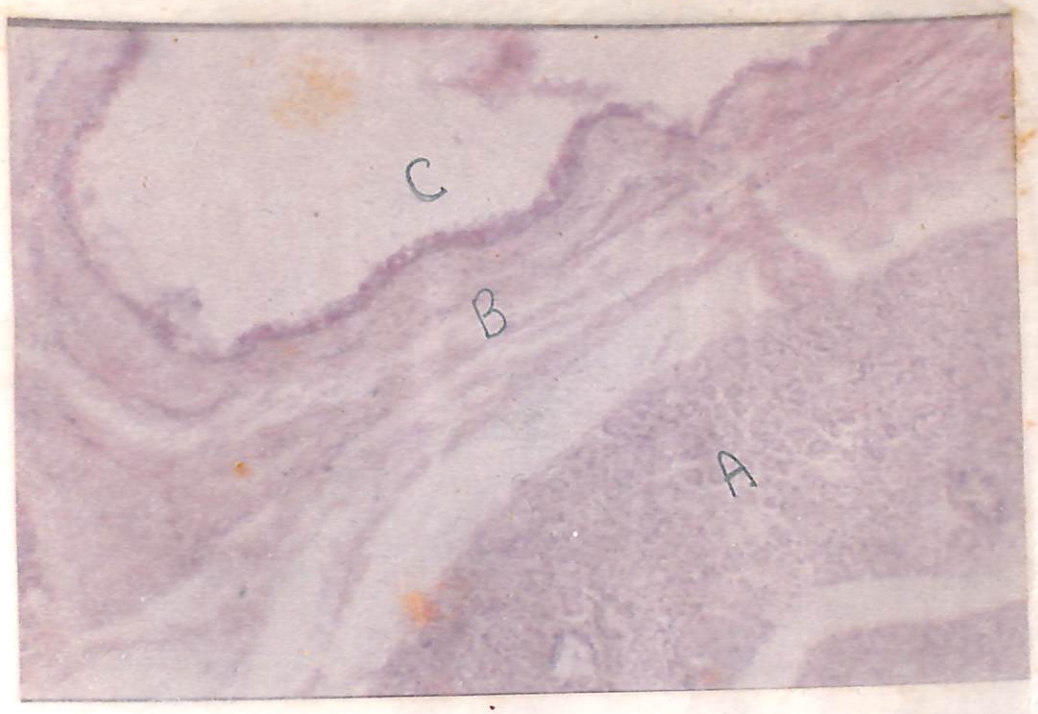
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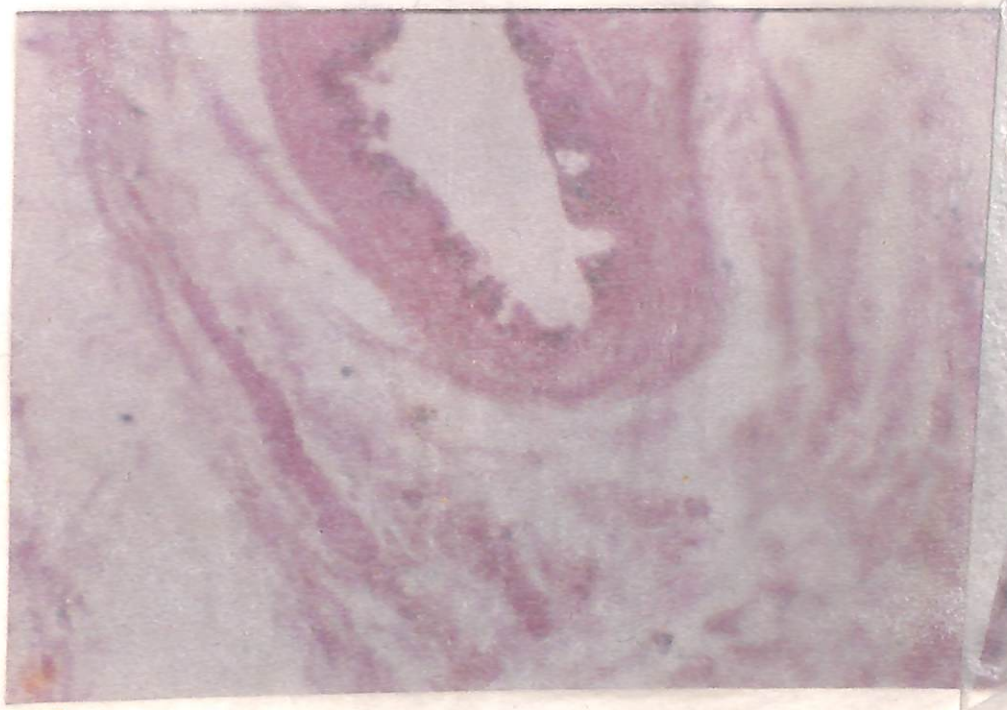

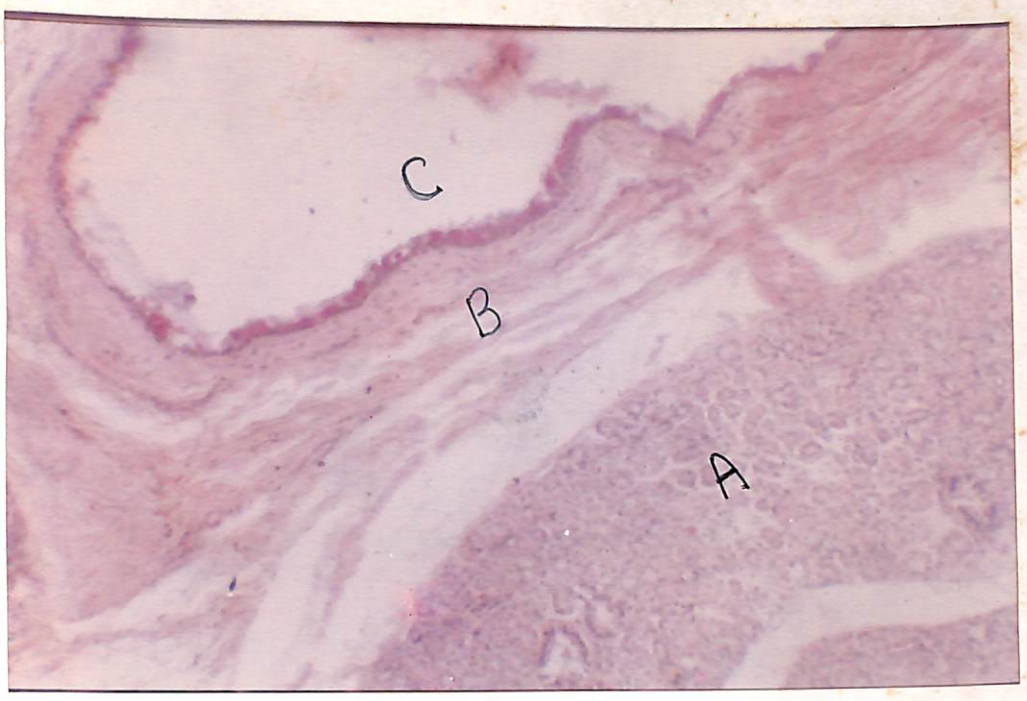
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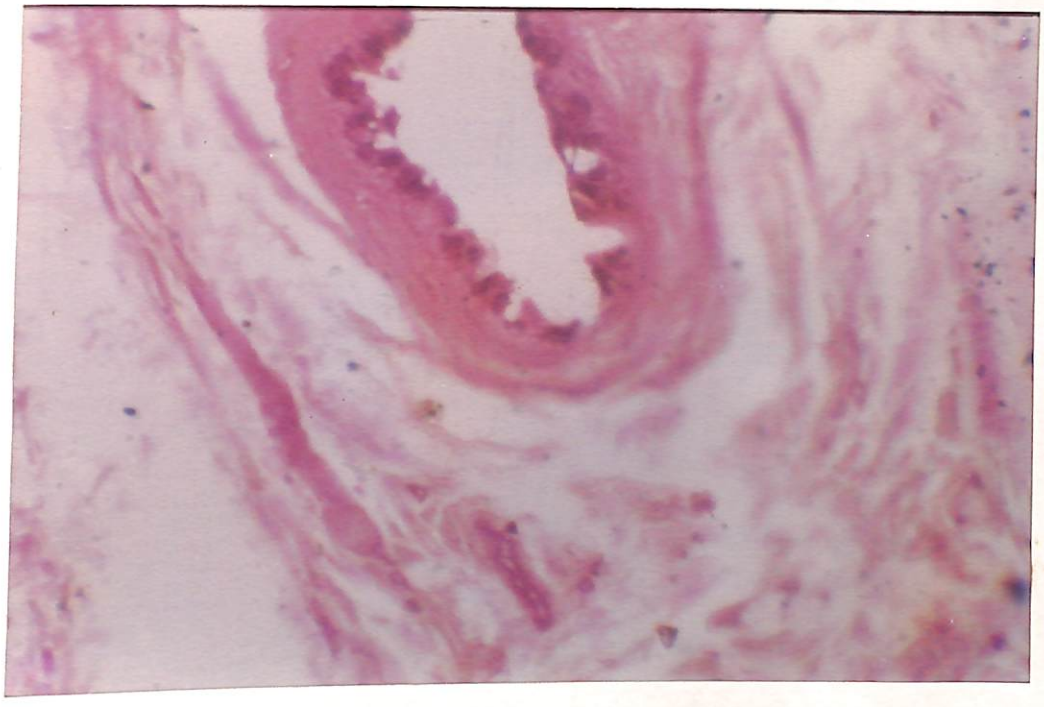

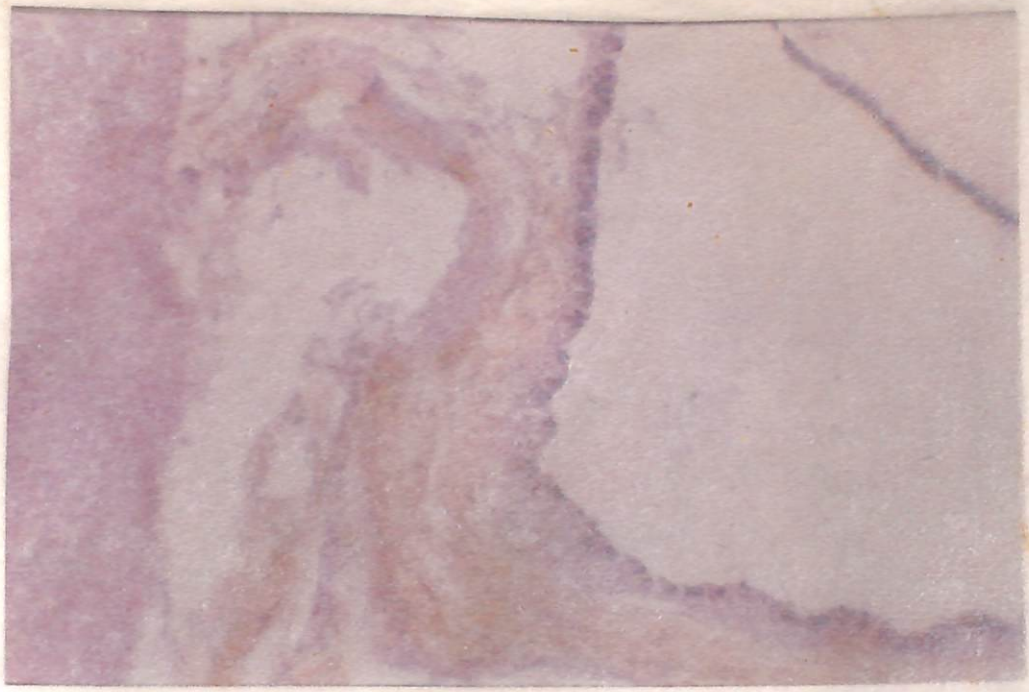

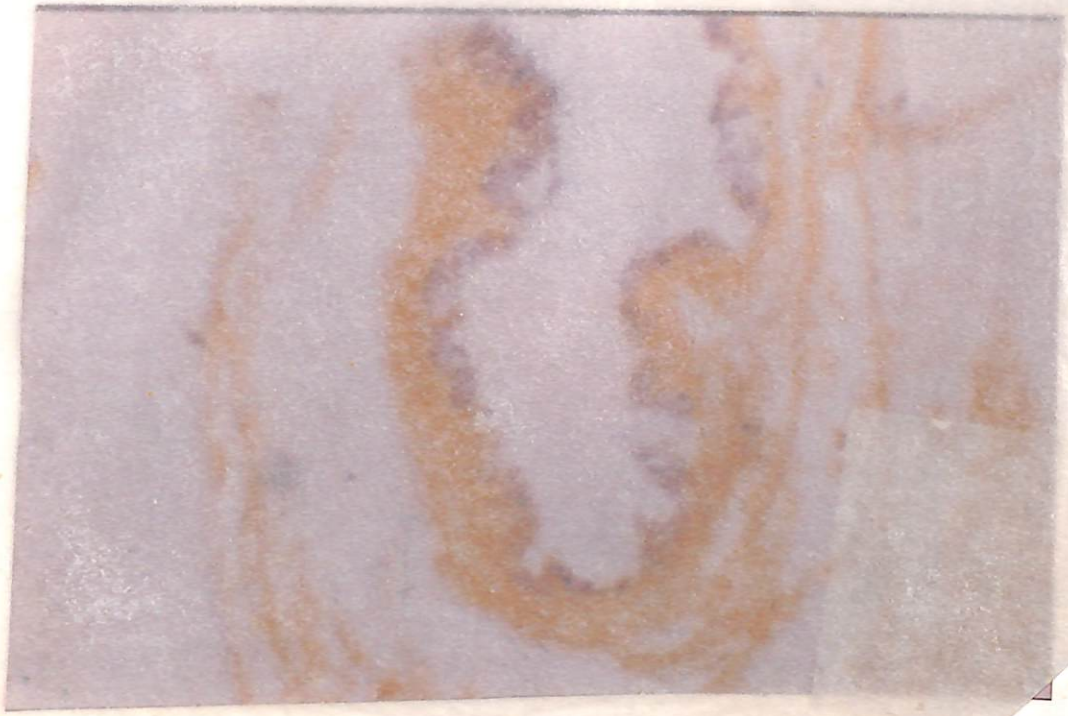

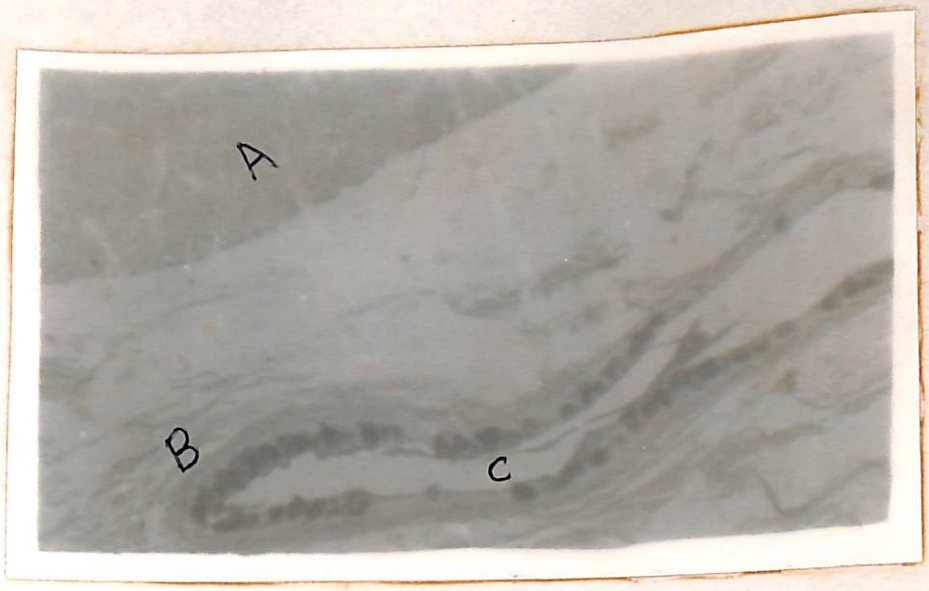
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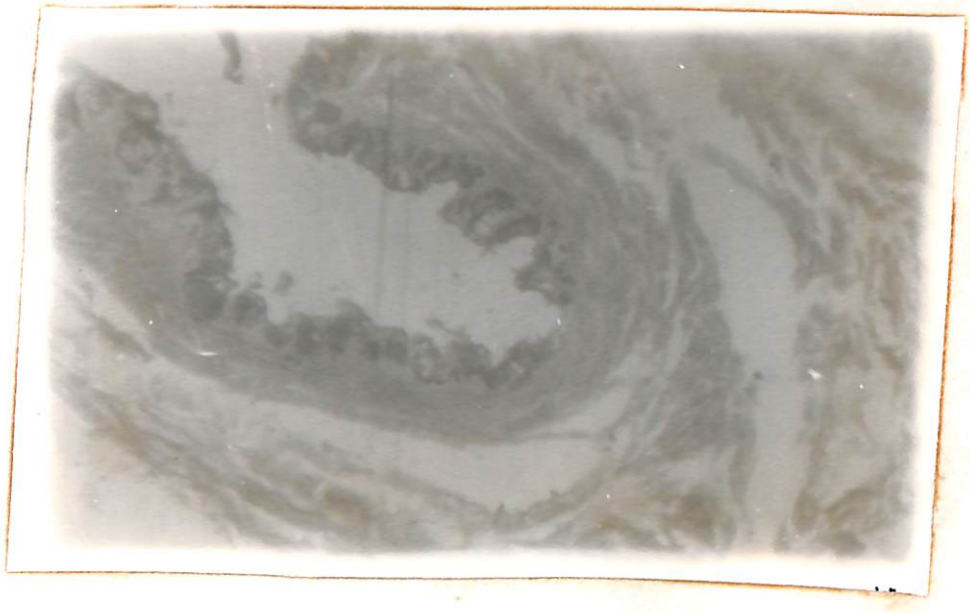

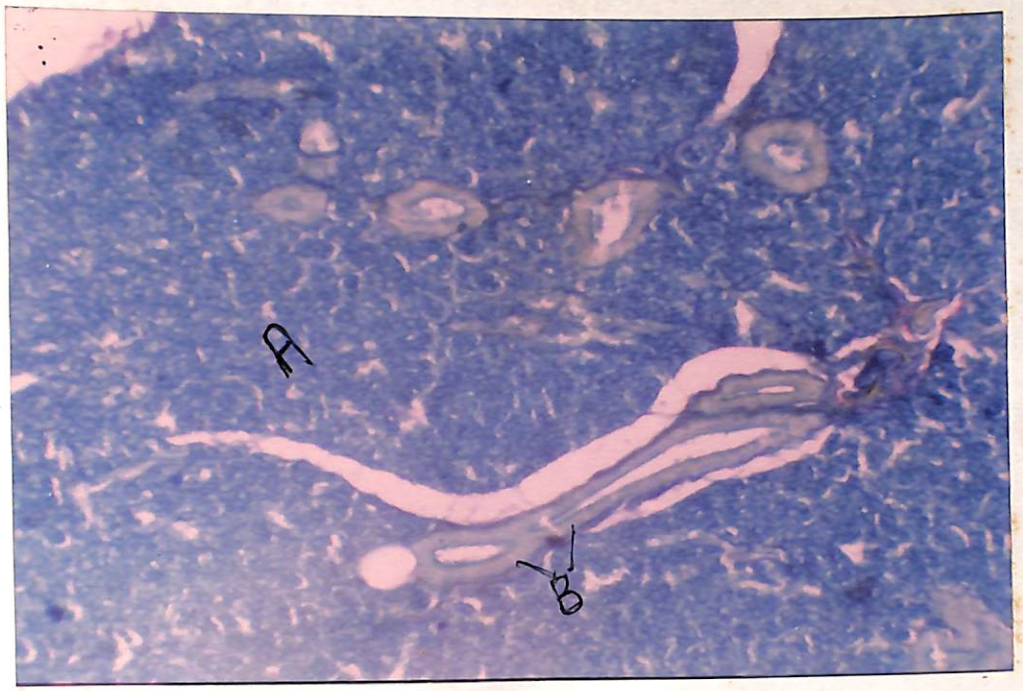
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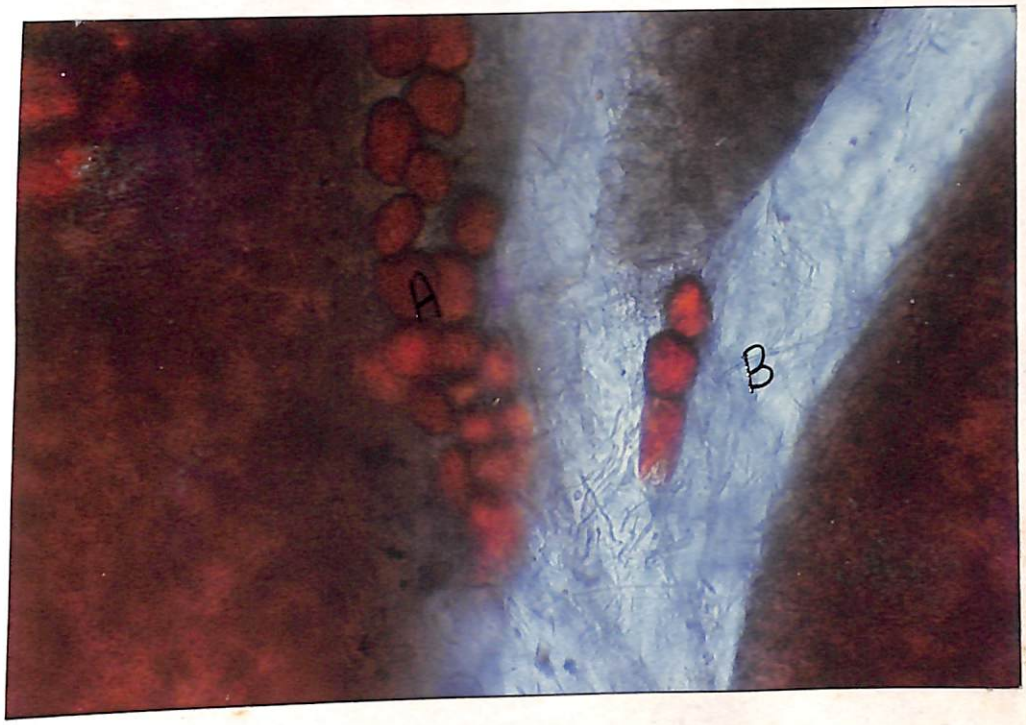
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