HISTOMORPHOLOGICAL STUDIES ON SUBMANDIBULAR AND SUBLINGUAL SALIVARY GLANDS IN RABBIT (*Oryctolagus cuniculus*)

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

(VETERINARY ANATOMY)

By

Madhuri Kumari

Reg No. - M/VAN/102/2000-2001

DEPARTMENT OF VETERINARY ANATOMY AND HISTOLOGY

BIHAR VETERINARY COLLEGE

PATNA (BIHAR)

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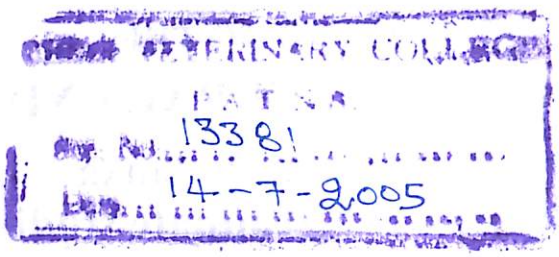
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DEDICATED TO THE MEMORY

OF MY FATHER

DEPARTMENT OF VETERINARY ANATOMY AND HISTOLOGY
BIHAR VETERINARY COLLEGE, PATNA-14
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR
PUSA (SAMASTIPUR)

CERTIFICATE-I

This is to certify that thesis entitled **"Histomorphological studies on submandibular and sublingual salivary glands in rabbit (*Oryctolagus cuniculus*)"** submitted in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Anatomy)** of the Faculty of post-graduate studies, Rajendra Agricultural University, Bihar is the record of bonafide research work carried out by **DR. MADHURI KUMARI,** Registration no. M/VAN/102/2000-2001 under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

A. Prasad
26.9.04

(A. Prasad)
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C E R T I F I C A T E - II

We, the undersigned, members of the Advisory Committee of **DR. MADHURI KUMARI**, Registration No. M/VAN/102/2000-2001 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 **"Histomorphological studies on submandibular and sublingual salivary glands in rabbit (*Oryctolagus cuniculus*)"** may be submitted by **DR. MADHURI KUMARI** in partial fulfilment of the requirements for the Degree.

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C E R T I F I C A T E - III

This is to certify that the thesis entitled **"Histomorphological studies on submandibular and sublingual salivary glands in rabbit (*Oryctolagus cuniculus*)"** submitted in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Anatomy)** of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar was examined and approved on 29/11/04

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Madhuri Kumari

Dated : 28-9-2004

(MADHURI KUMARI)

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INTRODUCTION

INTRODUCTION

The domestic or, laboratory rabbit *Oryctologus cuniculus* is the member of family leprodae and is an inhabitant of the area around Mediterranean sea and their inhabitation arised in spain as early as 1100 B.C. (Smallwood, 1998).

In India, rabbits are reared specially in north temperate zone. In recent years, rabbit rearing has become a minor agricultural industry as rabbitory. Since long, the rabbit has also been used as a laboratory or research animal in various fields of biomedical science. More than 400,000 rabbits are used annually for research purpose (Gillespie, 1998).

The rabbit also plays a major role in production of meat, pelt, fur or wool and is also used in making toys and novelties.

Now a days the rabbitory has become a greater economic unit. In India, Jammu & Kashmir alone provided over 30,000 gainful employment through this enterprise (Singh, 1998).

From the scientific and commercial point of view, rabbit has been recognized as one of the highly valuable livestock species and as such it is being included under the new course syllabi of the teaching Institute of Veterinary and Animal Science at both under graduate and post graduate level by the Veterinary council of India.

In mammals, the salivary glands are distinct anatomical features. The secretion of salivary gland furnishes both mechanical and chemical functions that help in mastication, swallowing and digestion of feed stuffs (Trautmann and Fiebiger, 1957). In certain domestic animals, saliva contain little

amylase, an enzyme that is capable for digesting or, hydrolyzing the starch (Frandson and Spurgeon, 1992).

A good number of histological and histochemical information about the different mammalian salivary glands are available that have been recorded by many research workers in India and abroad. But very little systematic informations are available on the microscopical features of submandibular and sublingual salivary gland in rabbit.

Considering the above facts, the present project has been under taken to study histomorphological features of submandibular and sublingual salivary glands in New zealand white rabbit. The results thus recorded may help the students, research workers and authors associated with Veterinary Anatomy and Histology in particular and allied science in general.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

SUBMANDIBULAR SALIVARY GLAND :

The submaxillary gland was the largest salivary gland. It was irregularly oval in shape and yellowish in colour. In dog and horse, it was much smaller than ox (Raghavan, 1964).

The mandibular gland appeared distinctly lobulated and was situated in a curve along the medial side of the angle of mandible in ox. In horse, sheep and goat, the gland was roughly triangular. In dog, it was rounded to oval in shape (Sisson, 1975).

Kishore *et al.* (1997) studied in the goat that the mandibular duct (Wharton's duct) left the middle of the concave dorsal border of the gland and passed dorsoanteriorly lateral to the diagastricus under the mucous membrane and opened on the ventral surface of the *cruncula sublingualis*.

HISTOLOGY :

SUPPORTING TISSUE :

Trautmann and Fiebiger (1957) observed that the salivary glands of domestic animals were enveloped by a connective tissue capsule which contained elastic fibers and some smooth muscle fibers, supplied with vessels and nerves.

Sognnaes and Moss (1966) found that the alveoli of submandibular gland of human were surrounded by elastic tissue fibers and the basement lamina upon which the gland cells rested and contained basket cells.

Venkatakrishnan and Mariappa (1969) observed the presence of connective tissue stroma in buffalo mandibular gland and the smooth muscle fibers.

Barnwal (1978) reported in calf that that the mandibular gland surrounded by loosely arranged connective tissue fibers. The interlobular duct was some where provided with large number of nerve bundles, blood vessels and lymphatics.

Copenhaver and Johnson (1978) reported in man and most other mammals that the serous, mucous alveoli and main duct of submandibular gland had richly cellular stroma and longitudinally disposed smooth muscle cells.

Leeson and Leeson (1979) studied that the lobar duct were associated with denser connective tissue network and frequently acquired a coat of smooth muscle fibers in the human submandibular gland.

Banks (1981) observed in most domestic animals and human that the lining epithelium of secretory portion of the submandibular gland encapsulated intimately with reticular fibers that were more dense in larger duct as the lobar connective tissue.

El Shafey *et al.* (1980) reported the encapsulated ganglia were present in the interlobular connective tissue, supplying nerve fibres to the ducts and blood vessels in goat mandibular gland.

PARENCHYMA :

SECRETORY ENDPIECES :

Trautmann and Fiebiger (1957) found that the submandibular salivary gland of domestic animals was the mixed type of gland and there were variation in the arrangement of serous and mucous cells.

Venlennep (1957) reported that the submandibular gland was mixed type of salivary gland with mucoserous demilunes in one humped camel.

Shakleford and Klapper (1962) reported that submandibular gland of cat, dog, cow and pig were predominantly mucous type whereas in the hamster, rat, mouse and rabbit, it was largely seromucous and purely serous in guinea pig. Serous secreting demilunes were found in man and rhesus monkey whereas mostly seromucous in carnivores.

Carleton and Short (1965) stated that the submaxillary glands in man and monkey were of mixed type with preponderance of serous cells. Crescents present at margins of mucous alveoli. In guinea pig, however glands were of serous type.

Senger and Singh (1970) studied that the mandibular salivary gland of buffalo contained more serous acini than mucous. The serous cells were either in the form of acini or demilunes.

Shakleford and Wilborn (1970) observed that all of the acini of mandibular gland in cat were mucous secreting and each acinus was capped with serous demilunar cells.

Ham and Leeson (1971) reported that the human submandibular gland were compound alveolar or tubuloalveolar gland. Although of the mixed type, the majority of their secretory units were of the serous variety. The mucous units were usually capped by serous demilunes.

Kangayama (1971) reported that the submandibular salivary gland of monkey was compound tubuloacinar type of gland in which serous cells were capped over the mucous one.

Bloom and Fawcett (1976) described the submandibular gland of human as a mixed gland in which serous cells were predominated. Some of the terminal secretory endpieces were exclusively serous.

Barnwal (1978) reported that the mandibular salivary gland in buffalo was a mixed compound tubuloacinar gland and was composed of serous and mucous acini. The mucous acini were capped with serous demilunes. The mucous acini predominated among the secretory endpieces.

Copenhaver and Johnson (1978) found that the submaxillary gland in man and most mammals was a mixed gland. The serous alveoli greatly outnumbered the mucous one, frequently the latter were capped by serous demilunes.

Leeson and Leeson (1979) reported that the human submandibular gland was compound tubuloalveolar or, mixed type of gland with serous, mucous and mixed acini.

Banks (1981) found the variation in distribution of serous and mucous cells of submandibular gland. It was serous in rodents, mucous in dog and cat, mixed in human, ruminants and horse in which either serous cells or, mucous cells intermingled with others and the serous demilunes capped the mucous endpiece. The stellate shaped basket cells or, myoepithelial cells were juxtaposed to the secretory epithelial cells of the alveoli.

Pinkstaff *et al.* (1982) reported that the submandibular gland of little brown bat was compound tubuloacinar mucous secreting glands. They contained some what atypical mucous secreting glands that often appears to be interposed between mucous tubule cells.

Asojo and Aire (1983) observed that the submandibular gland of giant rat contained only serous acini as in the guinea pig but unlike in many other mammals.

Nagai and Nagai (1985) observed the presence of myoepithelial cells in the parenchyma of human submandibular gland.

Nagato and Tandler (1986) found that the submandibular gland of two different species of macaques were identical in both species and predominantly serous but contained scattered mucous acini.

Stinson and Calhoun (1987) observed that the mandibular salivary gland in most of the domestic animal was a compound tubuloacinar gland and composed of mucous and serous acini. Mucous acini were predominated in dog and cat.

Hagelquivst *et al.* (1991) reported in rabbit that the submandibular salivary gland was heterocrine type and the secretory endpieces were formed mainly by morphologically two structures, proximally serous tubulei and distally the seromucous acini.

Sanders (1992) stated that the human submandibular glands were tubuloacinar and mixed type of glands although serous glands predominated. The secretory cells lied on the myoepithelial cells which were seen above the basement membrane.

Kishore *et al.* (1999) studied that the submandibular salivary gland in goat were compound tubuloalveolar gland of the mixed type.

DUCT SYSTEM :

Trautmann and Fiebiger (1957) observed in submandibular salivary gland of man and other domestic animals that the intercalated ducts were lined with simple low cuboidal epithelium and in the lobules they joined to form striated tubules or salivary ducts. The basement membrane of these ducts beared simple columnar epithelium composed of high strongly eosinophilic cells and these cells showed basal striation presumably caused by parallel rows of mitochondria.

Sognnaes and Moss (1966) observed in human submandibular gland that the main excretory duct was lined by stratified epithelium. The secretory ducts were well developed, the intercalated ducts were short and lined with simple cuboidal epithelium.

Suddick and Dowd (1969) observed in the rat submandibular gland that the intercalated, striated and the main excretory ducts were surrounded by denser capillaries network.

Bloom and Fawcett (1976) observed that the lining epithelium of larger ducts of submandibular gland in man and other domestic species were columnar and pseudostratified columnar epithelium which occasionally contained goblet cells.

Barnwal (1978) reported in the mandibular salivary gland of buffalo that the intercalated ducts were lined by cuboidal epithelium, striated by columnar epithelium and the main excretory duct was lined with stratified cuboidal epithelium. The epithelium varied from simple columnar to stratified cuboidal or, columnar in the interlobular ducts.

Copenhaver and Johnson (1978) found in man and most other mammals that the main duct of subamaxillary gland divided into interlobar ducts, which in turn divided to form interlobular ducts. The main duct lined by pseudostratified epithelium and continued in to the larger interlobular ducts which were lined by striated columnar epithelium.

Banks (1981) observed in equine submandibular gland that the intercalated duct connected alveoli to striated duct. The intercalated ducts were lined by low cuboidal epithelium and in striated duct, the striation of duct resulted from dense accumulation of mitochondria and numerous infoldings of plasmalemma.

Pinkstaff *et al.* (1982) observed that the intercalated duct composed of cuboidal or, low columnar epithelial cells, striated duct were composed of columnar cells whose apices buldged into the ductal lumina and excretory ducts also had bulging apices in the submandibular gland of little brown bat.

Ohtani *et al.* (1983) reported the denser subepithelial capillaries network around the excretory duct of submandibular salivary gland in rat.

Stinson and Calhoun (1987) reported in most of the domestic animals that the striated ducts of submandibular salivary gland were lined by cuboidal or, columnar epithelium with basal striation of mitochondria. The interlobular ducts were lined with columnar epithelium which transformed into two layered cuboidal and then in to stratified squamous epithelium.

Garginlo *et al.* (1992) observed the epithelial lining of main excretory duct of horse submandibular gland showed light, dark, basal and few goblet cells. The granules containing cells were occupied by rough endoplasmic reticulum and golgi apparatus.

HISTOCHEMISTRY :

Hill and Bourne (1954) observed the presence of lipid in the luminal border of duct showed intense reaction with sudan black in the rat submaxillary gland.

Leeson and Jacoby (1957) observed high activity of the alkaline phosphatase in the acini and intralobular ducts of rat submandibular gland.

Spicer and Duvenci (1964) reported the presence of neutral mucopolysaccharides and acid mucopolysaccharides in mucous acini and interlobular ducts of submandibular salivary gland, showed positive reactivity with PAS-AB and aldehyde fuchsin-alcian blue sequences in mouse, rat, syrian hamster, chinese hamster, guinea pig, rabbit and in human.

Leppi and Spicer (1966) observed the presence of equal proportion of sialomucin and sulfomucin in the mucous acini of submandibular gland of human, squirrel and rhesus monkey. The mucous cells showed presence of sialic acid.

Barnwal (1978) observed the presence of acid mucopolysaccharides and neutral/mucopolysaccharides in submandibular salivary gland of buffalo. The mucous and serous cells showed intense reaction of PAS and mild to moderate reactivity of colloidal iron. These both cells along with duct reacted moderately with mucicarmine and showed mild reaction for lipid or metachromatia with toluidine blue. Alkaline phosphatase were diffusely distributed throughout the gland.

El shafey *et al.* (1980) observed the presence of high concentration of mucosubstances in the mucous secretory units of goat submandibular gland.

Pinkstaff *et al.* (1982) stated that the mucous tubules cells contained sulfomucin and the demilunar cells contained moderate amount of nonsulfated acid mucosubstances and the all duct type contained PAS positive neutral mucosubstances in submandibular gland of little brown bat.

Asojo and Aire (1983) reported the distribution of sulphomucin, sialomucin and neutral mucin in the submandibular gland of giant rat. Moderate reaction occurred at the apical border of acinar cells with PAS, alcian blue (pH 2.5), AB-PAS, aldehyde fuchsin, toluidine blue, AF-AB staining sequences, were more marked and detected especially in the luminal border of ducts.

Ray and Pawar (1989) observed the positive reaction of PAS within serous, mucous ducts and demilunar cells of sheep submandibular gland. These reaction were the indicative of uniform distribution of neutral

mucopolysaccharides. Serous demilunes also showed positive reactivity with PAS-AB. The acinar cells were sudanophilic and also showed positive reaction with alkaline phosphatase.

Miyamoto and Miyamoto (1990) observed mucopolysaccharides in serous and mucous acini of submandibular gland in pre-and post-puberal pig. In pre-puberal pigs the serous cells contained mostly sulfosialomucin and the mucous cells mostly sialomucin while in the post-puberal pigs the serous cells increased in number and contained scarcely any sulfosialomucin while the mucous cells decreased in number and contained more sialomucin.

Hagelquivst *et al.* (1991) observed in rabbit submandibular salivary gland that the cells of the acini were strongly positive for alcian blue and negative for PAS and alcian blue (pH- 1.0) was indicative of production of acid glycoportein whereas the cells of serous tubulei were strongly PAS positive and negative for alcian blue (pH-2.5) indicating the presence of neutral glycoprotein.

Kishore *et al.* (1999) observed in the goat submandibular gland the mucous secretory units were strongly positive for PAS and alcian blue whereas the serous cells and ducts were positive for PAS and negative for alcian blue.

SUBLINGUAL SALIVARY GLAND :

The sublingual gland was placed beneath the mucous membrane of the floor of the mouth between the tongue and the horizontal ramus of the mandible and was yellowish in colour (Raghavan, 1964).

In all domestic animals except horse, the sublingual glands were of two types. The dorsally placed gland was polystomatic in type. Monostomatic gland was placed ventral to the polystomatic gland.

The polystomatic gland was composed of small tortuous duct (*ductus sublingualis minores*) as in horse which opened into the lateral sublingual recess.

The monostomatic sublingual salivary gland in ox, sheep, pig and dog had a single duct (*ductus sublingualis major*) opened along side the mandibular duct to *cruncula sublingualis* (Sisson, 1975).

Imai *et al.* (1982) prefered the minor sublingual salivary gland in man and Japanese macaques over the term polystomatic sublingual salivary gland because of the fact that minor sublingual salivary gland did not represent a single gland with several excretory ducts.

HISTOLOGY :

SUPPORTING TISSUE :

Carleton and Short (1965) stated that secretory acini of sublingual gland in man and most domestic animals were surrounded with capillary network and ramification of nerve and ganglia as fine varicose fibrils amongst the alveolar cells and the connective tissue around the largest duct contained few plain muscle cells.

Sognnaes and Moss (1966) reported the encapsulation of the ducts with connective tissue with few elastic fibres in the human sublingual gland.

Dellman (1971) observed that the sublingual gland of domestic animal was subdivided in to lobes and lobules. The lobules were separated by connective tissue within which secretory acini were surrounded by reticular connective tissue.

Barnwal (1978) observed the connective tissue capsule in the sublingual salivary gland of buffalo contained both elastic and few reticular fibres with rare occurrence of mast and plasma cells. Adipocytes were also present in stroma.

Copenhaver and Johnson (1978) found that the connective tissue septae were well developed in sublingual glands of man, dog, cat, rabbit and sheep.

Banks (1981) stated that the basement membrane of the ducts and secretory cells encapsulated within reticular connective tissue which were more dense in larger duct of ruminant, swine and rodent's sublingual salivary gland.

Taha *et al.* (1999) found the connective tissue septae around the serous endpieces in sublingual gland of one humped camel.

PARENCHYMA :

SECRETORY ENDPIECES :

Trautmann and Fiebiger (1957) reported that the sublingual gland of man and domestic mammals were mixed type of gland and the end of mucous tubules were capped by an outer group of serous cells or demilunes.

Carleton and Short (1965) reported that the human sublingual gland was mixed type in which mucous alveoli were the more numerous. Many of

these showed crescent at their margins and the mucous alveoli were larger or more uniform in shape than serous.

Sognnaes and Moss (1966) mentioned that the secretory endpiece of human sublingual salivary gland contained both mucous and serous cells or mixed, some serous cells capped over the mucous cells and form demilunes or, crescents.

Carpenter (1968) observed that the human sublingual gland possesses greater number of mucous acini. Some serous tubules were present but most of the serous cells were arranged as crescents around the mucous cells.

Senger and Singh (1970) observed that the sublingual gland of buffalo was predominantly of mucous type. The demilunes of serous cells were more frequently distributed.

Dellman (1971) stated that the sublingual gland in most of the domestic animals were mixed type or, seromucous type and the serous portion were generally restricted to demilunes around the mucous acini.

Ham and Leeson (1971) studied that the human sublingual salivary gland were compound tubuloalveolar and mixed type of gland in which there were majority of mucous type of alveoli.

Bloom and Fawcett (1976) reported in the man sublingual salivary gland that the mucous cells were more numerous than serous and many of them were mucoserous in character. For the most part they were arranged in thick demilunes.

Copenhaver and Johnson (1978) stated that the sublingual salivary gland was mixed gland in man, dog, cat, rabbit and sheep. In man, it was preponderantly mucous type and very few serous alveoli were seen. Serous

cells occur in the form of demilunes around mucous alveoli. Such demilunes were numerous and large.

Leeson and Leeson (1979) found that the human sublingual gland was compound acinar mucous gland although a few serous acini may be present.

Banks (1981) reported that the sublingual gland of horse, man and carnivores were mixed type of gland and mucous in ruminant, swine and rodents.

Imai *et al.* (1982) studied that the number of mucous cells in the sublingual gland of man and Japanese macaques was some what larger than that of the seromucous cells.

Asojo and Aire (1983) studied that the sublingual salivary gland of giant rat was seromucous type, in which mucous acini intermixed with few acini and the serous demilunes also commonly formed caps on the mucous acini.

Stinson and Calhoun (1987) studied that the sublingual gland was a compound branched tubuloacinar gland in domestic animals. These were almost entirely mucous with relatively few demilunes in cow, sheep and pig. In the dog and cat, these glands contained clusters of serous acini with well developed intercellular canaliculi.

Kishore *et al.* (1999) found that the sublingual salivary gland of goat were compound tubuloalveolar gland contained both serous and mucous secretory acini with some scattered serous demilunes and few entirely serous demilunes.

Taha *et al.* (1999) studied that almost all of the secretory endpiece were mucous yet small group of serous endpiece in the sublingual gland of one humped camel.

DUCT SYSTEM :

Carleton and Short (1965) found in man and most of the domestic mammals that the secretory duct of sublingual gland joined directly to the mucous alveoli by shorter and wider intermediate or junctional portion. The largest ducts were lined with continuation of the stratified epithelium.

Sognnaes and Moss (1966) observed the main excretory duct of human sublingual salivary gland was lined by pseudostratified columnar epithelium. The secretory ducts were short, lined with basally striated simple columnar epithelium and the slender intercalated ducts were absent.

Senger and Singh (1970) reported that the secretory duct connected the alveoli directly and the intercalated duct were absent in the sublingual gland of buffalo.

Dellman (1971) found in sublingual salivary glands of pig that the larger excretory ducts were located with in the interlobular or interlobar connective tissue capsule and they were lined with a continuation of the stratified epithelium.

Bloom and Fawcett (1976) observed in the human sublingual gland that most of the serous or seromucous cells undergo complete mucous transformation and the terminal portion joined directly on the striated tubules, represented by small groups of basally striated cells in the epithelium of interlobular ducts.

Barnwal (1978) reported in the buffalo that the intercalated ducts were essentially present in monostomatic sublingual salivary gland and were lined with cuboidal epithelium, interlobular ducts with cuboidal or, stratified squamous epithelium and the main excretory duct were lined with stratified cuboidal epithelium.

Copenhaver and Johnson (1978) stated that the larger salivary ducts of sublingual gland were lined with pseudostratified epithelium which was replaced by simple columnar epithelium in the smallest one.

Leeson and Leeson (1979) reported that the interlobular duct of human sublingual gland lied in relatively dense connective tissue, accompanied by small blood vessels. Fat cells were also present.

Stinson and Calhoun (1987) studied that the striated and intercalated ducts were present but not well developed in the sublingual gland of dog and cat however, in the horse, ruminants and pig, they were well developed. In most domestic animal, the interlobular ducts had at their origin, a low columnar epithelium that increased in height and became two layered in the larger ducts. The main duct was lined with stratified cuboidal epithelium with goblet cells occurred in the cow and pig.

Taha *et al.* (1999) observed in the sublingual salivary gland of one humped camel that the intralobular or, intercalated ducts were few and continued directly from excretory tubules. Interlobular excretory ducts were large and showed numerous goblet cells among the duct cells and these ducts were surrounded by many blood vessels.

HISTOCHEMISTRY :

Kawakatsu *et al.* (1959) observed diffuse or, moderate alkaline phosphatase activity around the base of the secretory cells in the sublingual salivary gland of rat, guinea pig, rabbit and dog.

Fava demoraes and Villa (1963) observed the two types of mucosubstances in the sublingual gland of dog, pig and man. The duct cells were nonreactive to PAS-AB stain. The secretion in the duct showed moderate to intense alcianophilia. The striated duct cells contained weakly

PAS positive granules. They were also mildly positive to mucicaramine and orthochromatic with toluidine blue.

Munger (1964) found that the PAS reaction were uniformly and intensely positive in the human sublingual gland and varies only in the number of PAS positive secretory granules in the apices of the cells.

Spicer and Duvenci (1964) observed the uniform distribution of acid mucopolysaccharides in mucous acini of sublingual gland in mouse, rat, syrian hamster, chinese hamster, guinea pig, rabbit and human showed positive reactivity with PAS-AB, aldehyde fuchsin-alcian blue sequences. The intralobular duct and serous demilunes were positive for neutral mucopolysaccharides with PAS-AB and their secretion showed orthochromatia in mouse and rabbit unlike other rodents or, lagomorph with Azure-A.

Leppi and Spicer (1967) observed that the mucous acini of the sublingual gland of ox, sheep and pig were strongly PAS positive (Digestion with diastase diminished the staining intensity). The seromucous demilunes in sheep and ox, were positive for sulfated mucosubstances and in cow they showed evidence of mucopolysaccharides containing sialic acid.

Barnwal (1978) reported the moderate to intense reaction of PAS in mucous cells in sublingual salivary gland of buffalo. These both cells also showed mild to moderate reaction of alkaline phosphatase and different intensity of colloidal iron reaction whereas metachromatia of toluidine blue and mild reaction of lipid involved only the mucous cells. The basal region of these cells also showed weak reaction for neutral fat and fatty acid and the luminal border of ducts showed weak reaction of PAS and mucicarmine.

Asojo and Aire (1983) reported the intense reaction of sulphomucin and sialomucin in the mucous acini and mild to moderate intensity of neutral mucin in the serous endpiece and demilunar cells of sublingual salivary glands of giant rat with toluidine blue, alcian blue (pH 2.5), AF-AB, AB-PAS, AB (pH 1.0), aldehyde fuchsin, colloidal iron staining sequences. Mild to moderate reaction were also detected mainly at the luminal border of the cells in all grades of ducts.

Kishore *et al.* (1999) reported the positive reaction with PAS and alcian blue in the mucous acini of goat sublingual gland. The serous acini also showed the positive reaction with PAS.

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MATERIALS AND METHODS

MATERIAL AND METHODS

The Histomorphological studies on submandibular and sublingual salivary glands were conducted on twelve adult and apparently healthy New zealand white rabbits, six from the each sex.

Tissue samples from different parts of both the glands were collected immediately after slaughter and were properly fixed in their specific fixatives (Luna, 1968).

The following fixative solutions were used during this present study.

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

After proper fixation various histological and histochemical procedures such as washing, dehydration, clearing, paraffin infiltration, blocking or, sectioning were adopted to stain the paraffin and frozen sections (Humason, 1967).

To observe histological and histochemical details of the parenchyma and stroma of glands, 5-6 micron (μm) thick paraffin sections were cut with the help of rotary microtome and 10-15 μm thick frozen sections for the demonstration of lipid.

The following stains and staining procedures were used to record the histological details :

1. Haematoxylin and eosin stain for routine observation (Luna, 1968).

2. Van Gieson stain for collagen and muscles fibers (Lillie, 1965).
3. Gomori's reticulin stain for reticular fibers (Humason,1967).
4. Mallory's modified trichrome stain for connective tissue fibers (Luna, 1968).
5. Verhoeff's elastin stain for elastic fibers (Humason, 1967).

The following stains and staining procedures were used to record the certain histochemical observations :

1. Periodic acid schiff stain for glycogen, mucin and mucoprotein, (Pearse, 1968).
2. Gomori's stain for alkaline phosphatase (Pearse, 1968).
3. Modified Mowry's colloidal iron stain for acid mucopolysaccharides (Humason, 1967).
4. Oil red O stain for lipid (Luna, 1968).
5. Toluidine blue method for mucin (Drury and Wallington, 1967).
6. Modified Mayer's mucicarmine stain for mucopolysaccharides and mucoprotein (Drury and Wallington, 1967).

The stained paraffin sections were mounted with DPX (Diesterine plastilizer xylene) and the frozen sections were mounted in glycerine jelly after staining.

The micrometry of different components of the glands were recorded with the help of caliberated occular micrometer.

Some of the important parameters from the parenchyma of submandibular and sublingual salivary glands were processed for statistical analysis and interpretation (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

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SUBMANDIBULAR SALIVARY GLAND :

There were two submandibular salivary glands in close proximity to each other in case of rabbit. These glands were irregularly oval in shape situated at the intermandibular space just cranial to caudal ends of the *pars molaris mandibularis*. Close observation of the gland revealed very faint surface lobulation. The mandibular duct from each gland travelled cranio dorsally to open at elongated *cruncula sublingualis* at the floor of the mouth cavity (fig.1).

The localization and gross morphological features of submandibular salivary gland in rabbit, were by and large in agreement with the observations made by Raghavan (1964); Sisson (1975) and Kishore *et al.* (1997) in different domestic animals.

HISTOLOGY :

SUPPORTING TISSUE : The submandibular salivary gland of rabbit was highly deficient in connective tissue components which formed the supporting tissue. A thin capsule was mainly formed by collagen fibers which was invested over the gland proper indistinctly (fig. 2). A few reticular fibers were distributed randomly in the capsule. The elastic fibers were lacking.

The connective tissue septae divided the parenchyma of the gland into lobes and lobules (fig. 2). The septae were usually indistinct at certain places where margin of lobulations were marked by linear spaces only (fig. 4). The interlobar connective tissue was marked by presence of coarse collagen fibres and reticular fibers encircling the lobar ducts (figs. 2, 3, 4). The elastic

fibers and smooth muscles fibers were altogether absent in the interlobar connective tissue septa.

The reticular fibers were widely distributed to constitute intensive meshwork in lobes and lobules to support the parenchymatous tissue (fig.3). Nerve fibers, blood vessels and lymphatics were usually observed in the interlobar connective tissue septae (figs. 4, 5).

The distribution of connective tissue fibers in the submandibular salivary gland of rabbit did not agree with the observations made by Trautmann and Fiebiger (1957) in domestic animals and Sognnaes and Moss (1966) in man who recorded distribution of elastic fibres in the supporting tissue of salivary gland. The absence of smooth muscle fibers in the supporting tissue of submandibular salivary gland of rabbit was also in disagreement with the findings of Venkatakrishnan and Mariappa (1969) in buffalo, Copenhaver and Johnson (1978) in man and some other mammals and Leeson and Leeson (1979) in human submandibular salivary gland. The distribution of blood vessels, nerve bundles and lymphatics in the supporting tissue of submandibular gland of rabbit, was in agreement with the findings of Barnwal (1978) and El-Shafey *et al.* (1981) who recorded similar structure in large ruminant and goat submandibular salivary gland respectively. The distribution of reticular fibers could be well correlated with the description made by Banks (1981) in the submandibular salivary gland of domestic animals.

PARENCHYMA :

The submandibular salivary gland of rabbit was typed as compound tubuloacinar mixed gland. The secretory endpieces presented two definite

portions namely tubular segments which continued to end in single acinus or in multiple acini (figs. 6, 8).

Trautmann and Fiebiger (1957) in domestic animals, Venlennep (1957) in one humped camel, Shackleford and Klapper (1962) in hamster, rat, mouse and rabbit, Carleton and Short (1965) in man and monkey, Bloom and Fawcett (1966) in man, Senger and Singh (1970) in buffalo, Shackleford and Wilborn (1970) in cat, Ham and Leeson (1971) in man, Kangayama (1971) in monkey, Barnwal (1978) in buffalo, Copenhaver and Johnson (1978) in man, Leeson and Leeson (1979) in man, Banks (1981) in ruminants and horse, Nagato and Tandler (1986) in macaques, Stinson and Calhoun (1987) in most of the domestic animal and Hagelquivst *et al.* (1991) in rabbit reported mixed variety of submandibular salivary gland, however Pinkstaff *et al.* (1982) in little brown bat and Asojo and Aire (1983) in giant rat observed mucous and serous type of submandibular salivary gland respectively.

The histomorphological characters of submandibular salivary glands in rabbit could be correlated with the findings of Kangayama (1971) in monkey, Barnwal (1978) in buffalo, Stinson and Calhoun (1987) in domestic animals, Pinkstaff *et al.* (1982) in little brown bat and Sanders (1992) in man who reported compound tubuloacinar type of submandibular salivary gland in respective species.

In contrast however, Ham and Leeson (1971) in man and Kishore *et al.* (1989) in goat described the submandibular salivary gland as compound tubuloalveolar gland.

GLANDULAR ENDPIECES :

The glandular endpieces were comprised of the glandular tubules and glandular acini. The glandular tubules continued with intercalated duct of the duct system. Other end of individual glandular tubule terminated either in to single acinus or in several acini (fig. 9). Histomorphologically, the glandular cells, lining the glandular tubules and the acini differed significantly (figs. 8,9,10). The acini were lined with cuboidal or pyramidal cells exhibiting distinct seromucous characters. The spherical nucleus was placed basally in the cells. The cytoplasm appeared foamy with few scattered eosinophilic cytoplasmic granules (figs. 8,9,10). The glandular cells of the tubule also appeared as cuboidal or pyramidal having spherical nucleus placed parabasally. These cells contained densely distributed acidophilic cytoplasmic granules giving the appearance of typical serous cells. With trichrome stain, the acinar cells appeared light coloured whereas tubular cells showed distribution of acid fuchsin positive cytoplasmic granules in the cells (figs. 11,12,13). In none of the glandular segments, the myoepithelial cells could be observed. None of the acini presented serous demilunes or crescents.

The present study on the submandibular salivary gland of rabbit did not agree with the pattern of distribution of serous and mucous acini in the submandibular salivary gland of different species (Trautmann and Fiebiger, 1957; Shackleford and Klapper, 1962; Carleton and Short, 1965; Senger and Singh, 1970; Shackleford and Wilborn, 1970; Ham and Leeson, 1971; Bloom and Fawcett, 1976; Barnwal, 1978; Leeson and Leeson, 1979; and Stinson and Calhoun, 1987).

Present findings, were in accordance with the findings of Hagelquivst *et al.* (1991) who also reported seromucous type of cells in glandular acini and serous type of cells in the glandular tubule of rabbit submandibular salivary gland.

The myoepithelial cells reported by Banks (1981) in domestic animals, Nagai and Nagai (1985) and Sanders (1992) in human submandibular salivary glands were not recorded in the submandibular salivary gland of rabbit.

Histometric observations on submandibular salivary gland of rabbit revealed that the diameter of glandular acini varied between 18.92 μm and 37.84 μm in both male and female rabbits. The average diameter of these acini measured 28.724 ± 2.472 μm in male and 27.692 ± 4.432 μm in female. Statistically, there was no significant difference in the diameter of acini between sexes at $P<0.01$ level. The diameter of glandular tubule measured between 18.92 μm and 41.28 μm in male whereas 18.92 μm to 37.84 μm in female rabbit. The average tubular diameter measured 27.692 ± 3.001 μm in male and 27.692 ± 2.965 μm in female. Statistically, diameter of glandular tubules of male and female rabbits did not differ significantly at $P<0.01$ level (Table-1).

DUCT SYSTEM :

The duct system of submandibular salivary gland appeared to be classical duct system comprised of intercalated duct, intralobular duct, interlobular duct, lobar duct and main excretory duct (figs. 9,10,12, 14,15,16,17, 18).

Trautmann and Fiebiger (1957) in domestic animals and Stinson and Calhoun (1987) in domestic animals also reported similar divisions of duct system in submandibular salivary glands.

The intercalated duct of submandibular salivary gland of rabbit was short and was lined with simple squamous or simple low cuboidal epithelium resting over a basement membrane. These ducts were interposed between tubular segments of secretory endpieces and intralobular duct (fig. 9). The cytoplasmic eosinophilia was prominent in the cells of lining epithelium of intercalated duct.

The findings were partially in agreement with the observations made by the Trautmann and Fiebiger (1957) in domestic animal, Sognnaes and Moss (1966) in man, Barnwal (1978) in buffalo, Banks (1981) in domestic animals and Pinkstaff *et al.* (1983) in little brown bat, who reported the lining epithelium of submandibular gland as low cuboidal or, low columnar epithelial cells.

The intralobular duct incase of submandibular salivary gland of rabbit presented occasionally secondary divisions. The primary segments of intralobular duct presented larger diameter than that of the less populated secondary division of intralobular duct. Such divisions were clearly observed in the section stained with colloidal iron stain (fig. 20). The intralobular duct was lined with high cuboidal or columnar epithelium resting over the distinct basement membrane. The cytoplasm was granular and eosinophilic. Vesicular nucleus was oval in outline and usually placed at the center of the cells (figs. 10,15). With trichrome stain, the cytoplasm appeared highly granular and acid fuchsin positive with slight tinge of orangophilia. The basal striations and adjacent myoepithelial cells were absent. Periductular

connective tissue presented vascularity containing orange-G positive RBCs (fig. 12).

Trautmann and Fiebiger (1957) in domestic animals, Barnwal (1978) in buffalo, Banks (1981) in domestic animals and Pinkstaff *et al.* (1982) in little brown bat reported the intralobular duct of submandibular salivary gland as secretory or striated duct. Since, basal striations were not observed in the intralobular duct of submandibular gland of rabbit, the secretory behaviour could be professed by the presence of coarse cytoplasmic granules in the lining epithelium surrounded by vascular network. Stinson and Calhoun (1987) also described the striated duct as salivary duct which was lined with simple cuboidal or columnar epithelium.

The micrometry of intralobular duct in submandibular gland along with statistical analysis as depicted in table-2 revealed that the diameter of intralobular duct from 36.12 μm to 61.92 μm with an average of 49.192 ± 2.314 μm in male rabbit whereas in the female rabbits it ranged from 36.12 μm to 56.76 μm with an average of 46.784 ± 2.031 μm.

The epithelial height in male rabbit ranged from 6.88 μm to 20.64 μm with an average of 13.76 ± 1.332 μm. In female rabbit, however the epithelial height ranged from 8.6 μm to 17.2 μm with an average of 13.072 ± 1.261 μm. Statistically, the diameter and epithelial height of intralobular ducts did not vary significantly in male and female rabbits ($P<0.01$).

The interlobular ducts were comparatively wider in diameter and were placed at the interlobular areas of the gland which received the intralobular ducts (fig 15). The epithelium was lined with simple columnar cells or high cuboidal cells with vesicular oval nucleus. The cytoplasm of epithelial cell was faintly acidophilic which showed variable positive reaction with acid

fuchsin and orange-G when stained with trichrome. Trautmann and Fiebiger (1957) in domestic animals, Barnwal (1978) in buffalo and Stinson and Calhoun (1987) in domestic animals also reported simple columnar epithelial lining in submandibular salivary gland.

The diameter of interlobular duct ranged from 48.16 μm to 77.4 μm in male rabbit with an average of 61.232 ± 2.617 μm. In female the diameter ranged from 25.8 μm to 75.65 μm with an average of 56.072 ± 4.53 μm. The epithelial height of interlobular duct in male ranged from 12.04 μm to 25.80 μm with an average of 18.404 ± 1.682 μm. Similarly, incase of female rabbit, the epithelial height varied from 8.6 μm to 29.24 μm with an average of 18.404 ± 2.446 μm as revealed in table-2.

The statistical analysis showed insignificant difference in the diameter and epithelial height of interlobular duct between the two sexes of rabbit ($P<0.01$).

The lobar ducts were the larger segments of excretory ducts displaced between the lobes which drained the interlobular ducts, the lobar ducts in turn to formed the main excretory duct (figs. 15,16,17). The lobar ducts at their initial parts close to the junction with interlobular duct were lined with high simple columnar epithelium with vesicular oval nucleus (fig. 15). The lobar ducts then passed usually in wavy manner surrounded by connective tissue, aggregation of the lymphatic tissue, lymphatic spaces, nerve bundles and ganglia (Fig. 17). At the mid part of the lobar duct, the epithelial lining was transformed into two layered stratified columnar epithelium which was more evident near the junction with main excretory duct. The cytoplasm of the epithelial cells was eosinophilic and with trichrome stain, it also

appeared slightly acid fuchsin positive but far lesser than the intralobular and interlobular ducts.

Bloom and Fawcett (1966) reported that larger duct of submandibular gland in man and other domestic animals were the columnar and pseudostratified epithelium.

The diameter of lobar duct of submandibular gland of male rabbit varied from 53.32 μm to 111.30 μm with an average of 81.7 ± 7.079 μm. In case of female, the diameter ranges from 55.04 μm to 111.8 μm with an average of 81.528 ± 6.024 μm. The epithelial height varied from 15.48 μm to 30.96 μm with an average of 20.124 ± 1.701 μm in male. Incase of female, however, the epithelial height varied from 18.92 μm to 30.96 μm with an average of 23.736 ± 1.597 μm. Statistical difference was insignificant as regards the diameter and epithelial height of lobar ducts in male and female as depicted in table no. - 2 ($P<0.01$).

Main excretory duct was formed by the union of lobar ducts and was lined with two layered stratified columnar epithelium to multilayered stratified columnar epithelium. The cytoplasm was acidophilic. The connective tissue around the main excretory duct presented fine capillary networks with occasional distribution of plasma cells. Rarely intraepithelial lymphocytes were seen migrating through the basal zone of the epithelium towards the lumen. The epithelium of main excretory duct abruptly transformed near the oral face of *cruncula sublingualis* into nonkeratinized stratified squamous epithelium (figs. 18, 19).

None of the animals, the epithelium of main excretory duct presented goblet cells. The lining epithelium of main excretory duct of submandibular salivary gland of rabbit as recorded, was in agreement with the observations

made by Trautmann and Fiebiger (1957) in domestic animals and Barnwal (1978) in buffalo submandibular salivary gland. Bloom and Fawcett (1976) in man and Garginlo *et al.* (1992) in horse recorded the presence of goblet cells in the epithelium of main excretory duct of submandibular salivary gland. In contrast to this, the goblet cells were lacking in the epithelium of main excretory duct of submandibular salivary gland of rabbit.

The subepithelial network of capillaries as observed in main excretory duct of rabbit's submandibular salivary gland was also reported by Suddick and Dowd (1969) and Ohtani *et al.* (1983) the main excretory duct of submandibular gland of rat.

HISTOCHEMISTRY :

The glandular endpieces of submandibular salivary gland reacted differently with several histochemical stains (Table-1). The acinar cells of submandibular salivary gland did not show positive reaction for PAS stain. The tubular cells were mildly reactive for PAS (Figs. 21,22). The vacuolar spaces formed by probably degeneration of glandular endpieces or, by realignment of glandular endpieces occasionally contained PAS positive colloidal mass (fig. 23). The glandular endpieces however did not reveal presence of glycogen when stained with PAS after saliva digestion. With toluidine blue technique (fig. 24), the tubular cells remained orthochromatic whereas the acinar cells presented mild metachromatia, suggestive for the presence of sulphated mucopolysaccharides and sialic acid associated mucoprotein. With colloidal iron technique, the cytoplasm of acinar cells appeared faintly greenish blue whereas that of tubular cells appeared greenish in colour (fig. 20). It was suggestive for mild presence of acid mucopolysaccharides in the acinar cells. When the section were stained with

mucicaramine, the cells of glandular acini as well as those of glandular tubule appeared faintly pink suggesting the mild presence of mucoprotein in association with mucopolysaccharides in these cells (fig. 25).

The present findings could be correlated with the findings of spicer and Duvenci (1964) who reported the presence of neutral mucopolysaccharides and acid mucopolysaccharides in submandibular salivary gland of different animals including rabbit. Leppi and Spicer (1966) in the submandibular salivary gland in man, squirrel and rhoesus monkey, Barnwal (1978) in buffalo, El-shafey *et al.* (1980) in goat, Pinkstaff *et al.* (1982) in little brown bat, Asojo and Aire (1983) in giant rat, Roy and Powar (1989) in sheep, Miyamotto and Miyamotto (1990) in pig, reported varying degree of histochemical reactivity of neutral and acid mucopolysaccharides in glandular cells of submandibular salivary gland. Hagelquivst *et al.* (1991) also reported PAS negative cells of glandular tubules in rabbit submandibular salivary gland.

With oil red O technique the glandular cells revealed the absence of lipid granules or, globules in the submandibular salivary gland (figs. 26,27).

The present findings could be compared with findings of Hill and Bourne (1954) who did not observe the presence of lipid in the glandular cells in the rat submandibular salivary gland. In contrast, Barnwal (1978) reported that the serous cells of submandibular gland of buffalo reacted moderately for lipids. Roy and Pawar (1989) also reported sudanophilic acinar cells in the submandibular salivary gland of sheep.

With Gomori's technique, the secretory cells of submandibular salivary gland of rabbit showed negative reaction for alkaline phosphatase (fig. 28). Although Barnwal (1978) in buffalo and Roy and Pawar (1989) in

sheep submandibular salivary gland, reported positive reaction for alkaline phosphatase.

Histochemically, the ductular epithelial cells were negative for neutral mucopolysaccharides and glycogen, when stained with PAS (Fig.21). The ductular epithelium also showed absence of acid mucopolysaccharides, when stained with colloidal iron technique (fig.20). The cells of the ductular epithelium remained orthochromatic when stained with toluidine blue technique. The mucicaramine stain did not show positive reaction for mucoprotein or mucin in the cells of the ductular epithelium (fig.24). With Gomori's alkaline phosphatase technique, mild positivity for alkaline phosphatase was recorded at the luminal margin of different segments of intralobular and interlobular ductular epithelium (fig. 28).

With oil red O technique the cells of ductular epithelium revealed variable positivity of oil red O in the submandibular gland of rabbit. Such positivity was most marked in the epithelium of interlobular ducts (figs. 26,27).

The present observation, was in the contrast of the findings of Spicer and Duvenci (1964) who recorded the presence of neutral and acid mucopolysaccharides in the epithelial cells of ducts of submandibular salivary glands in different animals including rabbit. The alkaline phosphatase positivity of the luminar border of ductular epithelium of the submandibular salivary gland of rabbit was, in agreement with the findings of Leeson and Jacoby (1957) in the intralobular duct of rat's submandibular salivary gland.

Oil red O positivity for lipid in the different segments of ductular epithelium of intralobular and interlobular in case of submandibular salivary

gland of rabbit could be correlated with the observations made by Hill and Bourne (1954) who recorded sudan black B positive lipids in the luminal border of ducts in the rat's submaxillary salivary gland.

SUBLINGUAL SALIVARY GLAND :

In case of rabbit, the sublingual salivary gland were located at two different places like several other domestic animals. Dorsocaudally, several groups of glandular tissues were localized under the mucous membrane at the floor of the mouth cavity which opened in the lateral sublingual papillae with the help of separate excretory ducts (fig. 1). These glandular masses were popularly termed as polystomatic salivary gland by several authors (Raghavan, 1964) and (Sisson, 1975). However Imai *et al.* (1982) preferred the term minor sublingual salivary gland in place of polystomatic salivary gland in case of man.

Cranially, In association with fibrous fold, the *plica sublingualis* comparatively less massive glandular aggregation was located as monostomatic salivary gland or, major sublingual salivary gland (fig. 1). The monostomatic sublingual salivary gland was drained with a single duct which opened at the *cruncula sublingualis* along the side of mandibular duct (Sisson, 1975).

HISTOLOGY :

SUPPORTING TISSUE :

Since, the both segments of sublingual salivary gland was embedded within the surrounding connective tissue at the floor of the mouth, the definite capsule was not clearly demarcated, however the connective tissue components divided the gland into lobes and lobules. These connective tissue strands were made up of collagen and reticular fibers (figs. 29,30,31,32). The elastic fibers were lacking. The connective tissue septations were much more prominent at the *plica sublingualis* which occasionally presented the transverse sections of main excretory duct of

major sublingual salivary gland (fig. 29). The connective tissue around the larger ducts usually presented lymphoid tissue, plasma cells, vascular network and fine nerve bundles (fig. 31). The present observation were in agreement with the observation of Carleton and Short (1965) who recorded presence of capillary network and nerves in the sublingual salivary glands however, plain muscle cells observed by them around the large ducts could not be recorded in the rabbit. The elastic fibers as recorded in human sublingual gland by sognnaes and Moss (1968) could not be discerned during present observation. The connective tissue septae and divisions of the glands into lobes and lobules. In case of rabbit sublingual salivary gland could be well correlated with the findings of Dellman (1971) in domestic animals, Copenhaver and Johnson (1978) in man and Taha *et al.* (1999) in the one humped camel.

The connective tissue capsule in the sublingual salivary gland of buffalo as observed by Barnwal (1978) could not be identified distinctly in case of rabbit.

PARENCHYMA : Histomorphologically, the sublingual gland of rabbit was characterized as compound branched tubuloacinar gland. According to the secretory nature of the glandular adenomeres of sublingual salivary gland of rabbit were of mixed type with predominantly mucous varieties (figs. 33,34,35,36).

Trautmann and Fiebiger (1957) in domestic animals, Carleton and Short (1965) in man, Sognnaes and Moss (1966) in man, Bloom and Fawcett (1966) in man, Carpenter (1968) in man, Senger and Singh (1970) in buffalo, Dellman (1971) in domestic animals, Ham and Leeson (1971) in man, Capenhaver and Johnson (1978) in man, dog, cat, rabbit and sheep, Leeson

and Leeson (1979) in man, Banks (1981) in horse, man and carnivores, Asojo and Aire (1983) in giant rat, Imai *et al.* (1983) in Japanese macaques, Stinson and Calhoun (1987) in domestic animals, Kishore *et al.* (1999) in goat and Taha *et al.* (1999) in one humped camel reported the sublingual salivary gland as mixed type of gland.

GLANDULAR ENDPIECES : The glandular endpieces were tubuloacinar type which were comprised of mucous and serous cells. The Kishore *et al.* (1999) however described the glandular endpieces of sublingual salivary gland of goat as tubuloalveolar in type.

In case of rabbit, the acini of sublingual salivary gland were composed of mucous cells having foamy cytoplasm with serous demilunes (figs. 34,35). Occasionally, small acini of serous varieties were also identified in the parenchyma of sublingual salivary glands (figs.36). The tubular part of the gland was essentially made up of mucous cells. The cells of serous demilunes appeared in association with mucous acini contained eosinophilic cytoplasm with oval nucleus (figs. 34,35). The isolated clusters of serous acini were not observed in the minor sublingual salivary gland.

The present findings were in agreement with findings of Trautmann and Fiebiger (1957) Carleton and Short (1965), Sognnaes and Moss (1966), Carpenter (1968), Dellman (1971), Copenhaver and Johnson (1978), Asojo and Aire (1983) and Kishore *et al.* (1999) who recorded the serous demilunes capping the mucous acini of sublingual salivary gland of different mammals. Taha *et al.* (1999) however reported that the sublingual salivary gland of the one humped camel was of mixed varities which almost contained mucous endpieces with small group of serous endpieces.

Micrometric observations as revealed in table no. 4 the glandular acini ranged their diameter from 34.40 μm to 58.48 μm in male rabbit whereas 36.12 μm to 61.92 μm in female rabbits. The average diameter was 48.16 ± 2.161 μm in male and 48.504 ± 2.41 μm in female.

Similarly, the tubular diameter in male varied between 25.8 μm to 51.6 μm with an average of 37.84 ± 2.543 μm. The tubular diameter incase of female rabbit, ranged between 24.08 μm and 49.88 μm with an average of 37.496 ± 1.943 μm.

Statistically, the difference between diameter of the acini between two sexes did not vary significantly. The tubular diameter also differed in-significantly between the male and female rabbit ($P<0.01$).

DUCT SYSTEM :

Both the minor and major parts of sublingual salivary gland of rabbit revealed intercalated duct, intralobular duct, interlobular duct, lobar duct and main excretory duct.

The distribution of intercalated ducts were comparatively less marked in the minor sublingual salivary gland when present, the intercalated duct was lined with simple squamous epithelium which join the glandular tubule with the intralobular duct (fig. 34).

The intralobular duct was lined with single layer of low cuboidal epithelium. The epithelial cells did not reveal basal striations (fig. 34). The interlobular ducts were lined with simple columnar epithelium which eventually joined the lobar duct. Lobar duct was lined with simple columnar or stratified columnar epithelium without any distribution of goblet cells (figs. 37,38,39).

The main excretory duct of minor salivary gland opened individually through several individual sublingual papillae. These excretory ducts of minor salivary gland or polystomatic sublingual salivary gland were lined with stratified squamous epithelium to continue with the epithelium of lateral sublingual recess of the mouth cavity (fig. 40). The main excretory duct was surrounded by loose connective tissue, lymph spaces and capillary network.

The main excretory duct of major sublingual salivary gland was lined with stratified columnar epithelium which continued in tortuous manner along the connective tissue septae of *plica sublingualis* associated with *cruncula sublingualis* to open at the floor of the mouth cavity (figs. 41,42).

Carleton and Short (1965) however, described in most of the domestic animals that the secretory ducts of sublingual glands joined directly to the mucous alveoli with the help of an intermediate on junctional portion. Sognnaes and Moss (1966) however, described that human sublingual salivary gland contained secretory ducts. The present findings, however in contrasts of the findings of Senger and Singh (1970) who reported the absence of intercalated duct in the buffalo sublingual salivary gland. Similarly, Bloom and Fawcett (1976) also observed that the terminal part of glandular adenomere directly join the striated duct. Barnwal (1978) also recorded the variable presence of intercalated duct in the polystomatic sublingual salivary gland, however the intercalated duct were essentially present in monostomatic sublingual salivary gland.

The present observation could also be compared with lining epithelium of ducts of sublingual salivary gland in man and domestic animals (Copenhaver and Johnson, 1978; Leeson and Leeson, 1979; and Stinson and Calhoun; 1987).

The distribution of numerous goblet cells as recorded by Taha *et al.* (1999) in intralobular excretory duct in sublingual salivary gland of one humped camel could not be recorded in rabbit sublingual salivary gland. The histometric observations (table-5) revealed that the diameter of intralobular duct varied from 18.92 μm to 65.36 μm in male rabbit with an average of 44.032 ± 14.557 μm. In female rabbit, however the diameter of intralobular duct measured from 36.12 μm to 60.20 μm with an average of 44.376 ± 2.111 μm. The epithelial height of intralobular duct ranged between 8.6 μm to 15.48 μm both in male and female rabbits. However, average epithelial height in male was 11.696 ± 0.802 μm whereas in female, the average height recorded as 12.212 ± 0.786 μm. Statistically, the diameter as well as epithelial height did not show sexual diamorphism at $P<0.01$. The diameter of interlobular duct, incase of male ranged from 41.28 μm to 86.00 μm with and average of 60.716 ± 5.037 μm. In female, however the diameter ranged from 34.40 μm to 77.40 μm with an average of 55.728 ± 4.617 μm.

The epithelial height of interlobular duct of sublingual salivary gland in male 8.6 μm to 22.36 μm and in female, 6.88 μm to 24.08 μm although the average epithelial height in male was recored 14.104 ± 5.037 μm and 13.932 ± 1.630 μm in female. The measurement of interlobular duct did not reveal sexual diamorphism. The average diameter of lobar duct of sublingual salivary gland in male rabbit ranged from 67.08 μm to 154.8 μm with an average of 90.644 ± 8.823 μm. In female, diameter measured from 58.48 μm to 153.08 μm with an average of 95.632 ± 10.024 μm. Statistically, the diameter of lobar duct in male and female rabbit did not vary significantly. The epithelial height of lobar duct ranged from 10.32 μm to 34.4 μm with an average of 15.996 ± 2.206 μm in male rabbit. In female rabbit, the epithelial

height ranged from 8.6 μm to 24.08 μm with an average of 15.48 ± 1.494 μm. Statistical analysis did not reveal significant difference between the epithelial height of lobar duct in the sublingual salivary gland of male and female rabbit ($P<0.01$).

HISTOCHEMISTRY :

The secretory endpieces reacted moderately PAS positive suggesting the presence of neutral mucopolysaccharides in the cells. However, the ductular epithelium showed occasional PAS positivity with variable amount of PAS positive substances in the lumen (fig. 43). The intensity of PAS positivity did not differ in the parenchyma after saliva digestion, suggesting the absence of glycogen in the cells of secretory endpieces and ductular epithelium of sublingual salivary gland in rabbit. With mucicarmine stain the glandular endpieces reacted mild to moderate intensity with negative reaction in the epithelium of ducts (figs. 44,45). The luminal margin of duct however, presented PAS positive materials when stained with colloidal iron technique, variable positivity for acid mucopolysaccharides was recorded in the glandular cells and ductular epithelium (figs. 46, 47). But when stained with toluidine blue technique, the glandular cells reacted intensely showing strong metachromatia (figs. 48,49). The positive reaction with colloidal iron stain and the metachromatia in the glandular cells were suggestive for acid mucopolysaccharides containing sialomucin or sulphomucin in variable proportion.

The present observations could be well correlated with findings of Fava demoraes and Villa (1963) who recorded that the cells of striated duct were weakly PAS positive containing mildly positive mucicarmine granules which reacted orthochromatically when stained with toluidine blue.

The PAS reactivity as well as the reaction for acid mucopolysaccharides in rabbit sublingual gland appear to be in agreement with the findings of Munger (1964) in human sublingual salivary gland and Spicer and Duvenci (1964) in different mammalian including rabbit's sublingual salivary gland.

The negative reaction for glycogen in the sublingual salivary gland of rabbit was however, in contrast to the findings of Leppi and Spicer (1967) who recorded the glycogen after digestion of PAS positive substances with the help of diastase. They however, further reported positive reaction for sulphated mucosubstances containing sialic acid in the sublingual salivary gland of cow.

Positive reactions for PAS and colloidal iron stain along with metachromatic reaction with toluidine blue in the sublingual salivary gland of rabbit, were in agreement with the findings of Barnwal (1978) who also recorded similar reactions in the mucous cells of sublingual salivary gland of buffalo. Asojo and Aire (1983) in giant rat and Kishore *et al.* (1999) in goat sublingual salivary gland also reported the presence of acid and neutral mucin in the glandular cells.

With Gomori's alkaline phosphatase stain mild positivity of alkaline phosphatase was recorded at the basal border of the glandular cells and the luminal border of the ducts of sublingual salivary gland of rabbit (fig. 50). Kawakatsu *et al.* (1959) also observed diffuse or, moderate alkaline phosphatase activities around the base of secretory cells in the sublingual salivary gland of rat, guinea pig, rabbit and dog. Barnwal (1978) also reported the positive reaction for alkaline phosphatase in the serous and mucous cells of sublingual salivary gland in buffalo.

With oil red O stain the distinct lipid droplet were not observed in the interstitium, however, mild oil red O positivity was observed in the glandular adenomeres of sublingual salivary gland of rabbit (fig. 51).

Barnwal (1978) showed mild presence of lipid in the glandular cells in the sublingual salivary gland of buffalo.

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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Histomorphological studies on submandibular and sublingual salivary glands of New zealand white rabbits were conducted. During the study, the tissue samples were collected from six male and six female rabbits.

After proper fixation microscopical slides were procured and stained for histological and certain histochemical studies. Certain histometrical data were also recorded for statistical analyses.

SUBMANDIBULAR SALIVARY GLAND :

The submandibular salivary glands were irregularly oval and were situated at the intermandibular space near the caudal end of *pars molaris mandibularis*. The main excretory duct opened at elongated *cruncula sublingualis* near the cranial floor of the mouth cavity.

Histologically, the submandibular salivary gland of rabbit was highly deficient in connective tissue components. A thin capsule was mainly made up of collagen fibers with a few reticular fibers. The elastic fibers were absent. The interstitium of lobes and lobules was mainly made up of reticular fibers with scanty collagen fibers. The elastic fibers and smooth muscle cells were altogether absent.

The submandibular gland of rabbit was typed as compound tubulo acinar mixed gland. The glandular endpieces comprised of glandular tubules and glandular acini.

Histomorphologically, the cells of glandular tubules and acini differed from each other. The acini were lined with cuboidal or pyramidal cells with spherical nucleus and foamy cytoplasm with few scattered eosinophilic cytoplasmic granules suggestive for seromucous characters. The cells of the

glandular tubule appeared as cuboidal or pyramidal cells having spherical nucleus with acidophilic cytoplasmic granules giving the appearance of a typical serous cells. The myoepithelial cells around the glandular epithelium were lacking.

The average diameter of glandular acini measured 28.724 ± 2.472 μm in male and 27.692 ± 4.432 μm in female. The average diameter of glandular tubule measured 27.692 ± 3.001 μm in male and 27.692 ± 2.965 μm in female. Statistically, the difference was insignificant between the acinar diameter of male and female rabbit. Similarly, there was no significant difference between the tubular diameter of two sexes.

The duct system of submandibular gland presented intercalated duct, intralobular duct, interlobular duct, lobar duct and main excretory duct. The intercalated duct was lined with simple squamous or simple low cuboidal epithelium. The intralobular duct occasionally showed secondary divisions. Intralobular ducts were lined with high cuboidal or columnar epithelium with eosinophilic granular cytoplasm. The basal striations and myoepithelial cells were lacking. The average diameter of intralobular duct measured 49.192 ± 2.314 μm in male and 46.784 ± 2.031 μm in female rabbit. The epithelial height measured 13.76 ± 1.332 μm in male and 13.072 ± 1.261 μm in female. Statistically, no sexual variation was recorded in the diameter and epithelial height of intralobular duct at $P<0.01$ level. The interlobular ducts were comparatively wider in diameter with a similar type of epithelial lining. The acidophilic character of cytoplasm was however, lesser. The average diameter of interlobular duct measured 61.232 ± 2.617 μm in male and 56.072 ± 4.53 μm in female. Statistically, no sexual variation was noted at $P<0.01$ level. The lobar ducts were lined with simple high columnar

epithelium which transformed in to two-layered stratified columnar epithelium near the junction of main excretory duct. The cytoplasm of the epithelial cells appeared to be faintly acidophilic. The average diameter of lobar duct measured 81.7 ± 7.079 μm in male and 81.528 ± 6.024 μm in female. Statistically, no sexual variation was noticed. The main excretory duct was lined with stratified columnar epithelium with acidophilic cytoplasm. The epithelium abruptly transformed near the oral face of *Cruncula sublingualis* into nonkeratinized stratified squamous epithelium. The epithelium did not show the presence of goblet cells.

Histochemically, the glandular acinar cells were negative for PAS, glycogen, alkaline phosphatase and lipid. They however, reacted mildly for colloidal iron, tolouidine blue and mucicarmine stain. The glandular tubular cells however, reacted mildly for PAS and mucicarmine stain. The cells were negative for glycogen, colloidal iron and toluidine blue stains. They were also lacking alkaline phosphatase and lipid droplets. The ductular epithelium mildly showed positive reaction for alkaline phosphatase at intralobular and interlobular duct segments. The epithelium of intralobular duct showed mild reaction for lipid whereas interlobular ductular epithelium reacted moderately for lipid stain. The epithelial cells of intercalated duct did not reveal the positive reaction for histochemical stains undertaken during the course of investigation.

SUBLINGUAL SALIVARY GLAND :

In rabbits, the sublingual salivary glands were located at two different places. Dorsocaudally, several groups of glandular tissues were located under the mucous membrane which opened into the lateral sublingual papillae with the help of separate excretory ducts. They were grouped

together as minor sublingual salivary glands or polystomatic sublingual salivary gland.

Ventrocranially, the major sublingual salivary gland was located whose main excretory duct opened at *cruncula sublingualis* thus considered to be monostomatic sublingual salivary gland.

Histologically, the definite capsule was not clearly identified for these glands as they were embedded in the surrounding connective tissue at the floor of mouth cavity however, septation divided the glands into lobes and lobules. The interstitium was made up of collagen and reticular fibers. Elastic fibers were lacking. The glandular endpieces were tubuloacinar type with mixed variety of cells. The acini of the sublingual salivary glands were composed of mucous cells with serous demilune. The acini of serous variety were occasionally identified in the parenchyma. The glandular tubules were essentially made up of mucous cells. The minor salivary gland did not present the serous acini.

Micrometric observations revealed average diameter of the glandular acini as 48.16 ± 2.161 μm in male and 48.504 ± 2.41 μm in female. Similarly, tubular diameter of male presented average of 37.84 ± 2.543 μm in male and 3.496 ± 1.943 μm in female. Statistically, there was no significant variation between the sexes within the measurement of these two traits.

Both minor and major sublingual salivary glands revealed standard subdivisions of duct system however, presence of intercalated duct was comparatively less in minor sublingual salivary glands. Intercalated duct when present, was lined with simple squamous epithelium. The intralobular duct was lined with simple low cuboidal epithelial cells without showing

basal striations. The interlobular duct was lined with simple columnar epithelium whereas the lobar duct presented simple or stratified columnar epithelium without the presence of goblet cells. The main excretory duct of minor sublingual salivary gland was lined with stratified squamous epithelium whereas main excretory duct of major sublingual salivary gland was lined with stratified columnar epithelium.

Histometrically, the diameter of intralobular duct measured 44.032 ± 14.557 μm in male and 44.376 ± 2.111 μm in female. The diameter of interlobular duct measured 60.716 ± 5.037 μm in male and 55.728 ± 4.617 μm in female. The diameter of lobar duct in male, measured as average of 90.644 ± 8.823 μm and in female 95.632 ± 10.024 μm. The sexual diamorphism could not be observed in the diameters of these ducts.

Histochemically, secretory endpieces reacted moderately for PAS showing intense metachromatia with toluidine blue. The cells were lacking in glycogen but were reacted mild to moderately with mucicarmine stain. With colloidal iron stain, cells were occasionally reacted mildly for acid-mucopolysaccharides. The glandular cells were also reacted mildly for lipid droplets. The basal border of acini or tubule reacted mildly with Gomori's stain for alkaline phosphatase.

The ductular epithelium, however revealed occasional positivity for PAS which did not reveal the presence of glycogen when stained after saliva digestion. The ductular epithelium also reacted mildly with colloidal iron stain but were negative for toluidine blue reaction, mucicarmine and lipid stains. The luminal border of ductular epithelium reacted mildly for alkaline phosphatase.

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TABLES

Table 1. : showing mean diameter ± S.E. with CV % of the glandular tubules and acini in the submandibular salivary gland of rabbit.

Structures	Male rabbit		Female rabbit	
	Mean ± S.E. (μm)	CV %	Mean ± S.E. (μm)	CV %
Acini	$28.724^{h} \pm 2.472$ (18.92 – 37.84)	16.235	$27.692^{h} \pm 4.432$ (18.92 – 37.84)	28.894
Tubules	$27.692^{h} \pm 3.001$ (18.92 – 41.28)	25.079	$27.692^{h} \pm 2.965$ (18.92 – 37.84)	25.010

Mean under each trait bearing same supercript (row wise) do not differ significantly ($P<0.01$).

Figure under parentheses denote the range of measurements.

Table 2. : Showing certain micrometric mean ± S.E. with CV % in the different segments of excretory duct system of submandibular salivary gland in rabbit.

Structures	Male rabbit		Female rabbit	
	Mean ± S.E. (μm)	CV %	Mean ± S.E. (μm)	CV %
Intralobular duct				
Diameter	$49.192^{h} \pm 2.314$ (36.12 – 61.92)	14.878	$46.784^{h} \pm 2.031$ (36.12 – 56.76)	13.730
Epithelial height	$13.760^{h} \pm 1.332$ (6.88 – 20.64)	30.610	$13.072^{h} \pm 1.261$ (8.6 – 17.2)	30.507
Interlobular duct				
Diameter	$61.232^{h} \pm 2.617$ (48.16 – 77.40)	13.515	$56.072^{h} \pm 4.530$ (25.8 – 75.65)	25.549
Epithelial height	$18.404^{h} \pm 1.682$ (12.04 – 25.80)	28.901	$18.404^{h} \pm 2.446$ (8.6 – 29.24)	42.034
Lobar duct				
Diameter	$81.7^{h} \pm 7.079$ (53.32 – 111.30)	27.397	$81.528^{h} \pm 6.024$ (55.04 – 111.80)	23.367
Epithelial height	$20.124^{h} \pm 1.701$ (15.48 – 30.96)	26.739	$23.736^{h} \pm 1.597$ (18.92 – 30.96)	21.275

Mean under each trait bearing same superscript (row wise) donot differ significantly ($P<0.01$).

Figure under parentheses denote the range of measurements.

Table 3 : Showing different histochemical staining reactions in the glandular endpiece and ductular epithelium of submandibular salivary gland of rabbit.

Structures	PAS	PAS with saliva digestion	Colloidal iron technique	Metachromatia with toluidine blue technique	Mucicarmine	Gomori's Akpase	Oil red 'O' technique
Glandular endpiece							
Acinar cells	-	-	+	+	+	-	-
Tubular cells	+	+	–	-	+	-	-
Ductular epithelium							
Intercalated	–	–	–	–	–	–	–
Intralobular	–	–	–	–	–	+	+
Interlobular	–	–	–	–	–	+	+
Negative (–); Mild (+); Moderate (++)							

Table 4. : Showing mean diameter ± S.E. with CV % of the glandular tubules and acini in the sublingual salivary gland of rabbit.

Structures	Male rabbit		Female rabbit	
	Mean ± S.E. (μm)	CV %	Mean ± S.E. (μm)	CV %
Acini	$48.160^{h} \pm 2.161$ (34.40 – 58.48)	23.791	$48.504^{h} \pm 2.410$ (36.12 – 61.92)	27.520
Tubules	$37.840^{h} \pm 2.543$ (25.80 – 51.60)	29.048	$37.496^{h} \pm 1.943$ (24.08 – 49.88)	22.190

Mean under each trait bearing same superscript (row wise) do not differ significantly ($P<0.01$).

Figure under parentheses denote the range of measurements.

Table 5. : Showing certain micrometric mean ± S.E. with CV % in the different segments of excretory duct system of sublingual salivary gland in rabbit.

Structures	Male rabbit		Female rabbit	
	Mean ± S.E. (μm)	CV %	Mean ± S.E. (μm)	CV %
Intralobular duct				
Diameter	$44.032^{h} \pm 14.557$ (18.92 – 65.36)	104.530	$44.376^{h} \pm 2.111$ (36.12 – 60.20)	15.041
Epithelial height	$11.696^{h} \pm 0.802$ (8.6 – 15.48)	21.691	$12.212^{h} \pm 0.786$ (8.6 – 15.48)	20.373
Interlobular duct				
Diameter	$60.716^{h} \pm 5.037$ (41.28 – 86.00)	26.236	$55.728^{h} \pm 4.617$ (34.40 – 77.40)	26.196
Epithelial height	$14.104^{h} \pm 5.037$ (8.6 – 22.36)	112.946	$13.932^{h} \pm 1.630$ (6.88 – 24.08)	71.830
Lobar duct				
Diameter	$90.644^{h} \pm 8.823$ (67.08 – 154.80)	30.779	$95.632^{h} \pm 10.024$ (58.48 – 153.08)	33.143
Epithelial height	$15.996^{h} \pm 2.206$ (10.32 – 34.4)	43.610	$15.480^{h} \pm 1.494$ (8.6 – 24.08)	30.536

Mean under each trait bearing same superscript (row wise) do not differ significantly ($P<0.01$).

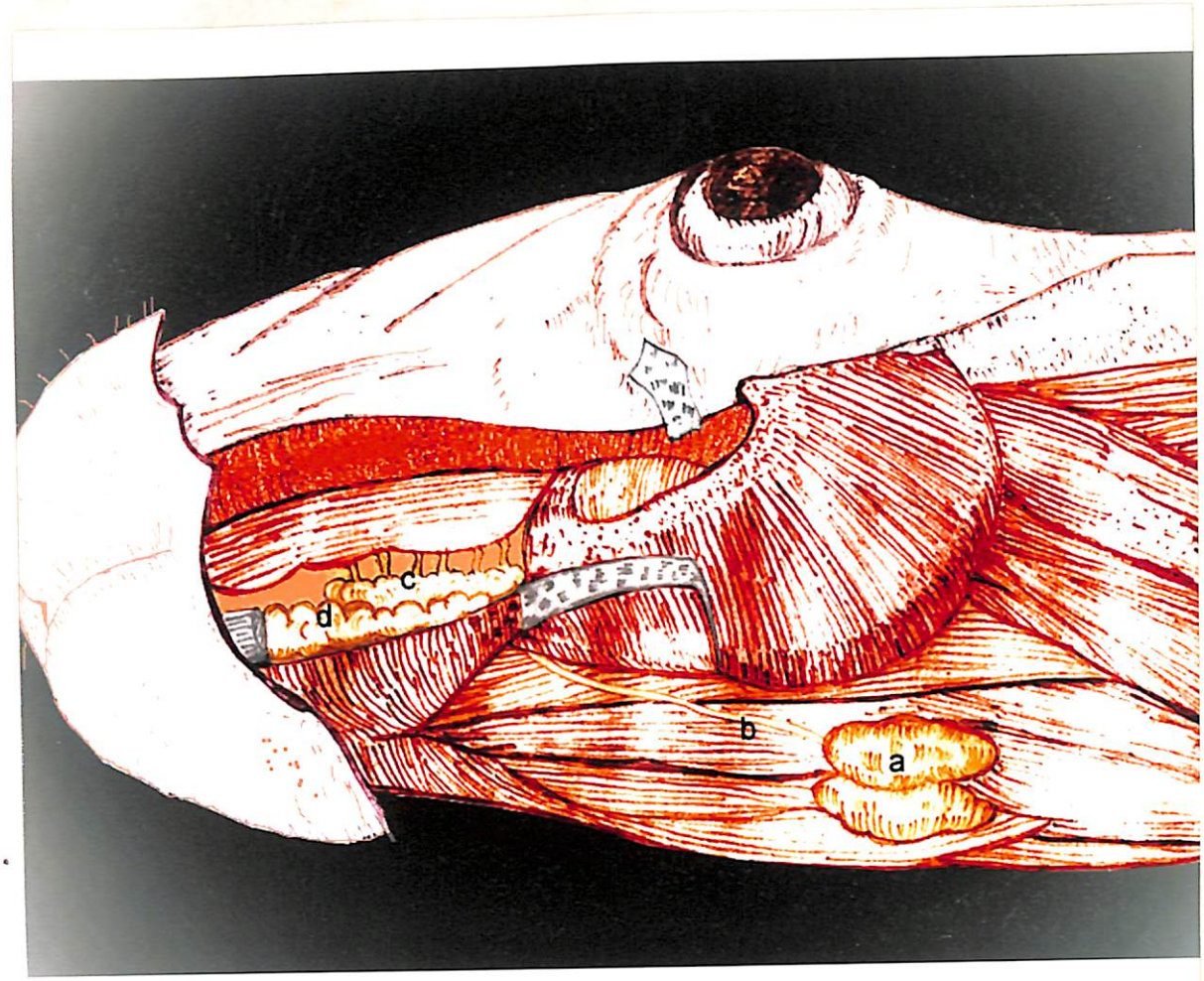
Figure under parentheses denote the range of measurements.

Table 6. : Showing different histochemical staining reactions in the glandular endpiece and ductular epithelium of sublingual salivary gland of rabbit.

Structures	PAS	PAS with saliva digestion	Colloidal iron technique	Metachromatia with toluidine blue technique	Mucicarmine	Gomori's Akpase	Oil red 'O' technique
Glandular endpiece							
Acinar cells	++	++	± to +	+++	+ to ++	(at basal border) +	+
Tubular cells	++	++	± to +	+++	+ to ++	(at basal border) +	+
Ductular epithelium							
Interalobular	±	-	+	-	-	(at luminal border) +	-
Interlobular	±	-	+	-	-	(at luminal border) +	-
Lobar	±	-	+	-	-	(at luminal border) +	-

Negative (-); Occasional (±) ; Mild (+); Moderate (++); Strong or, Intense (+++).

GROSS FIGURE AND PHOTO MICROGRAPHS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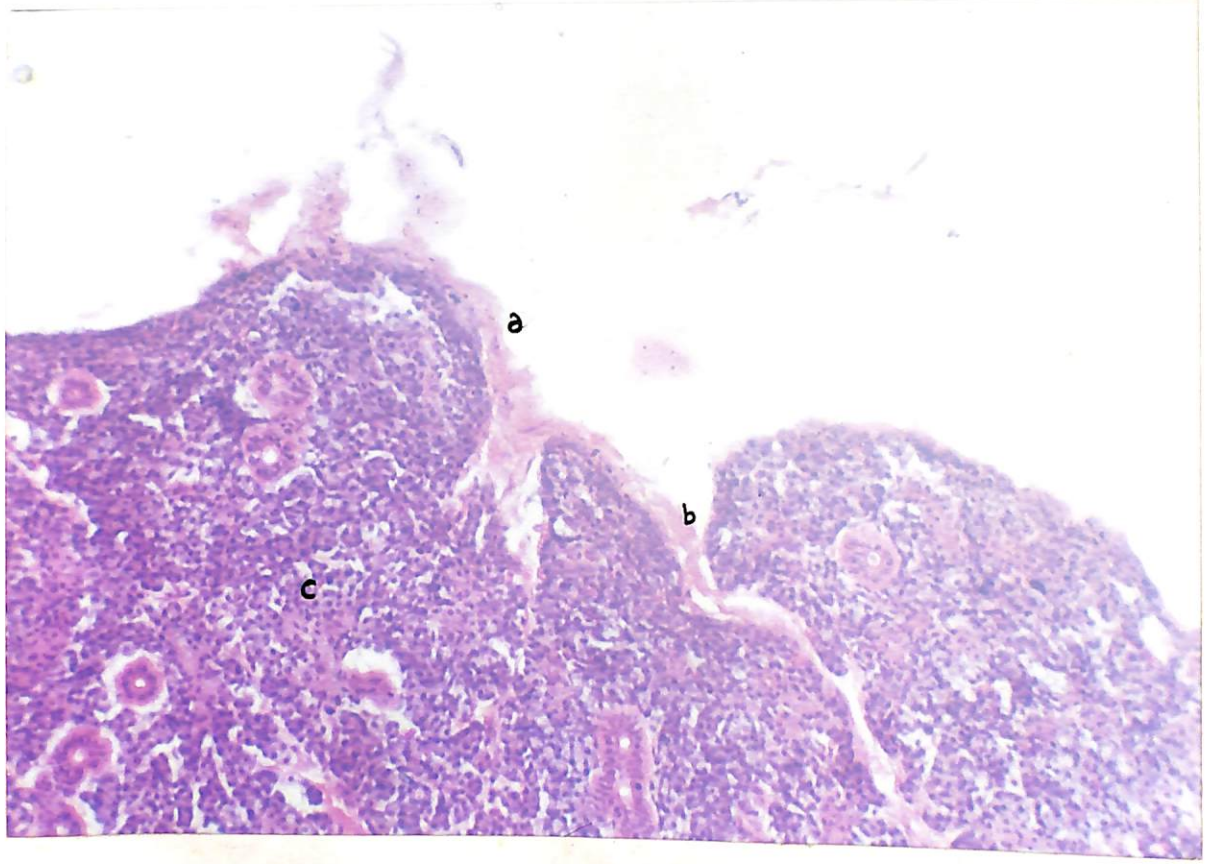
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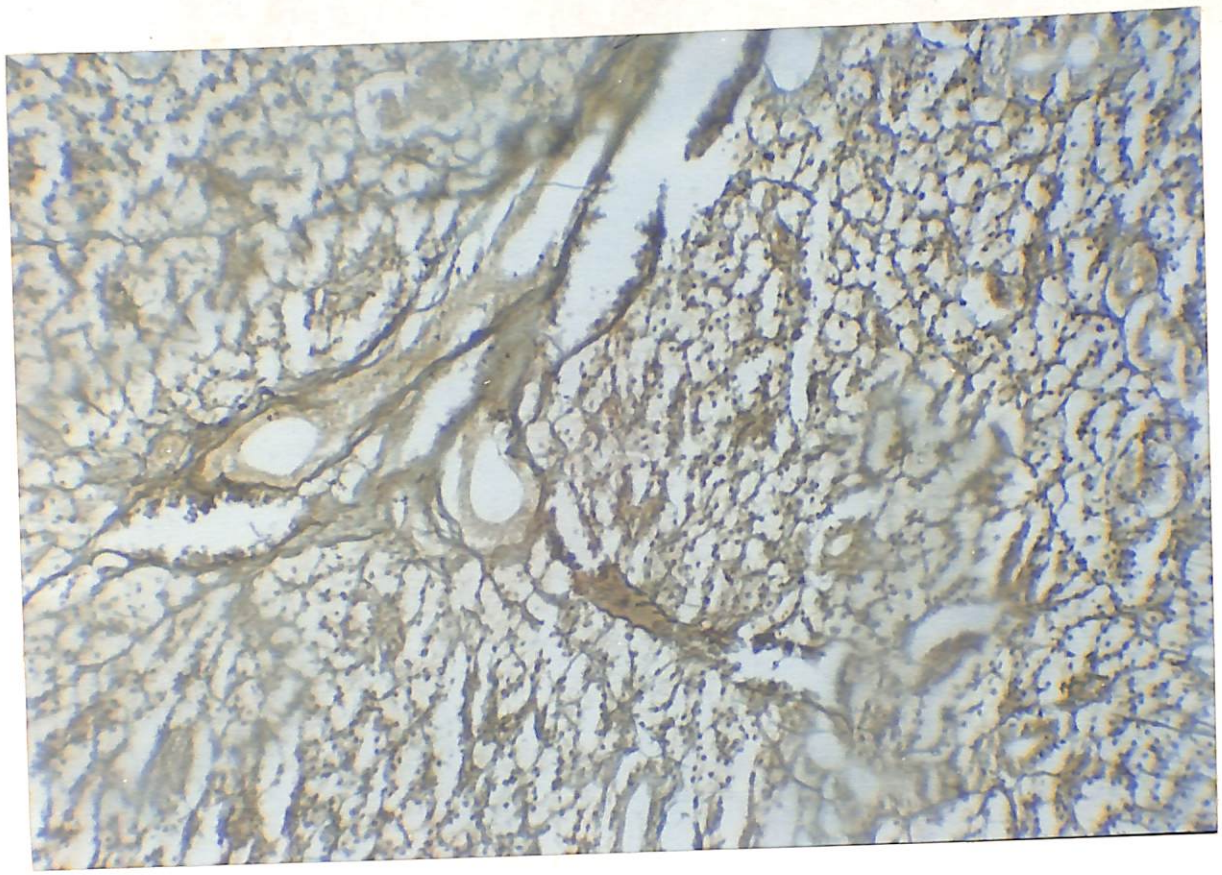

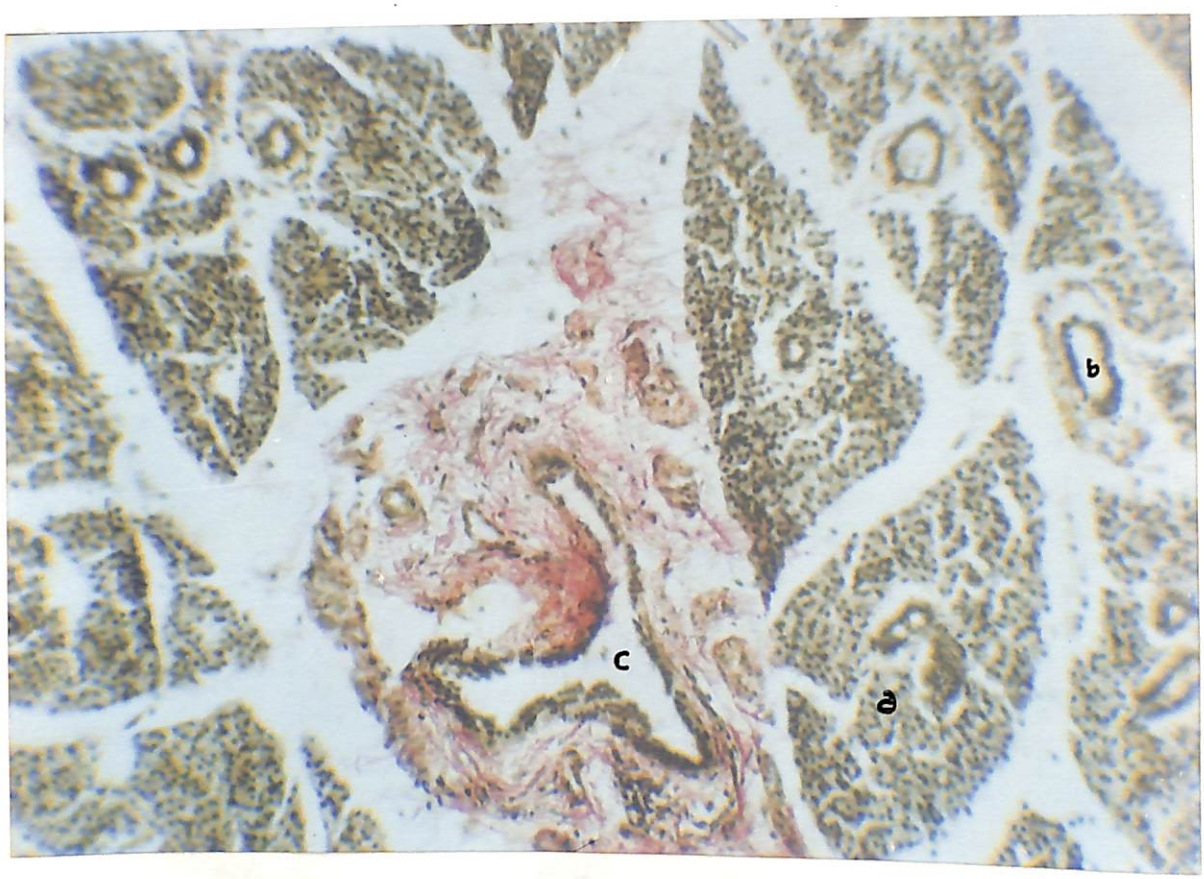
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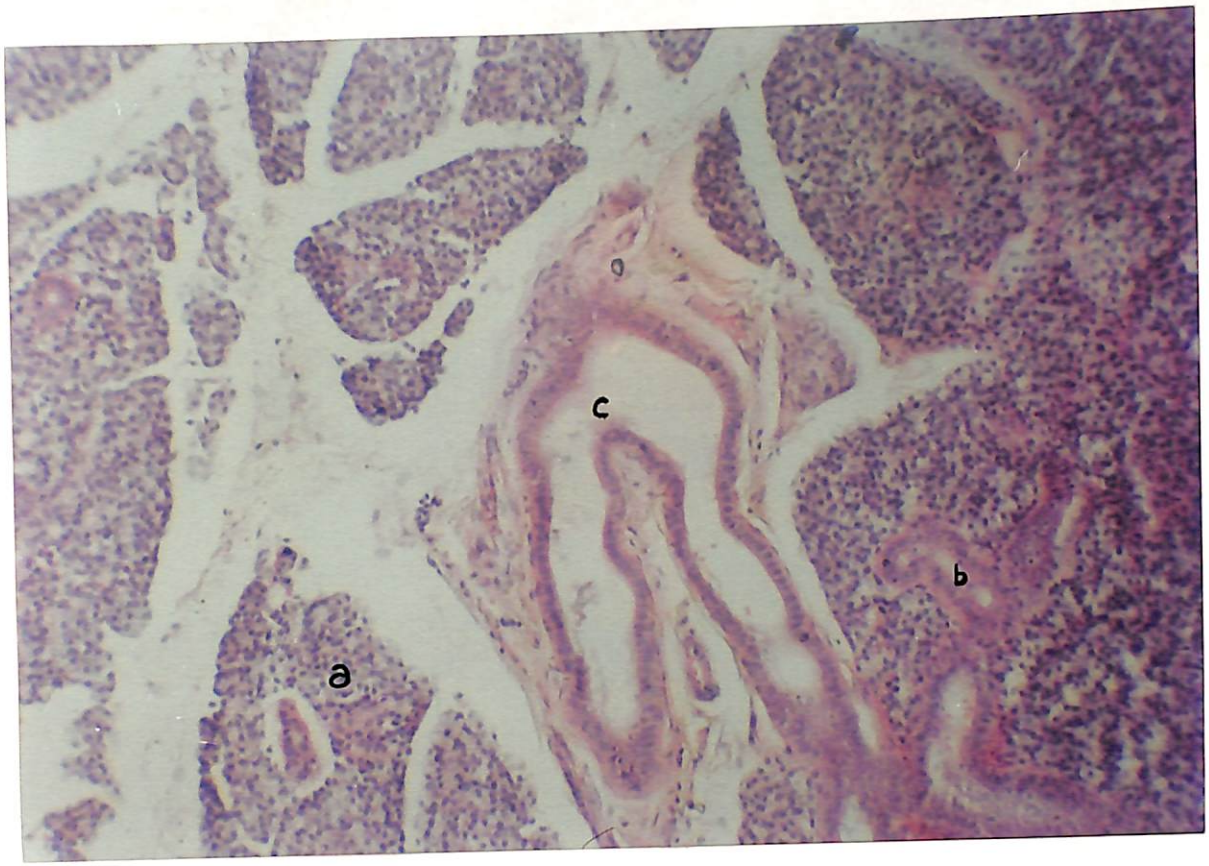
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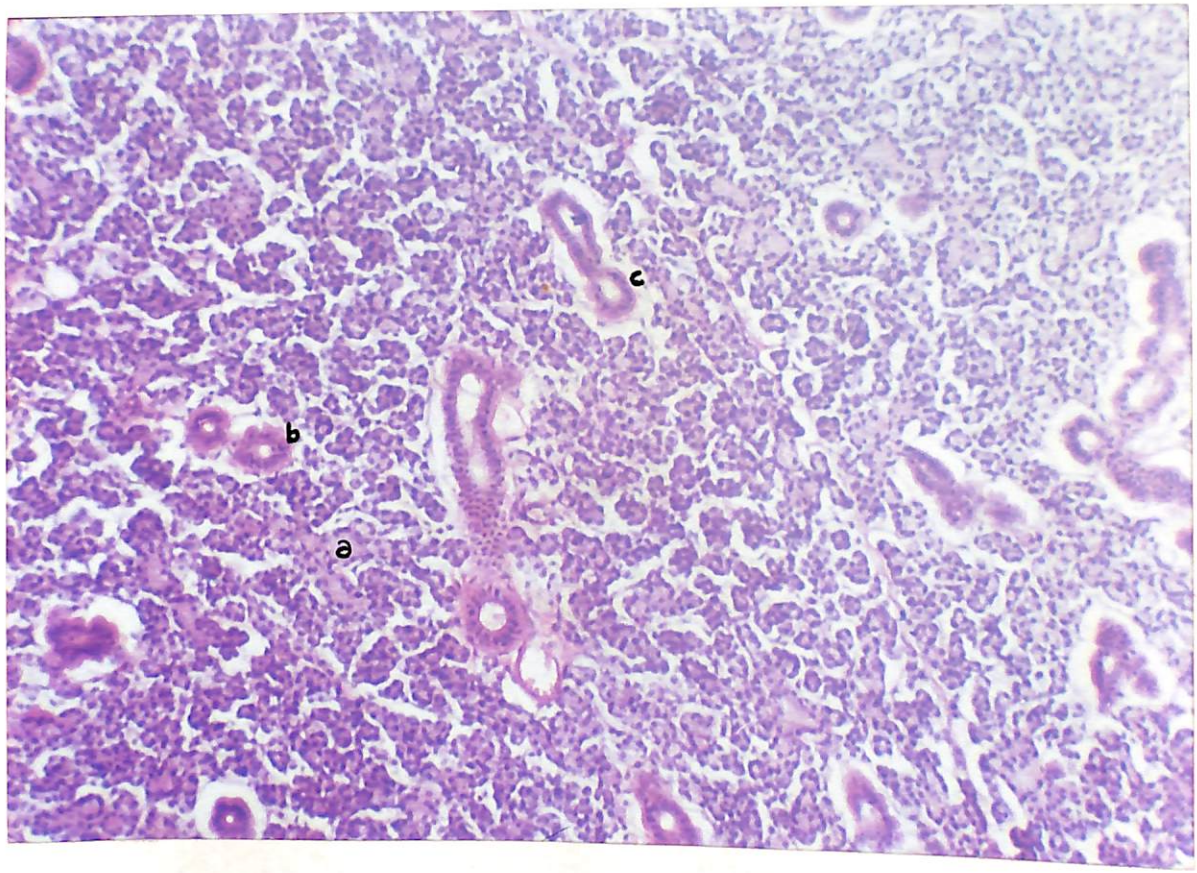
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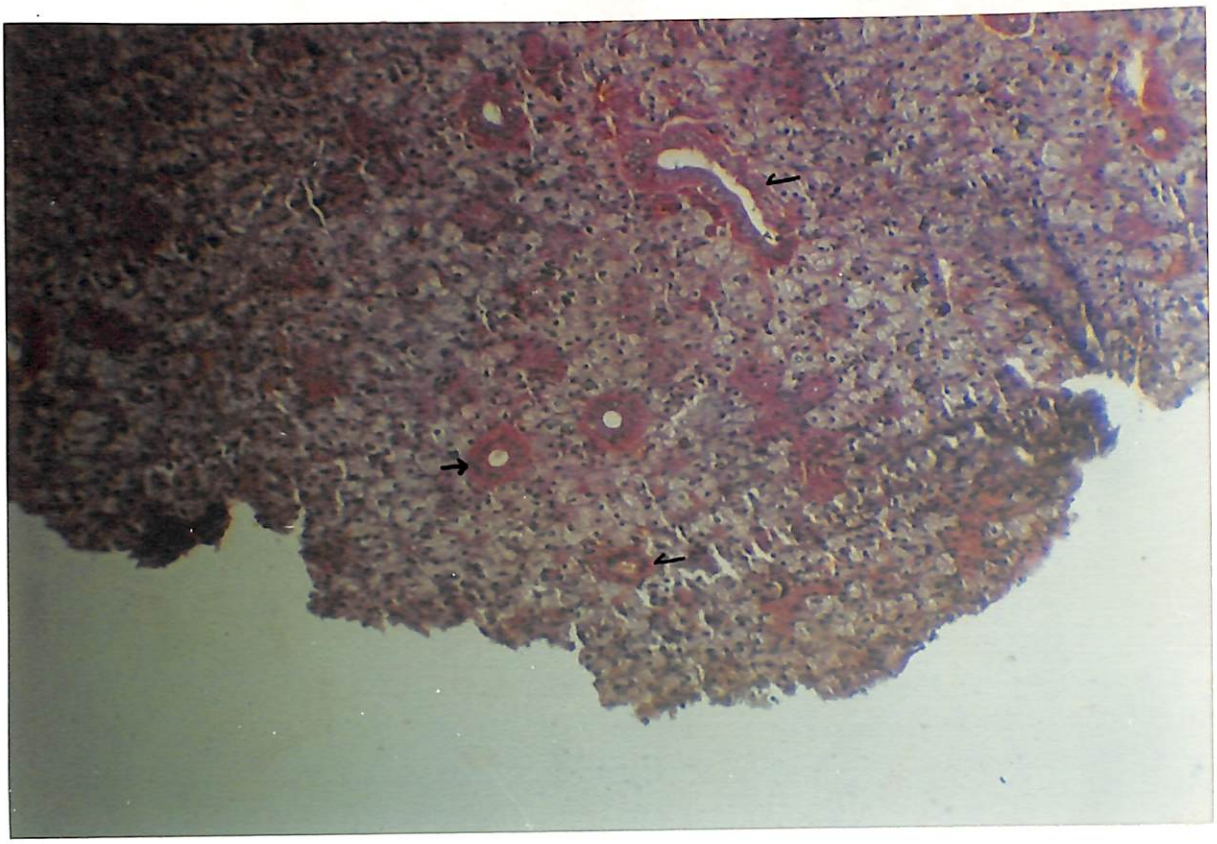

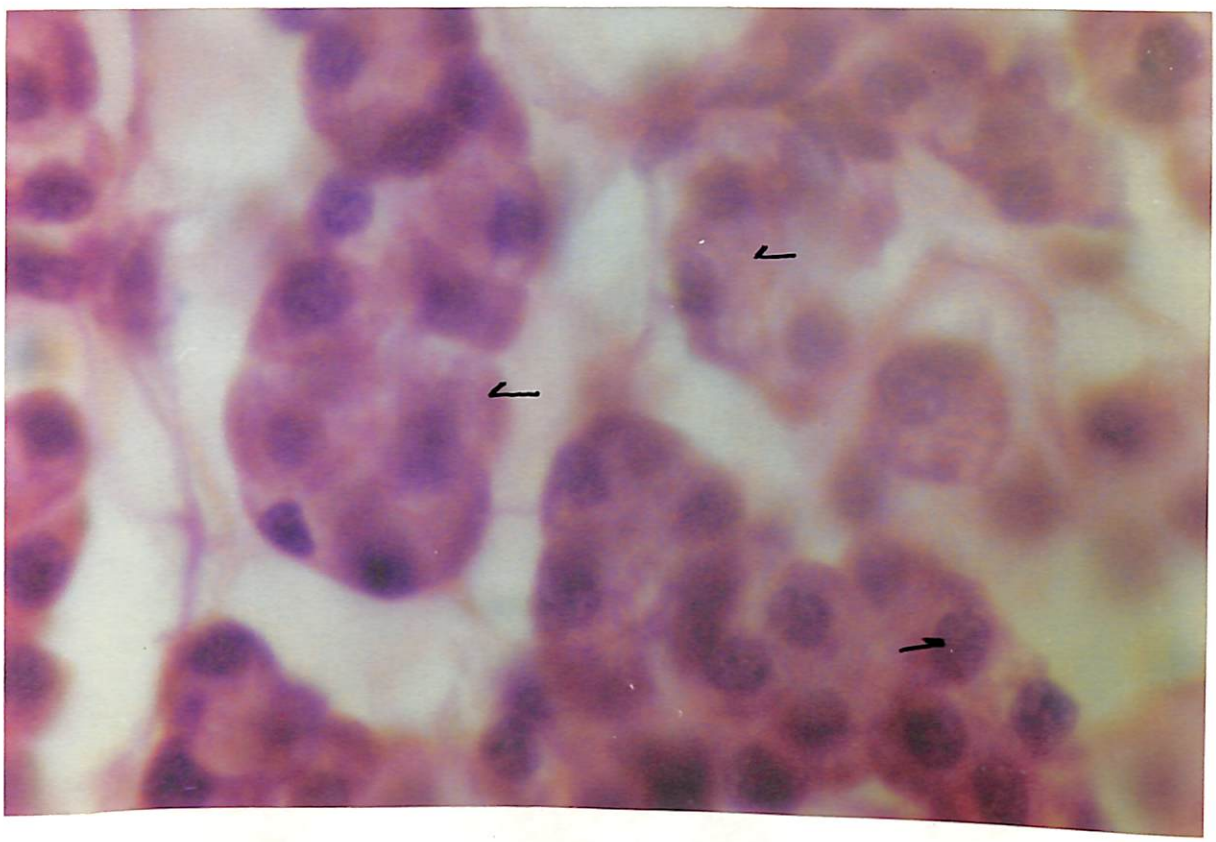

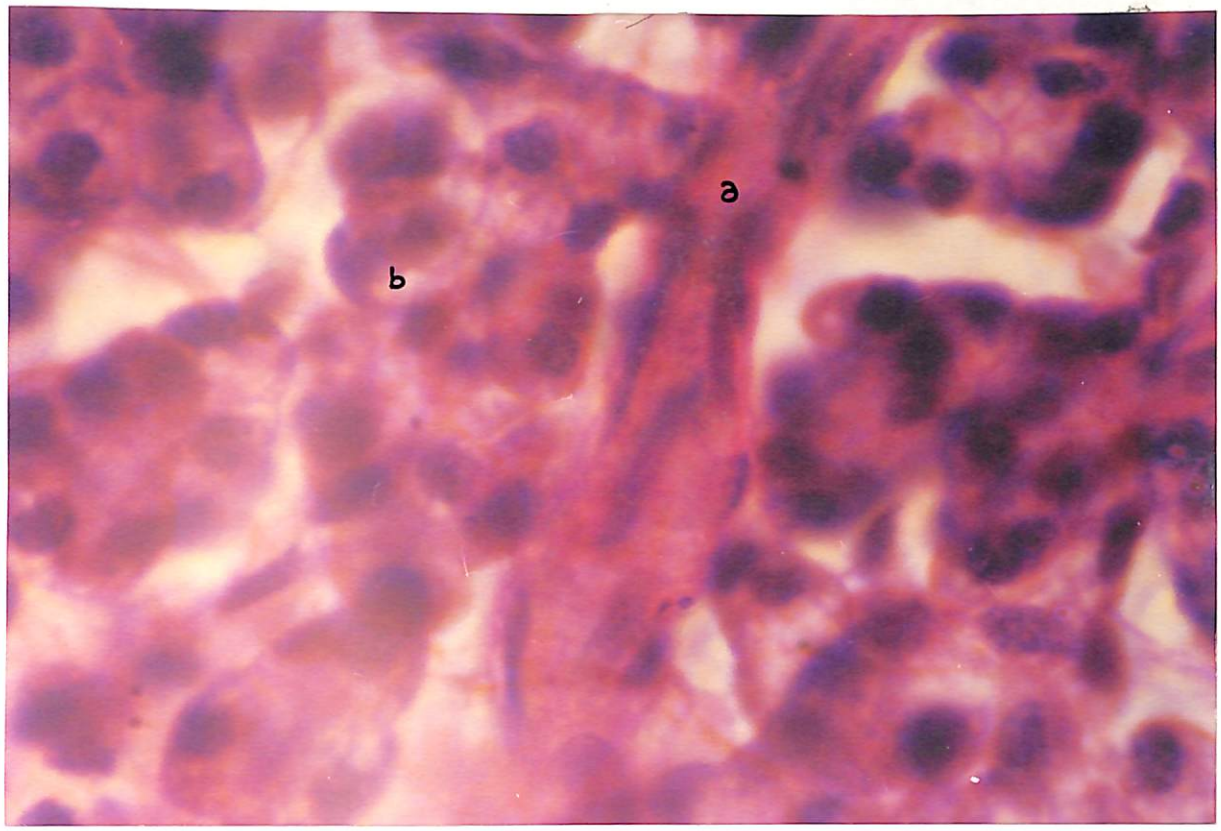
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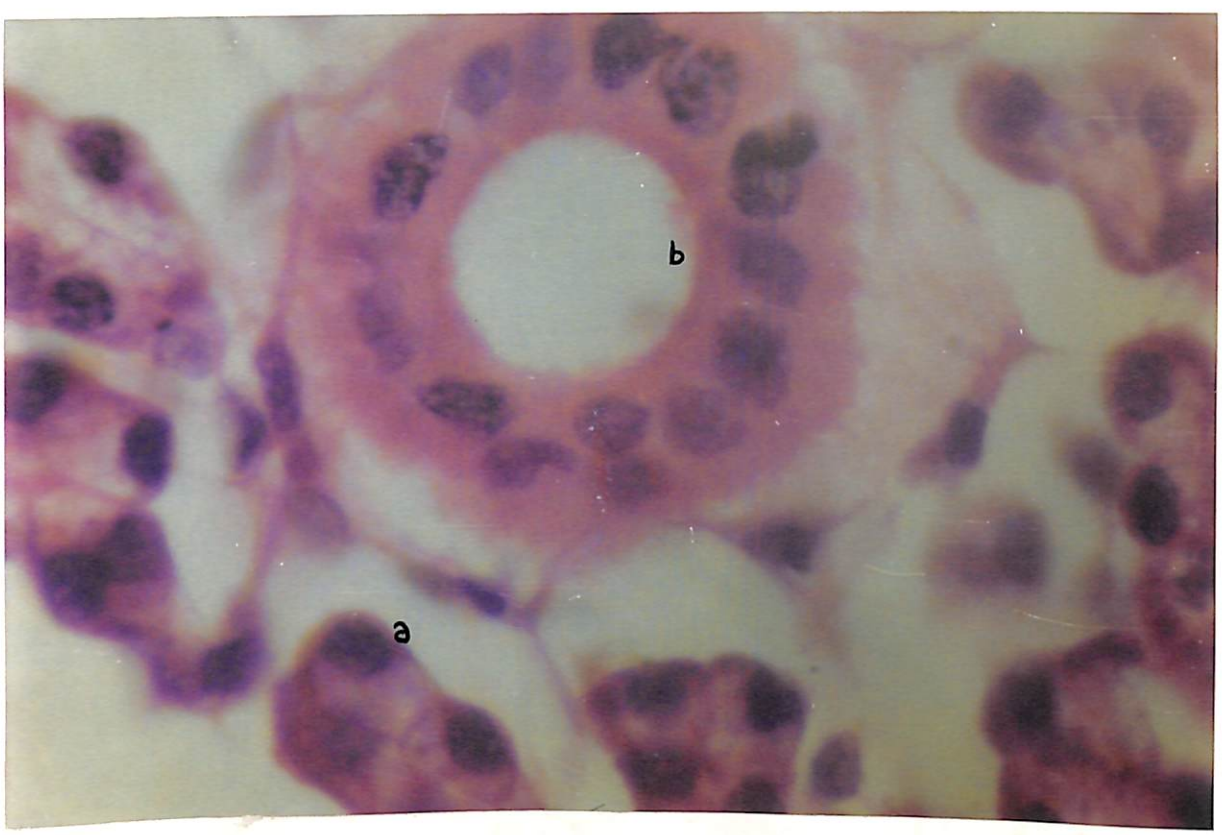
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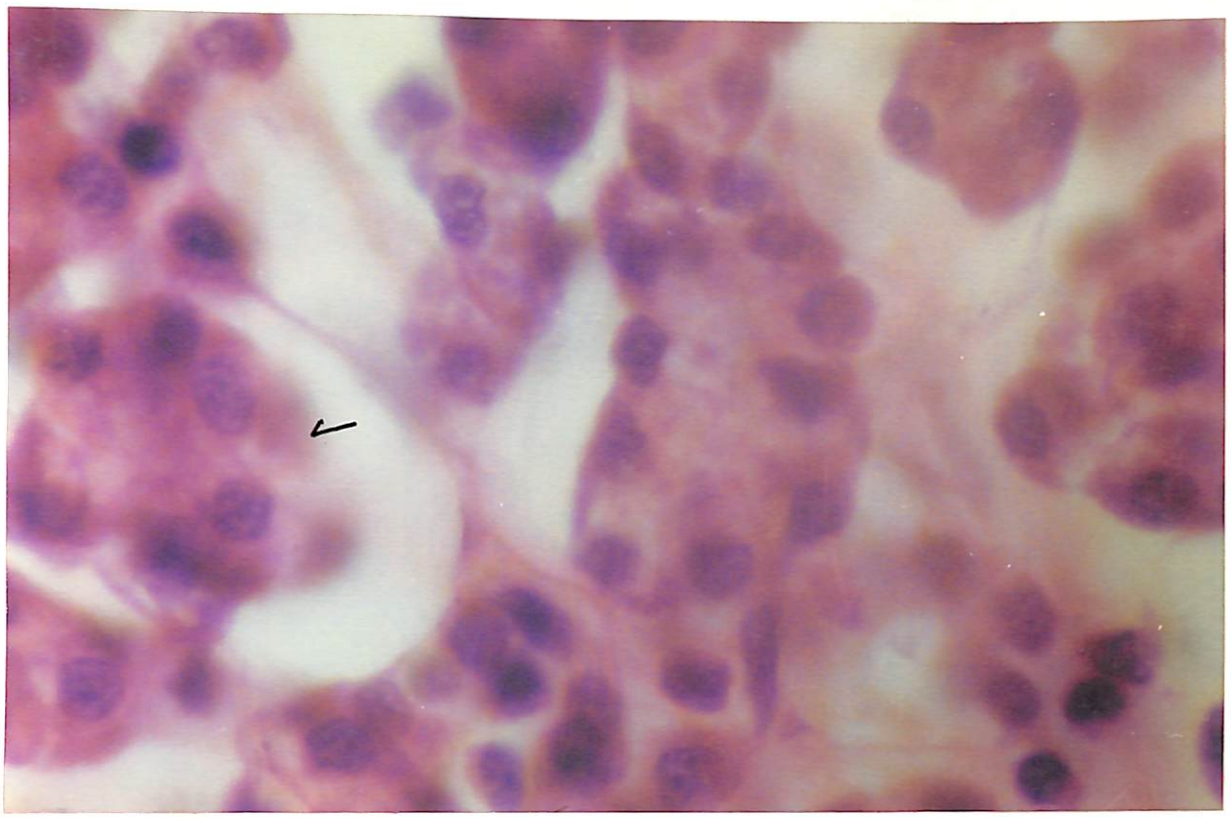

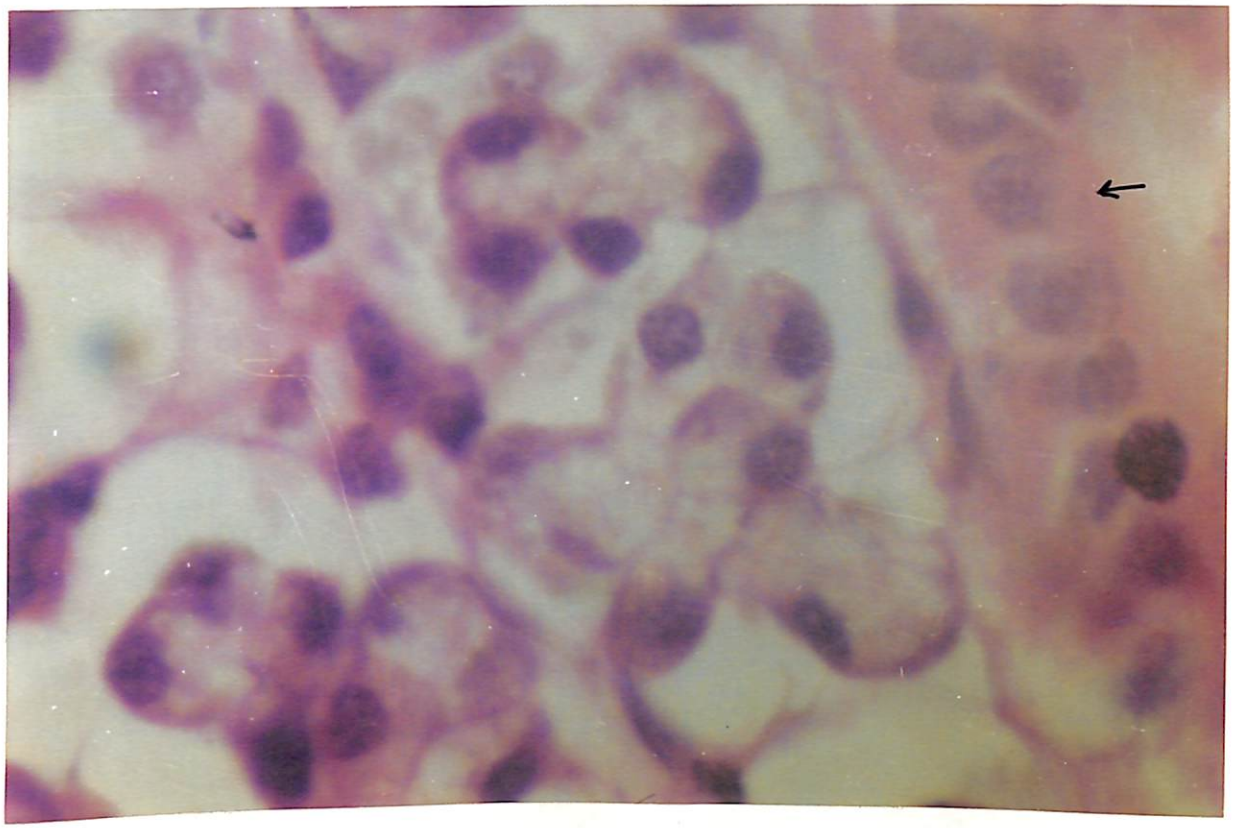

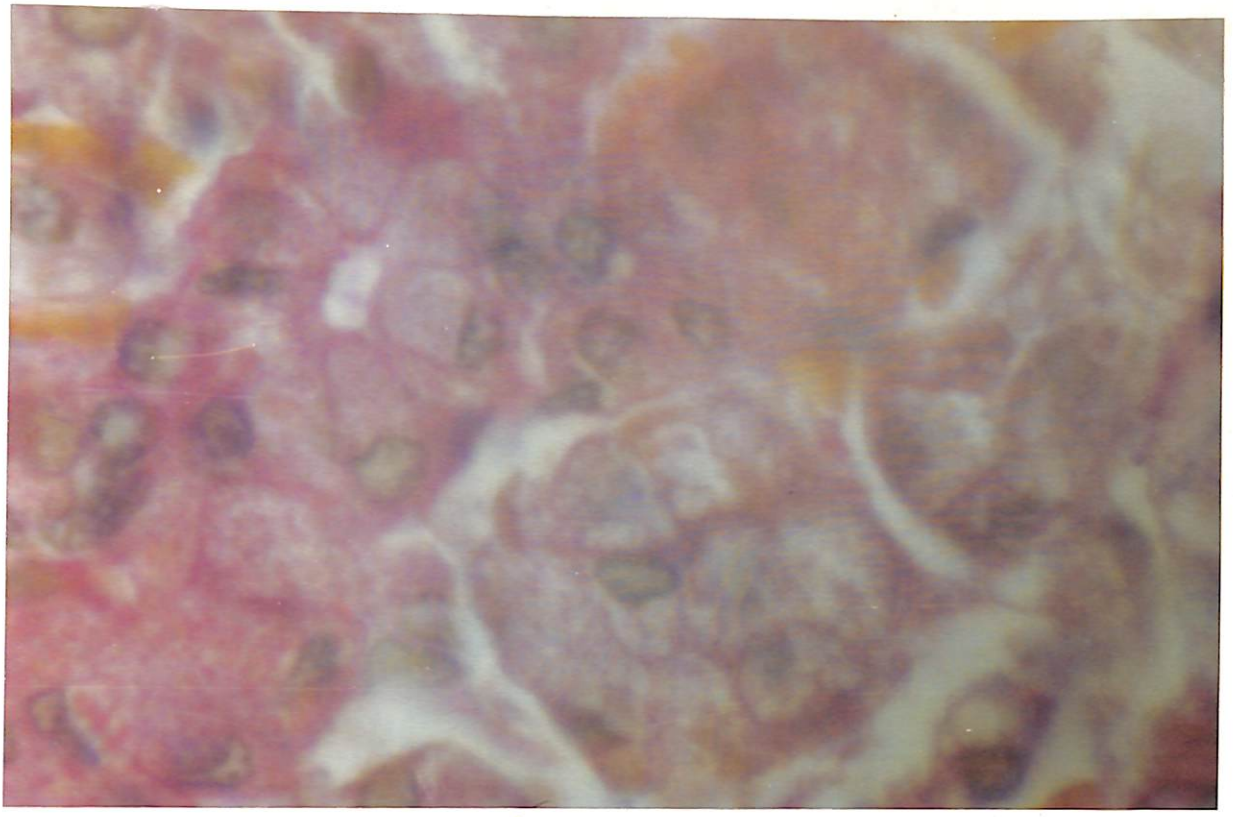

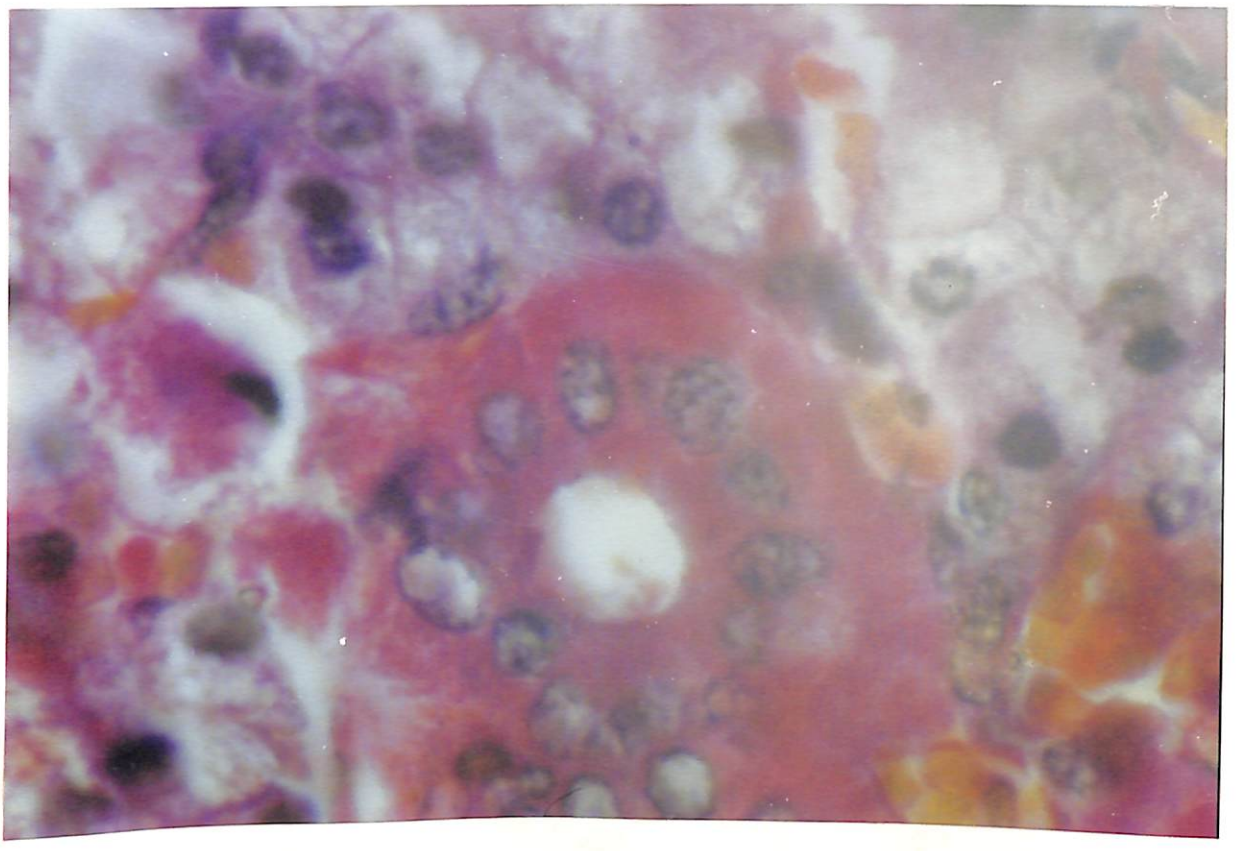

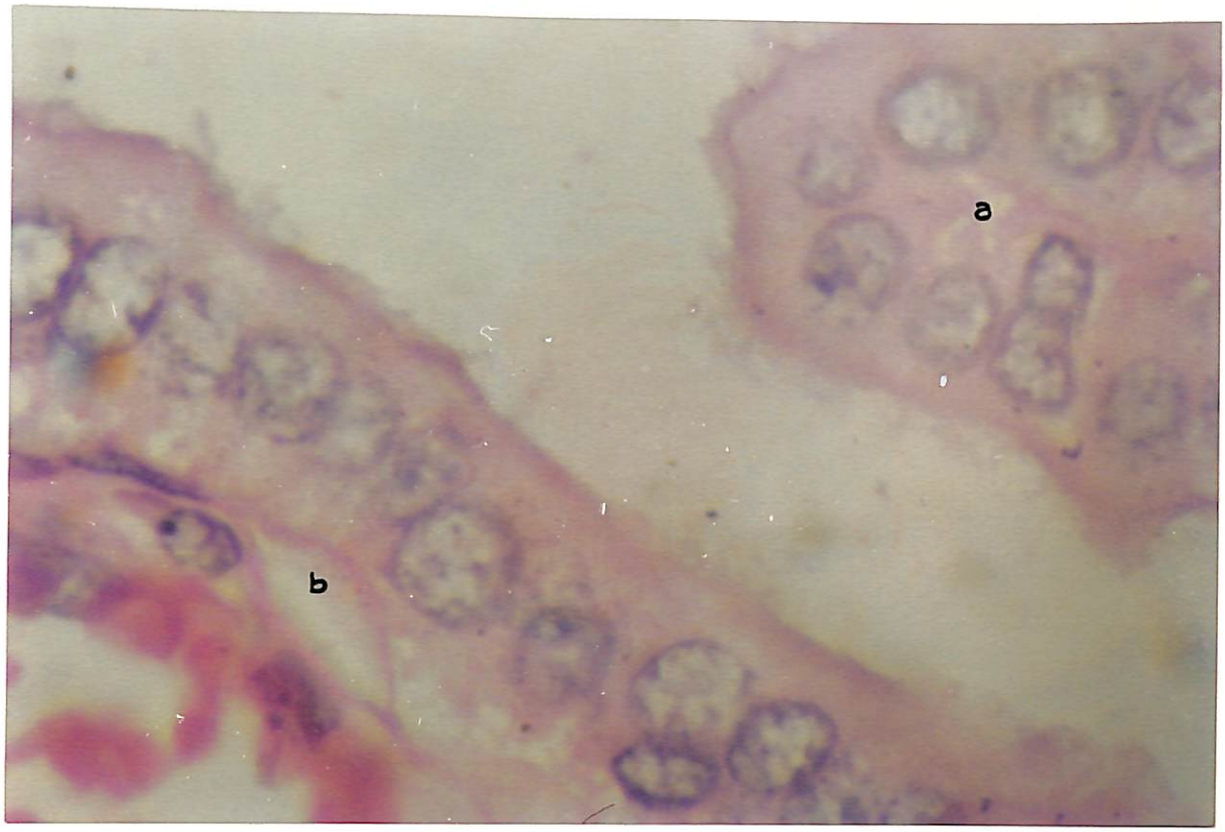
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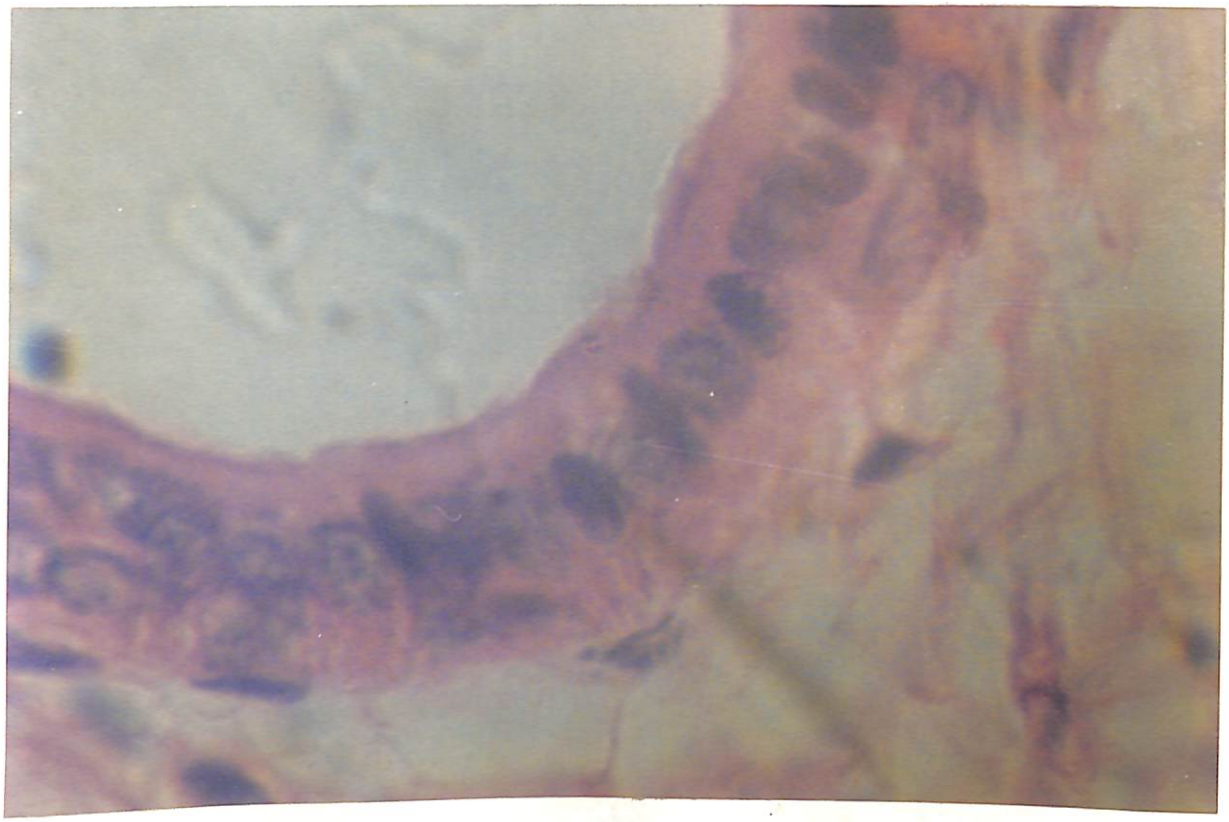

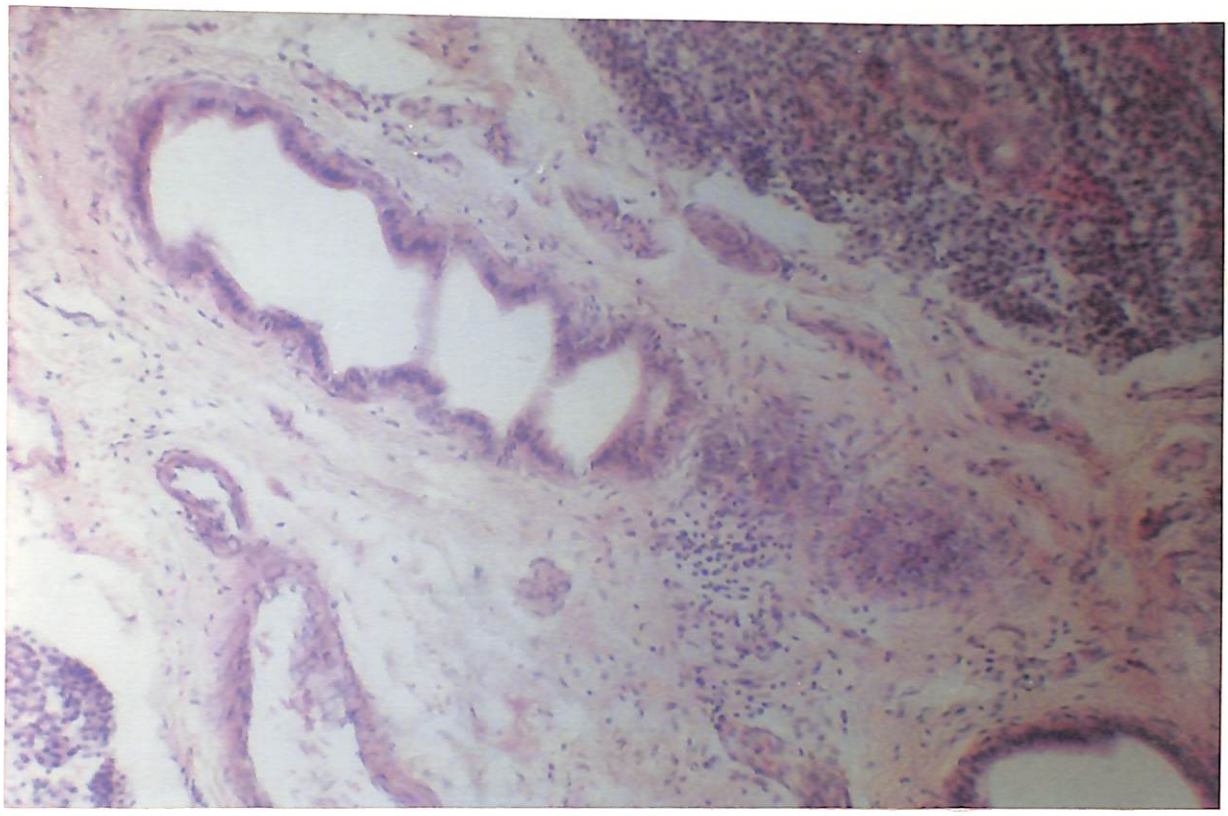

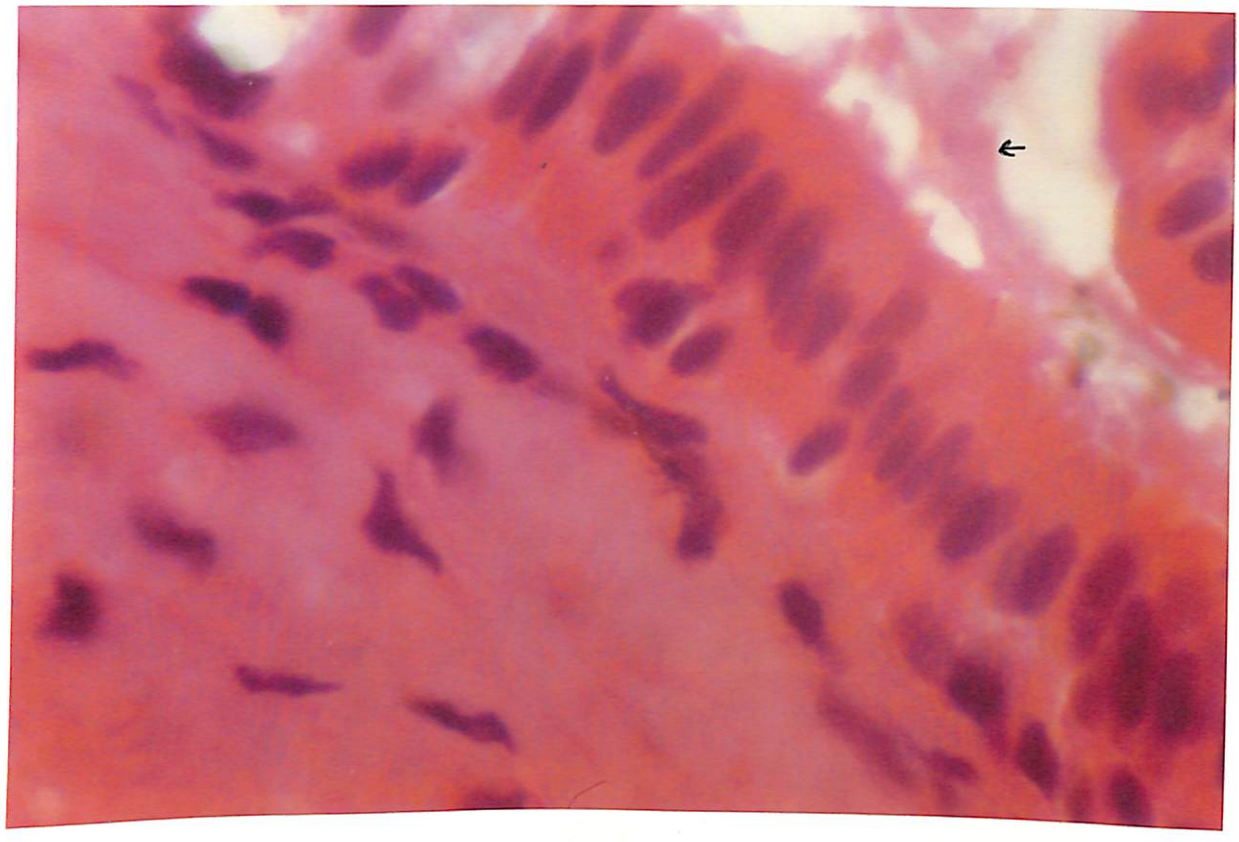

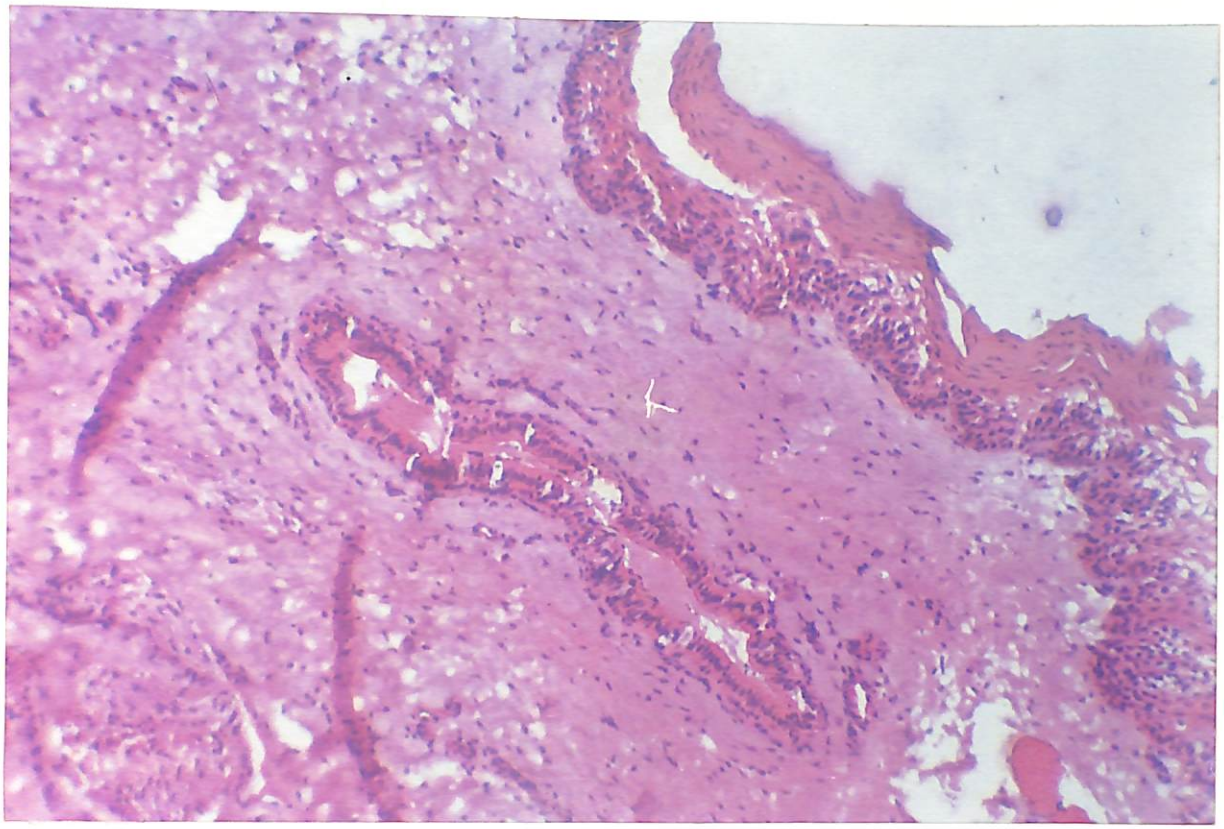

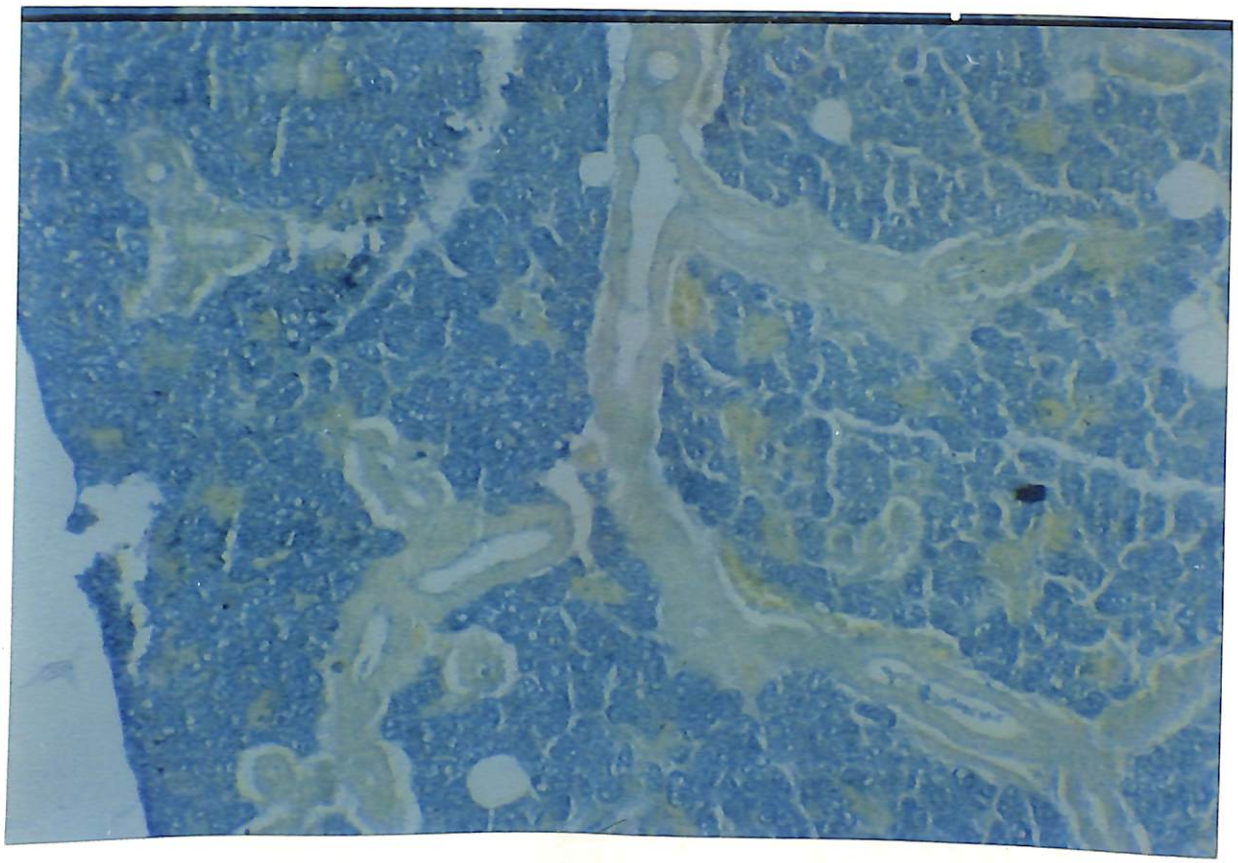

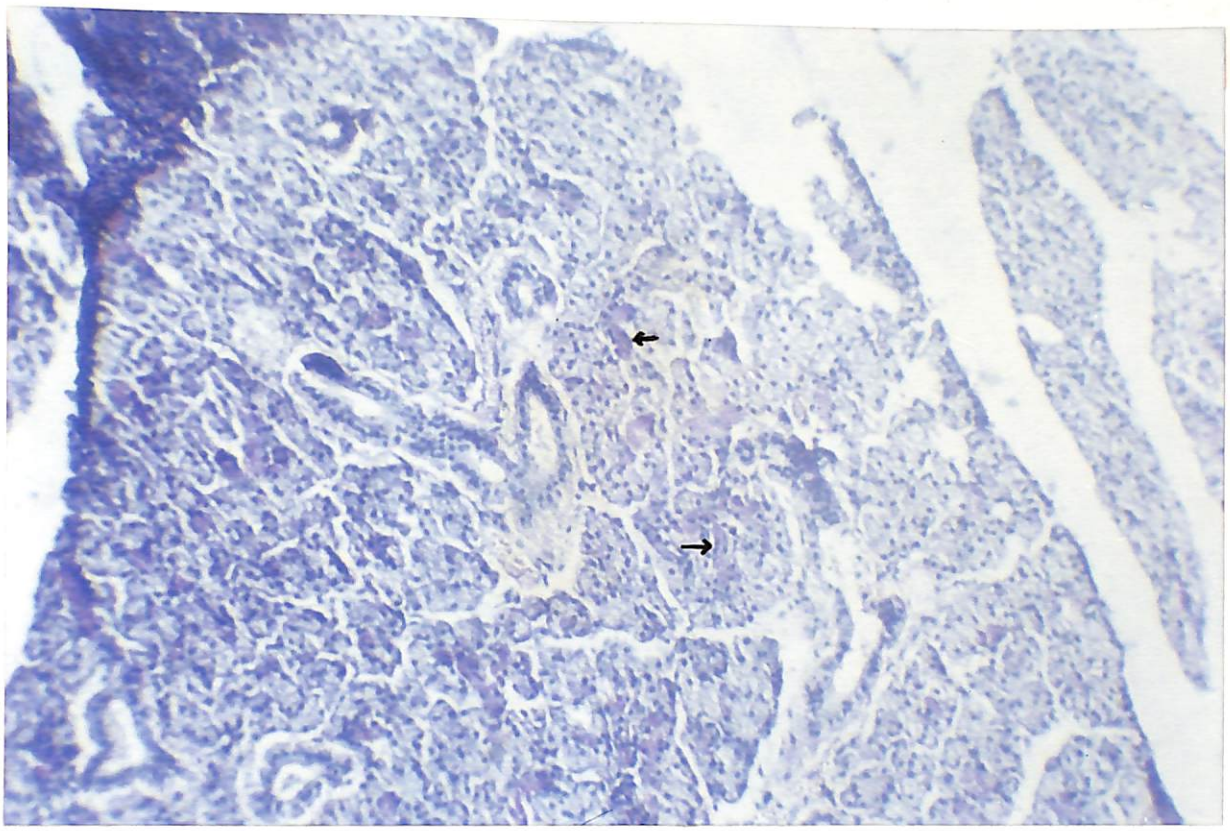

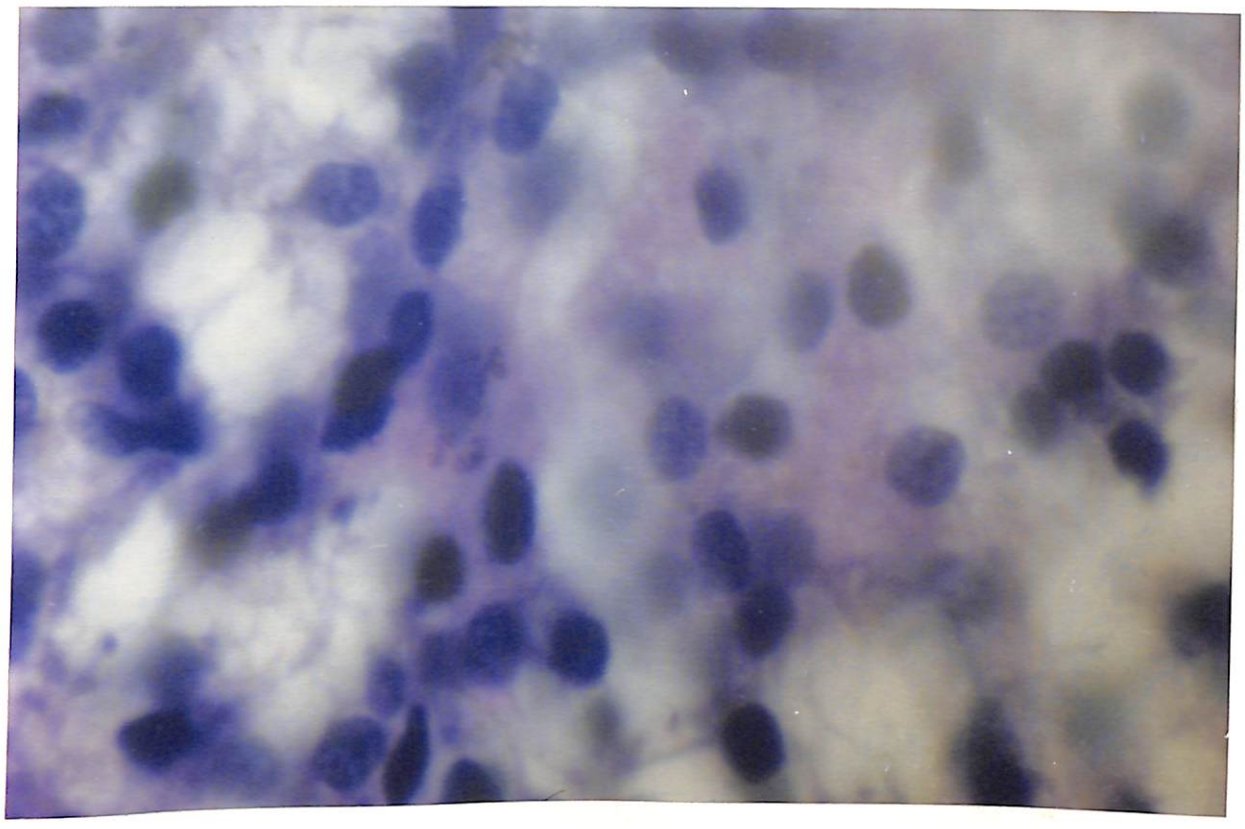

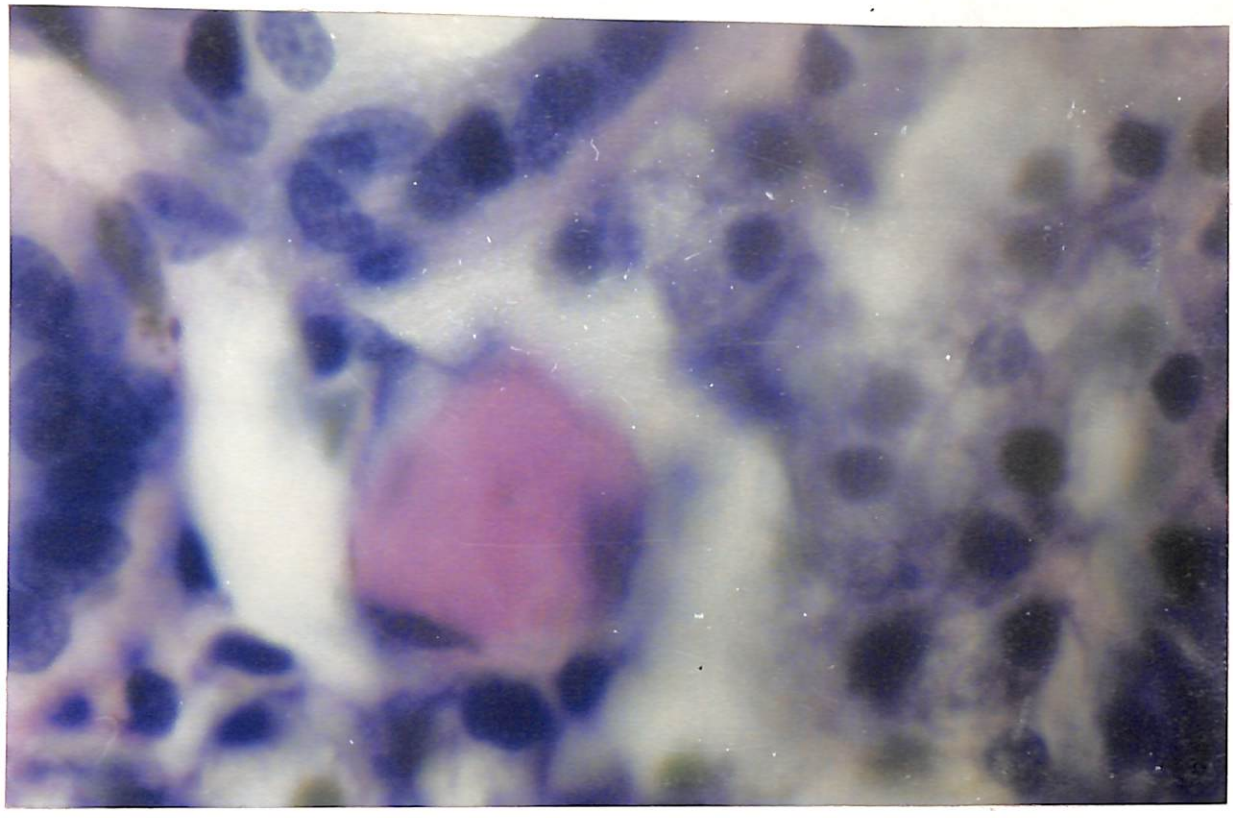

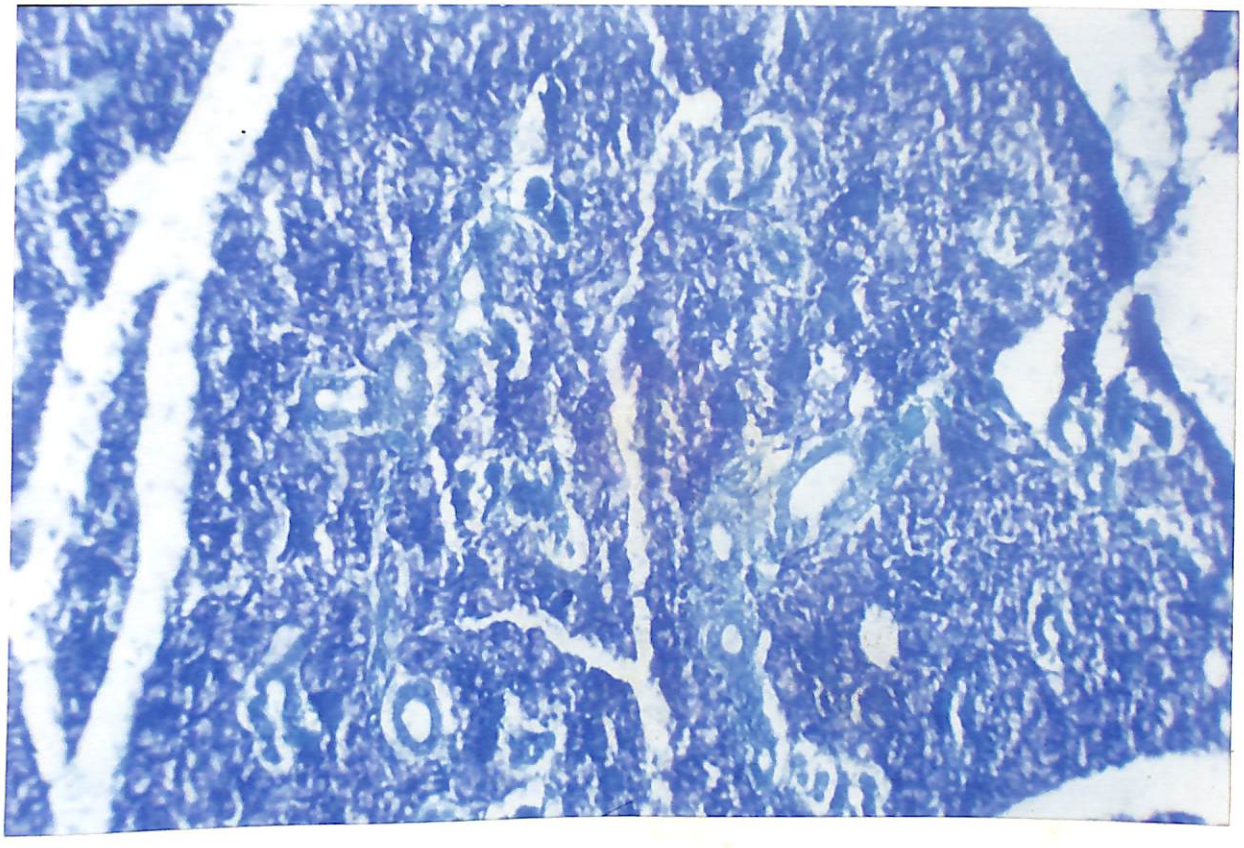

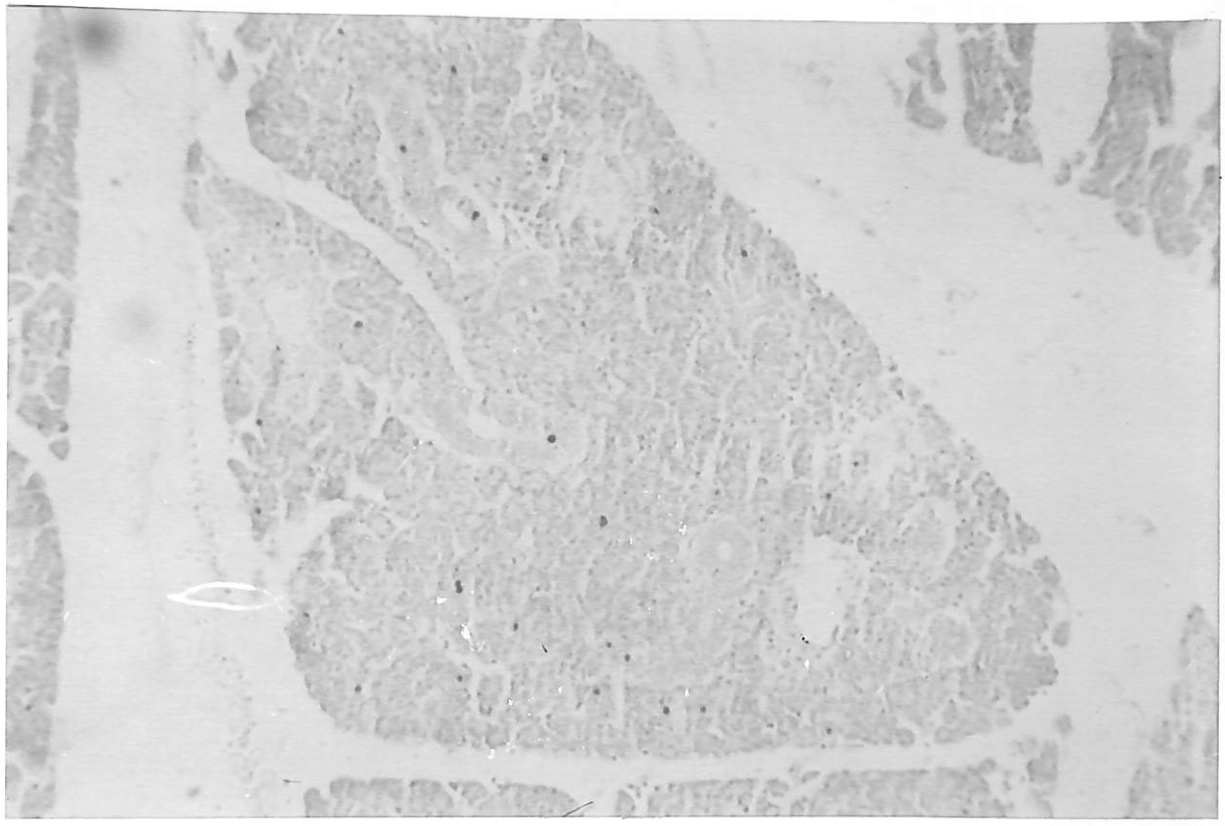

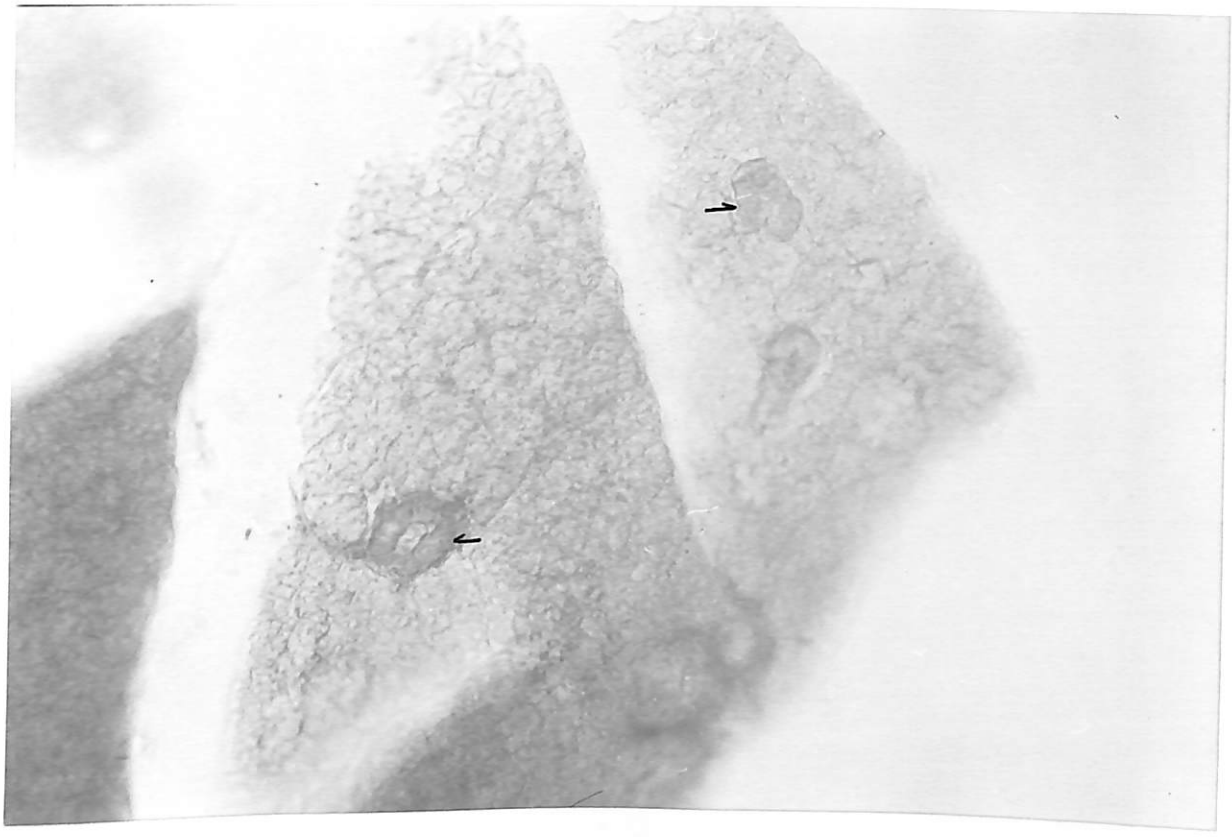

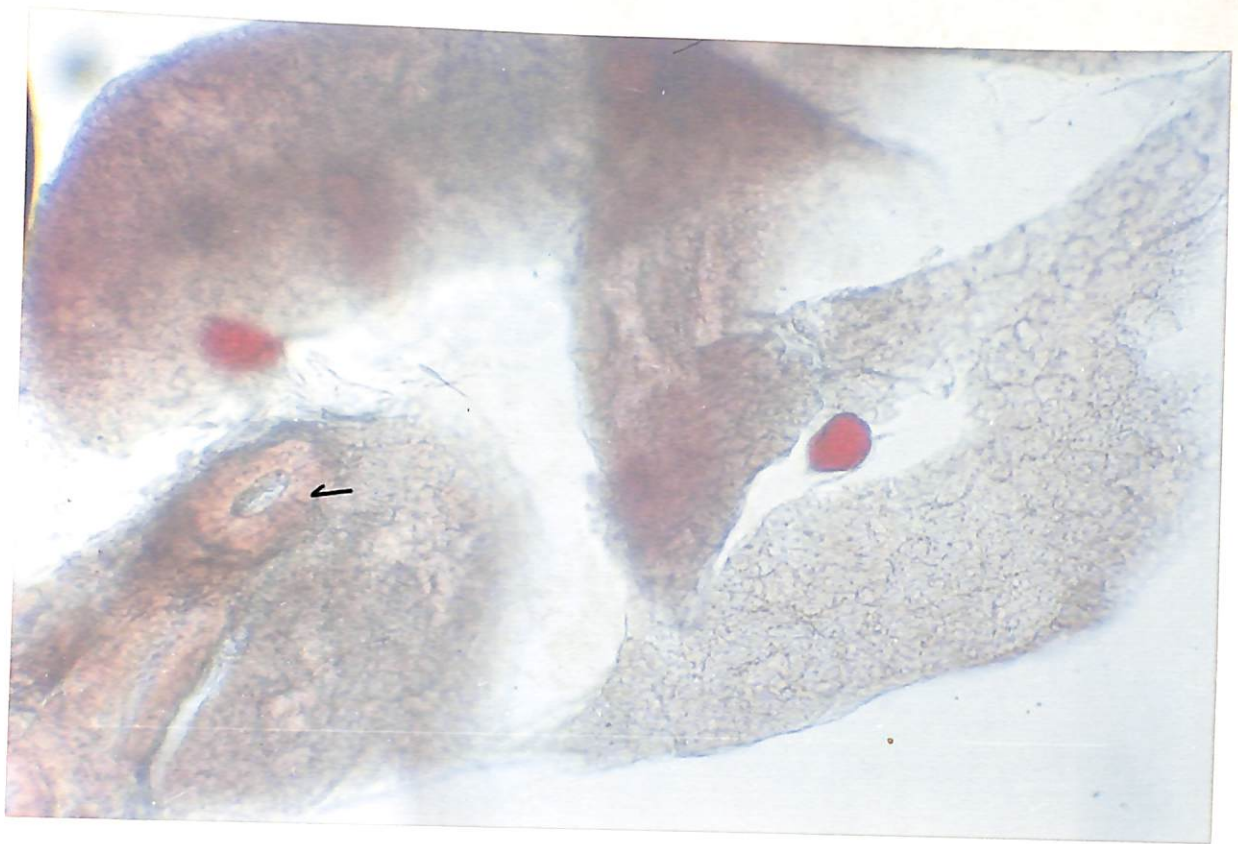

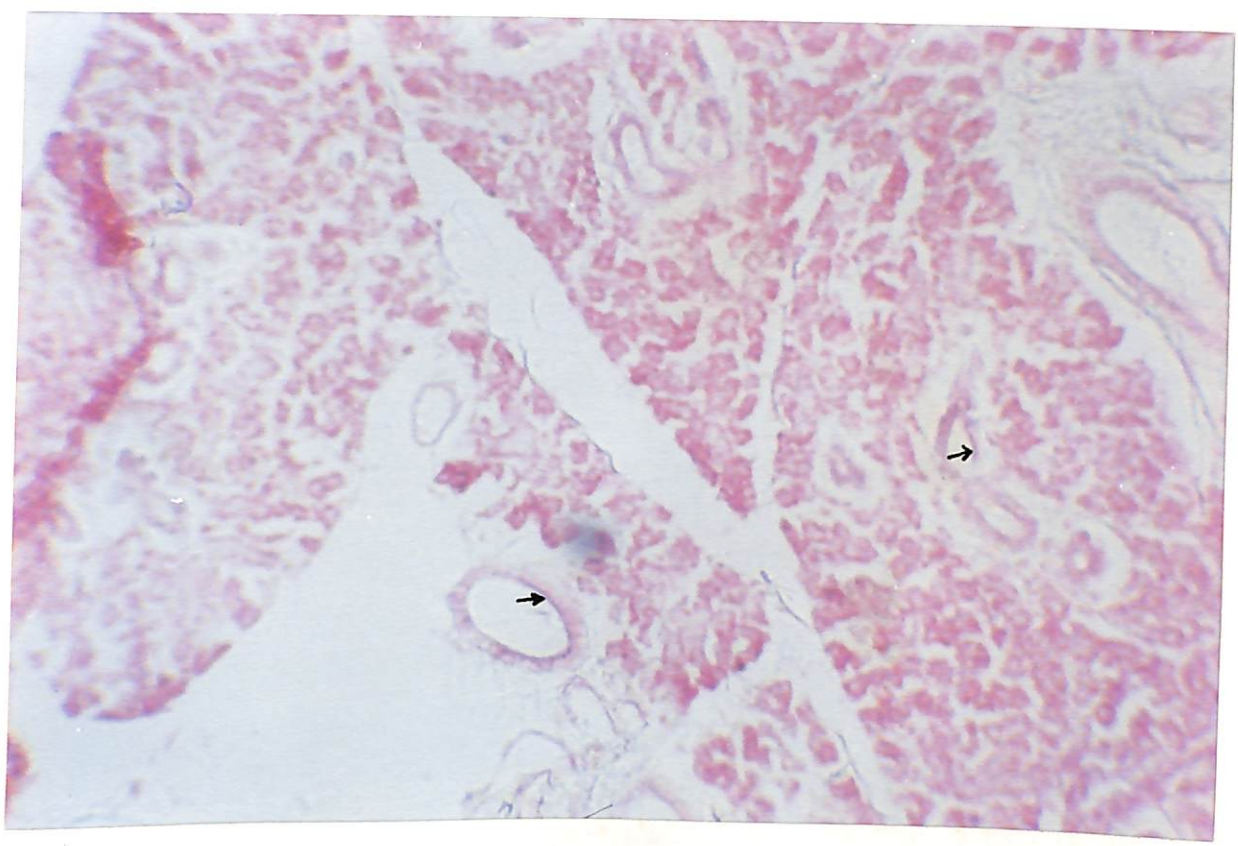

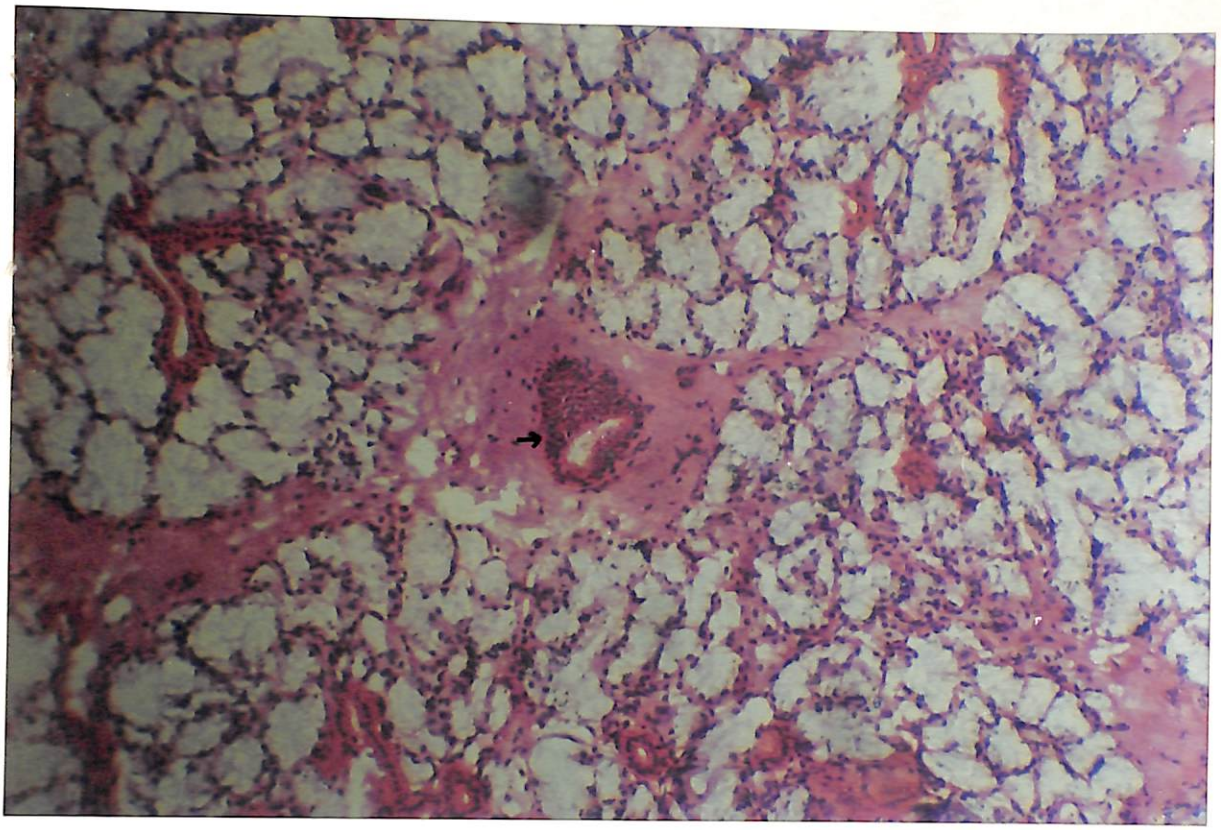

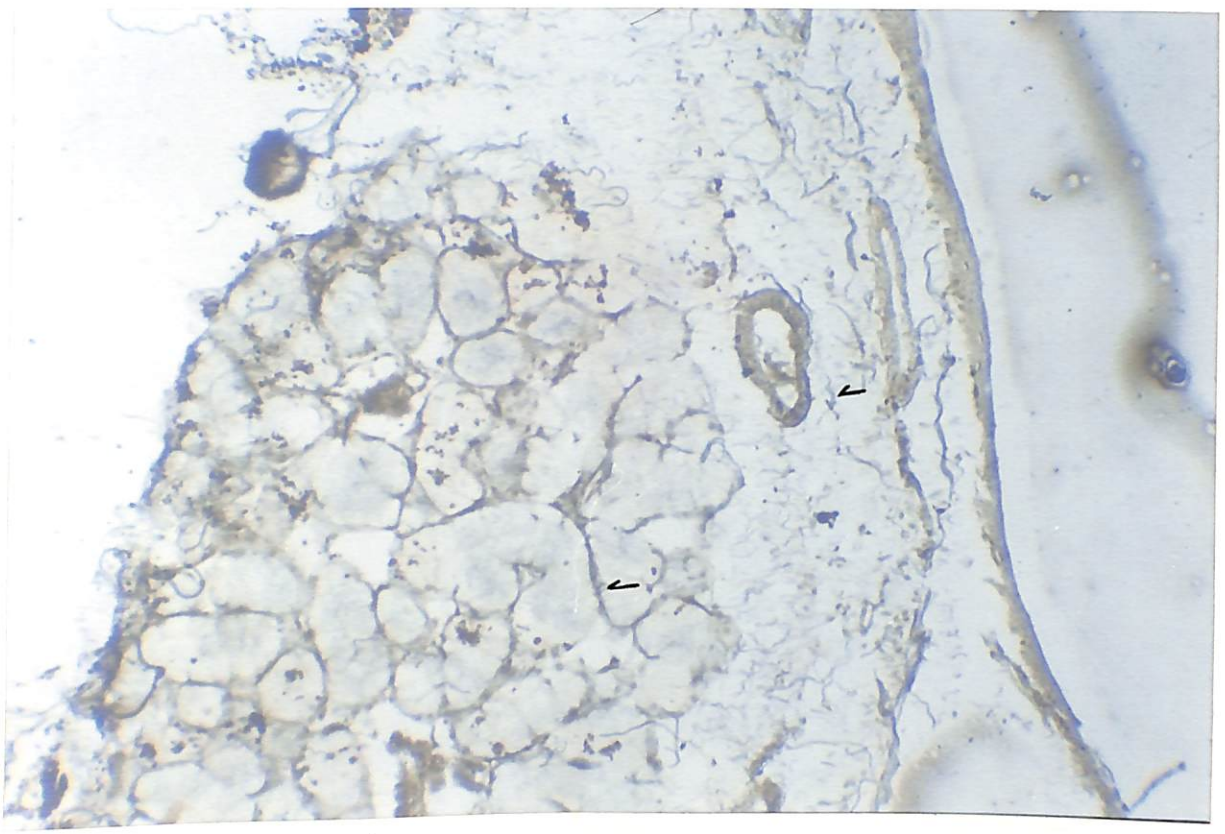

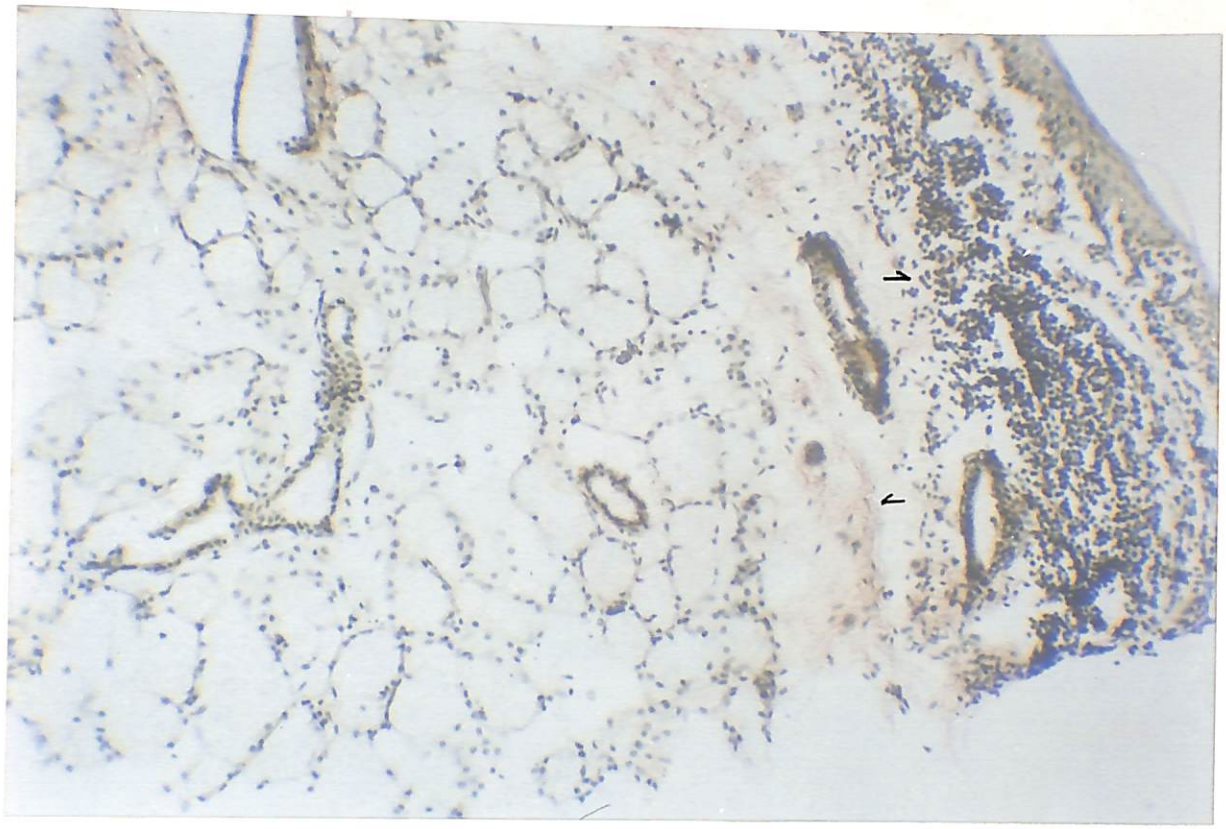

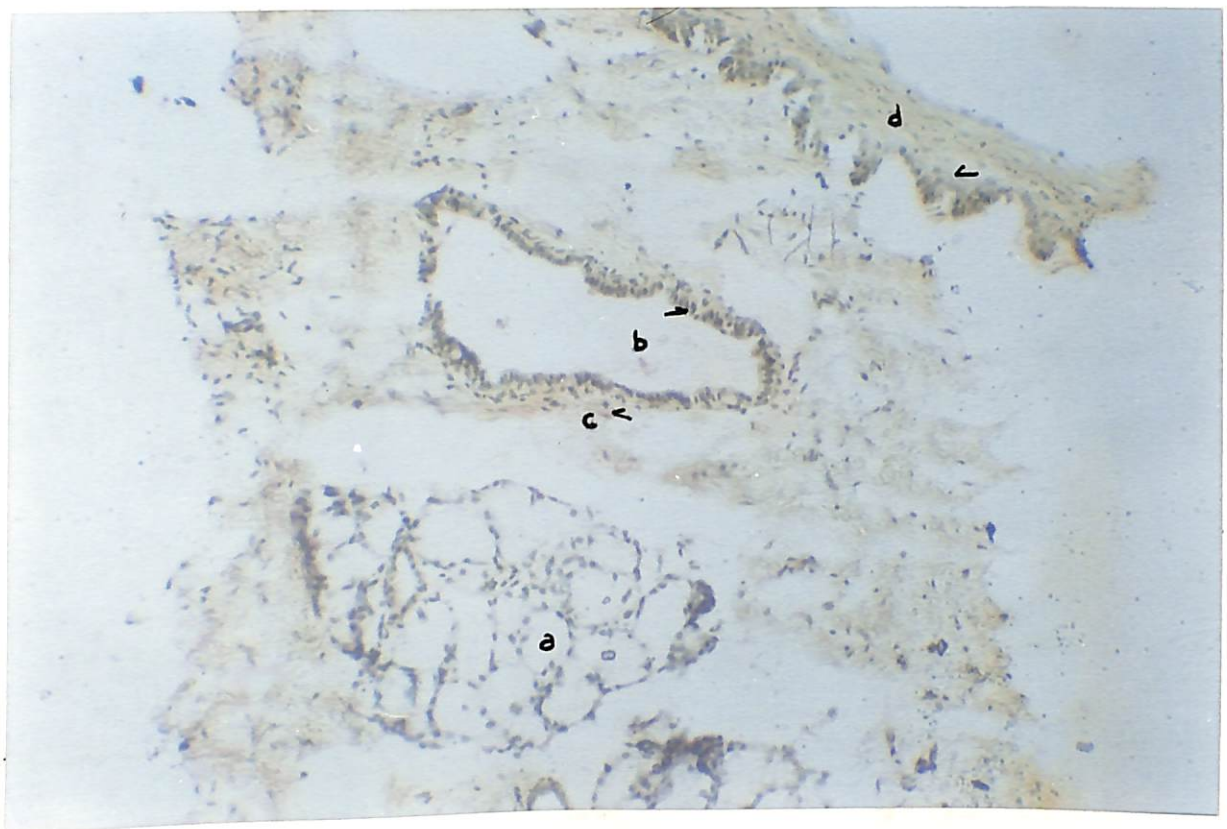
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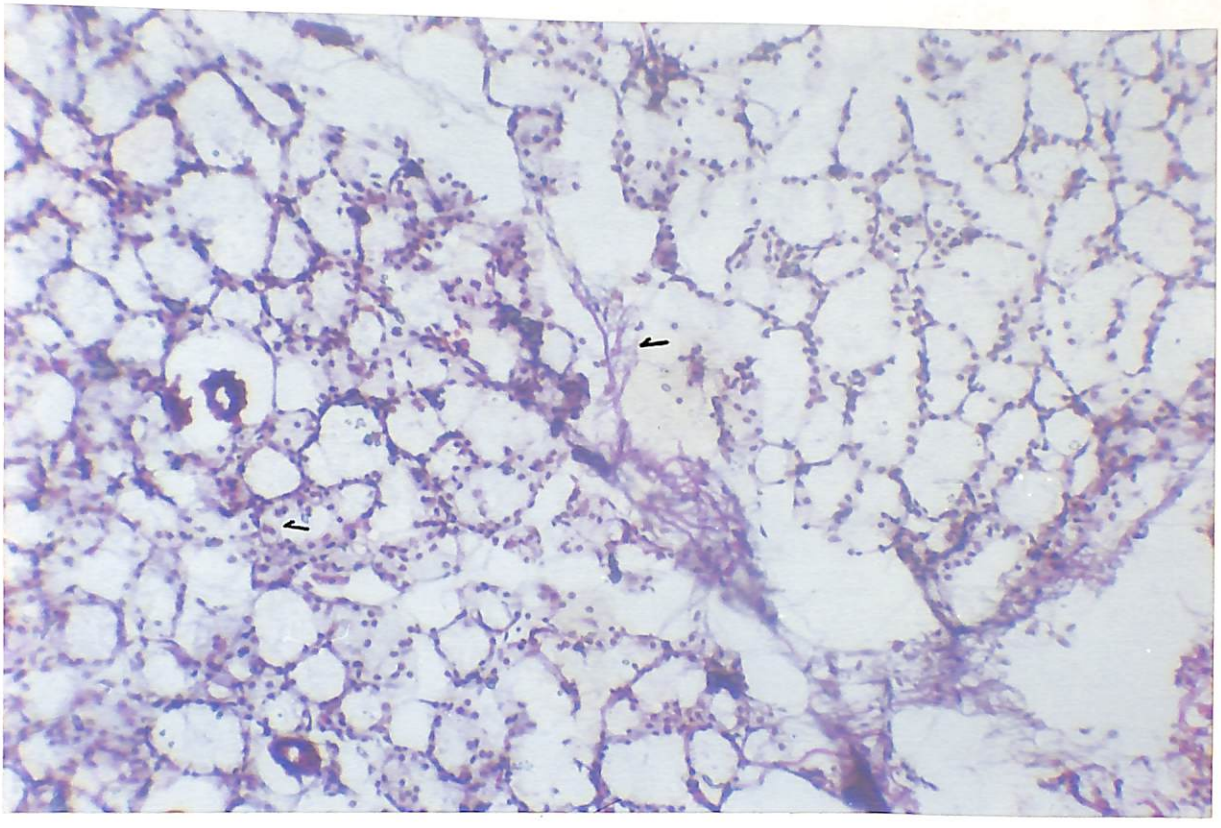

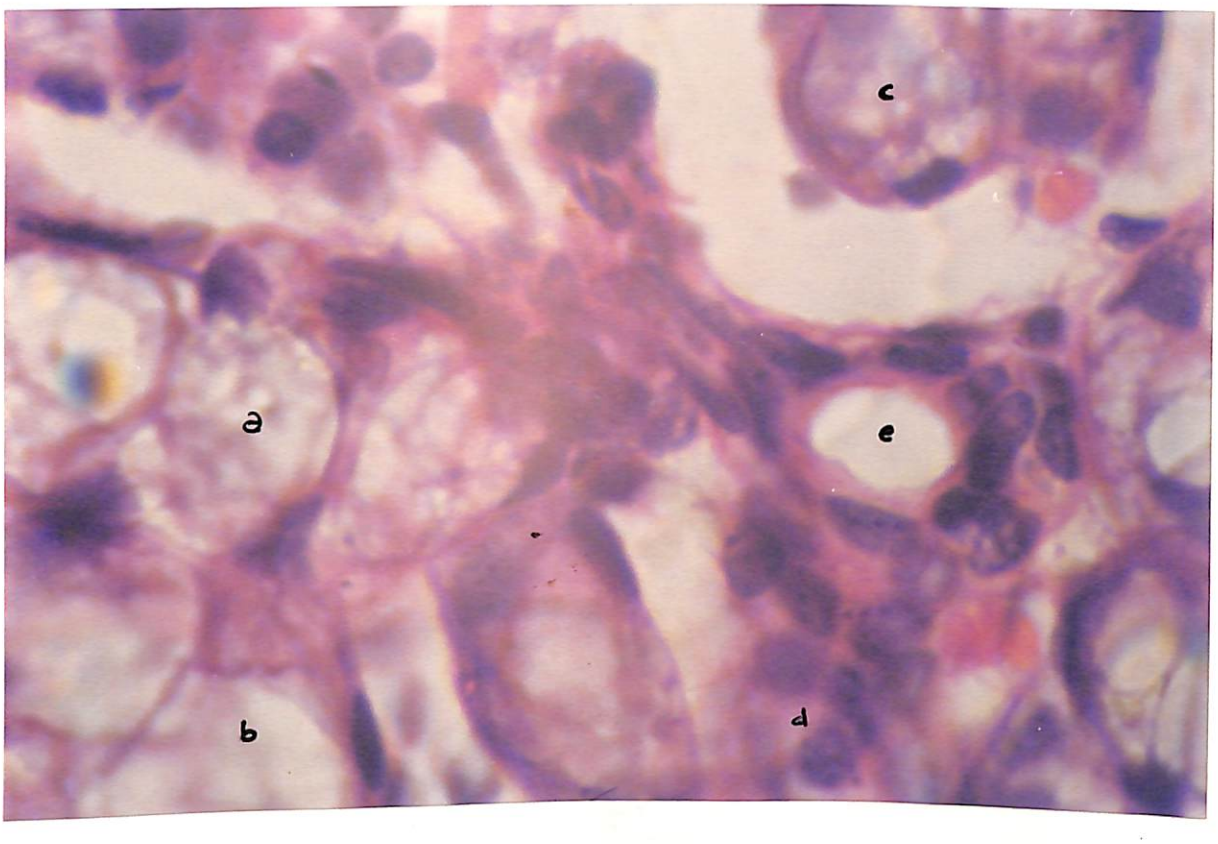
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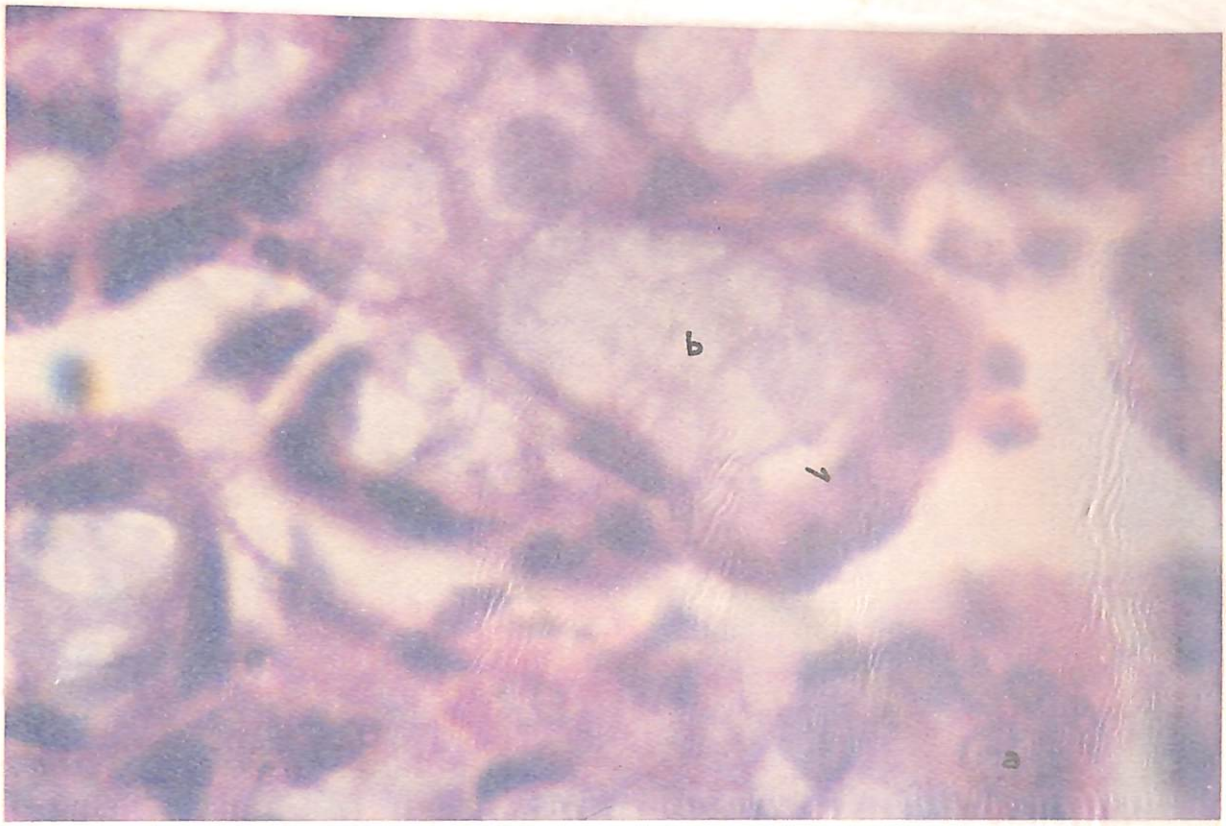
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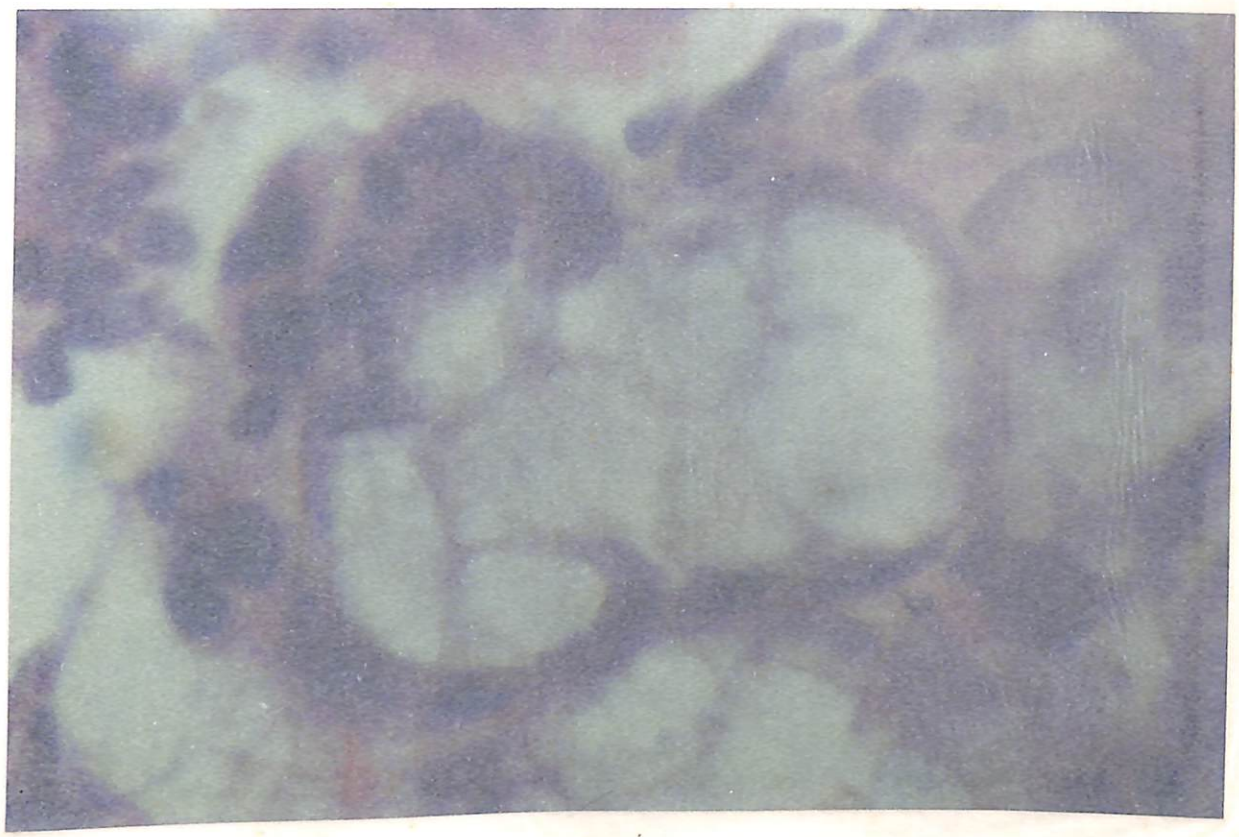

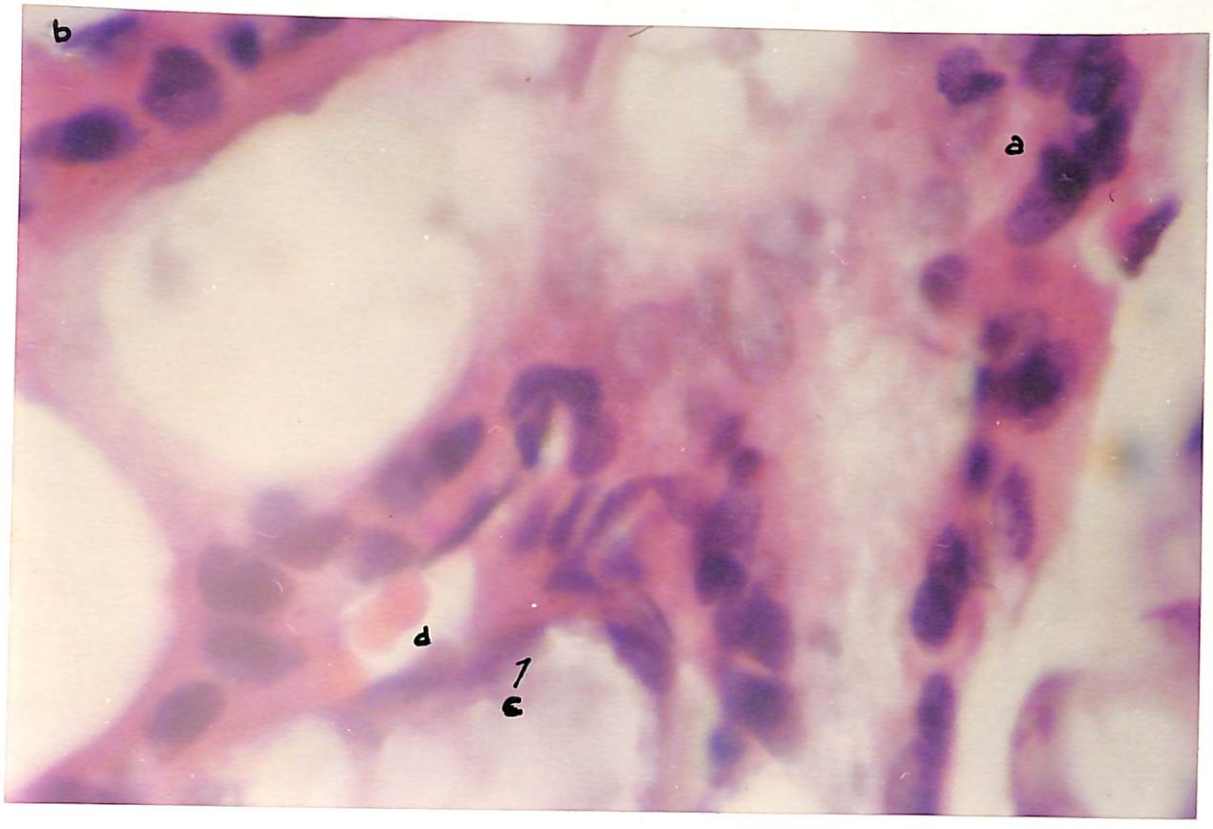
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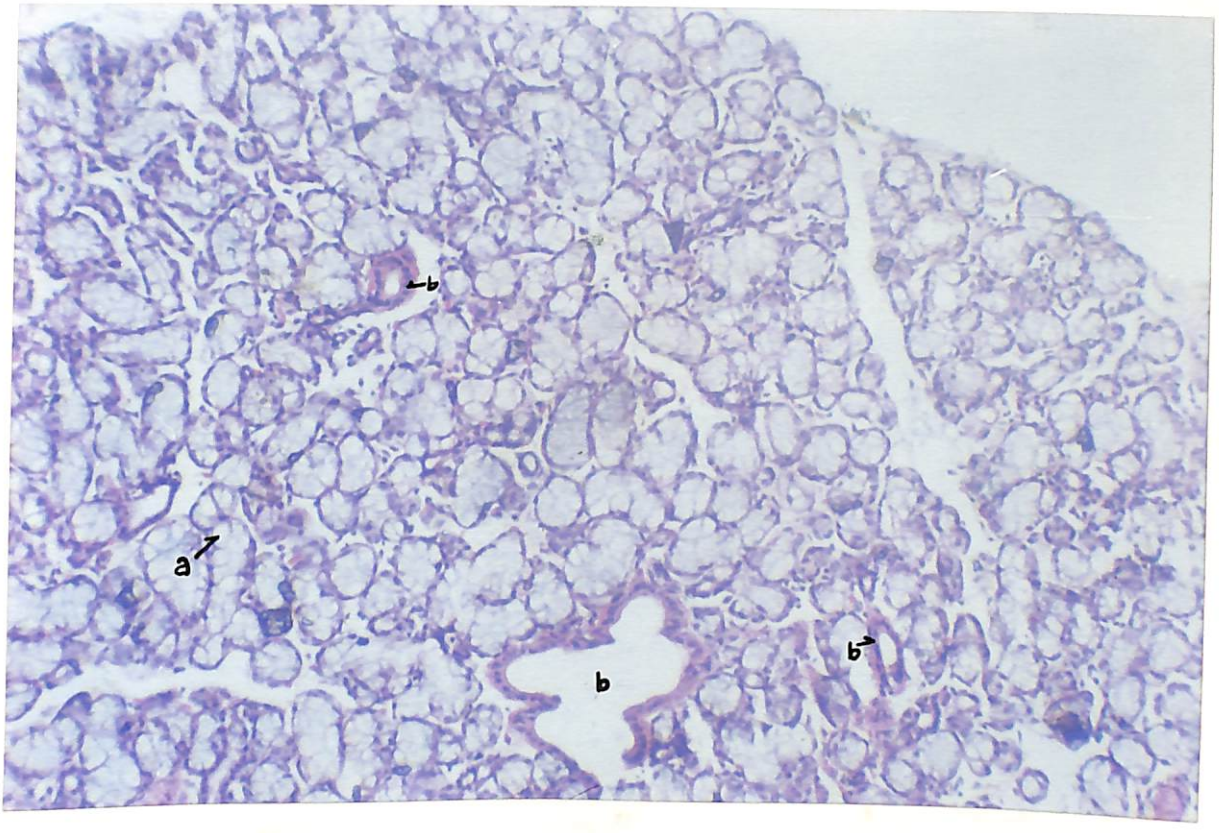
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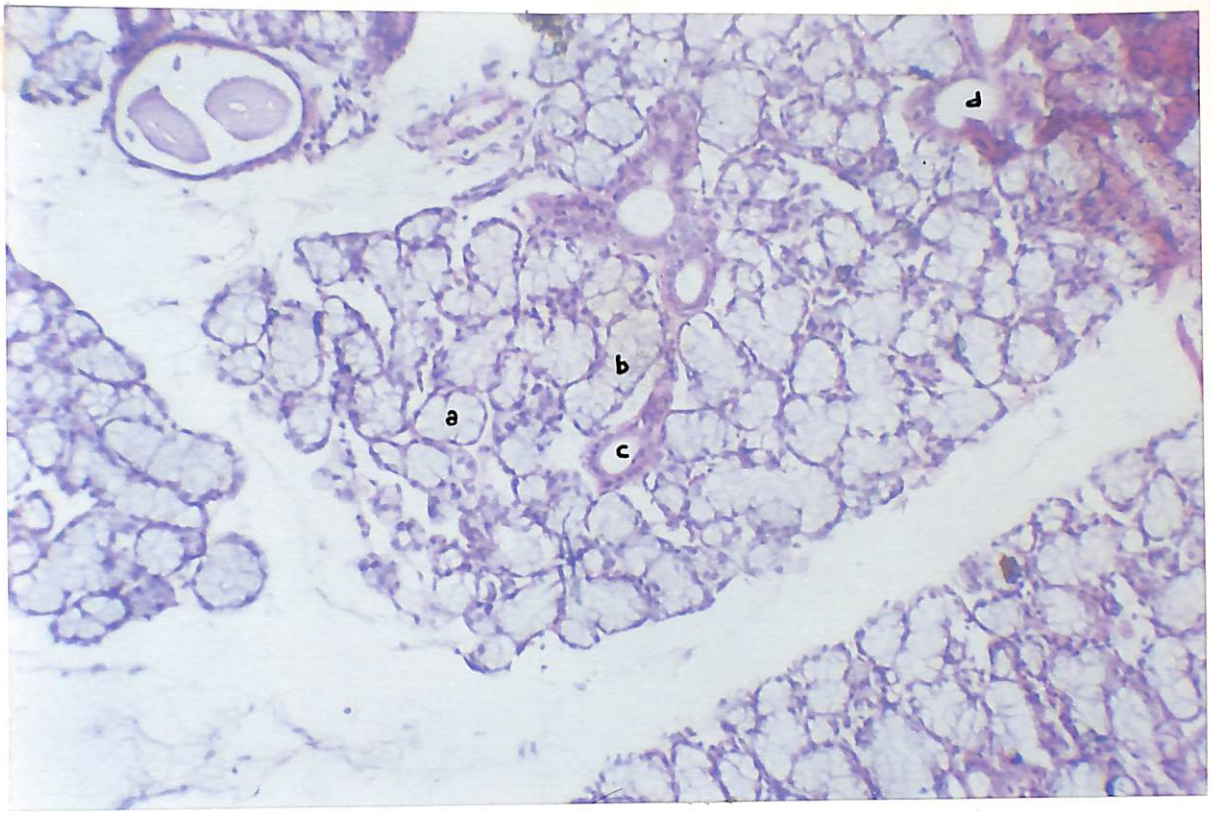
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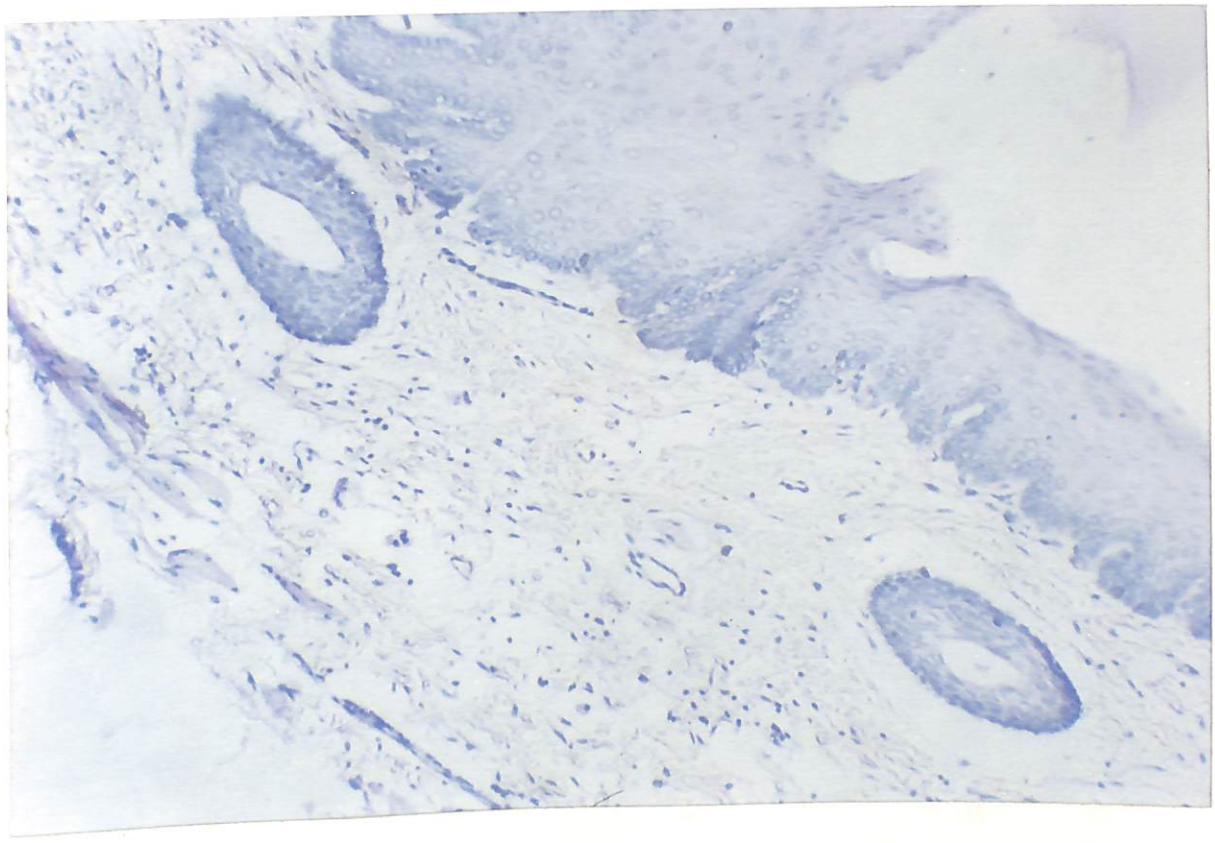

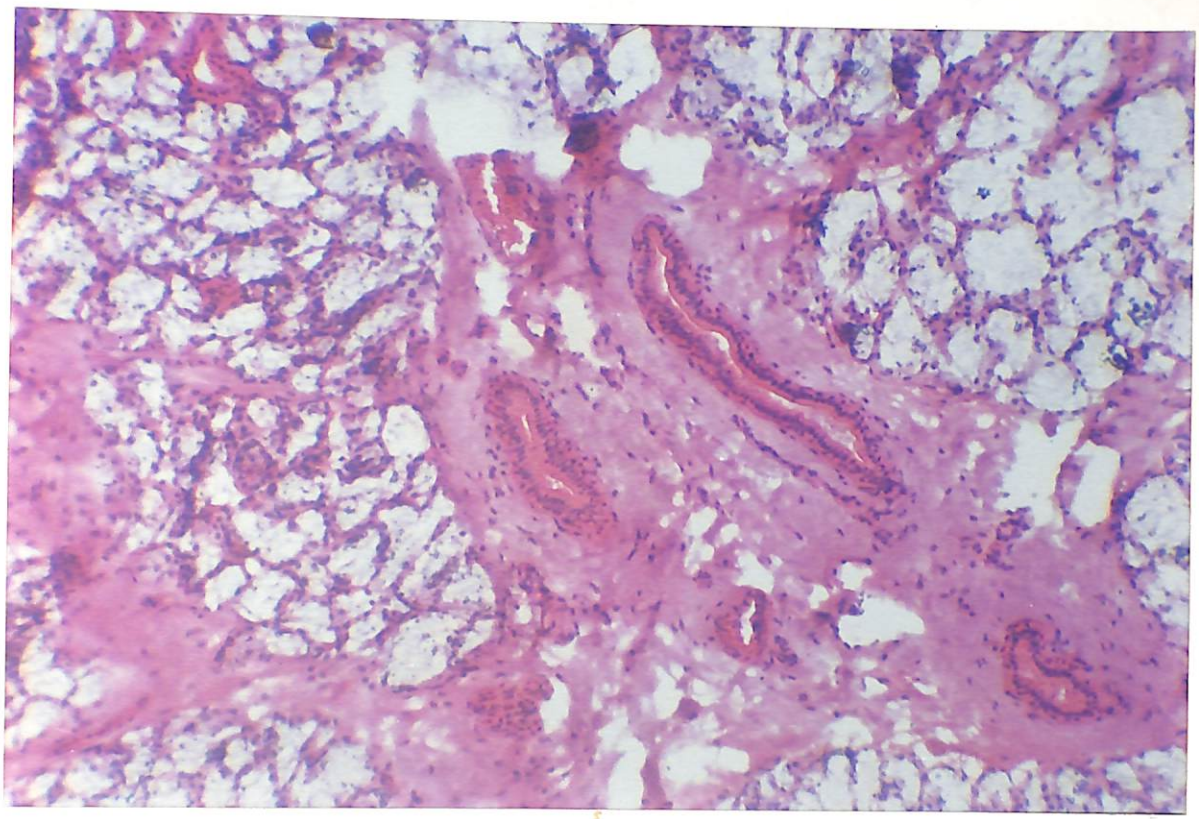

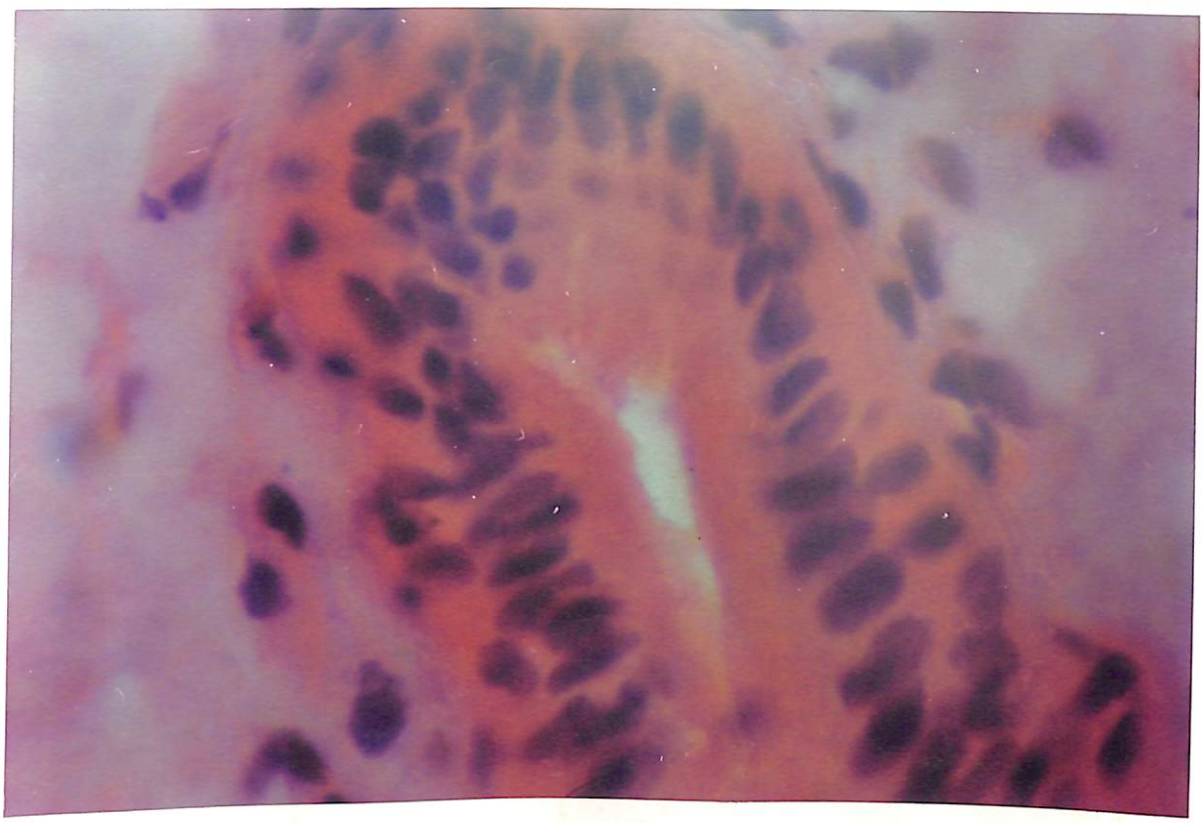

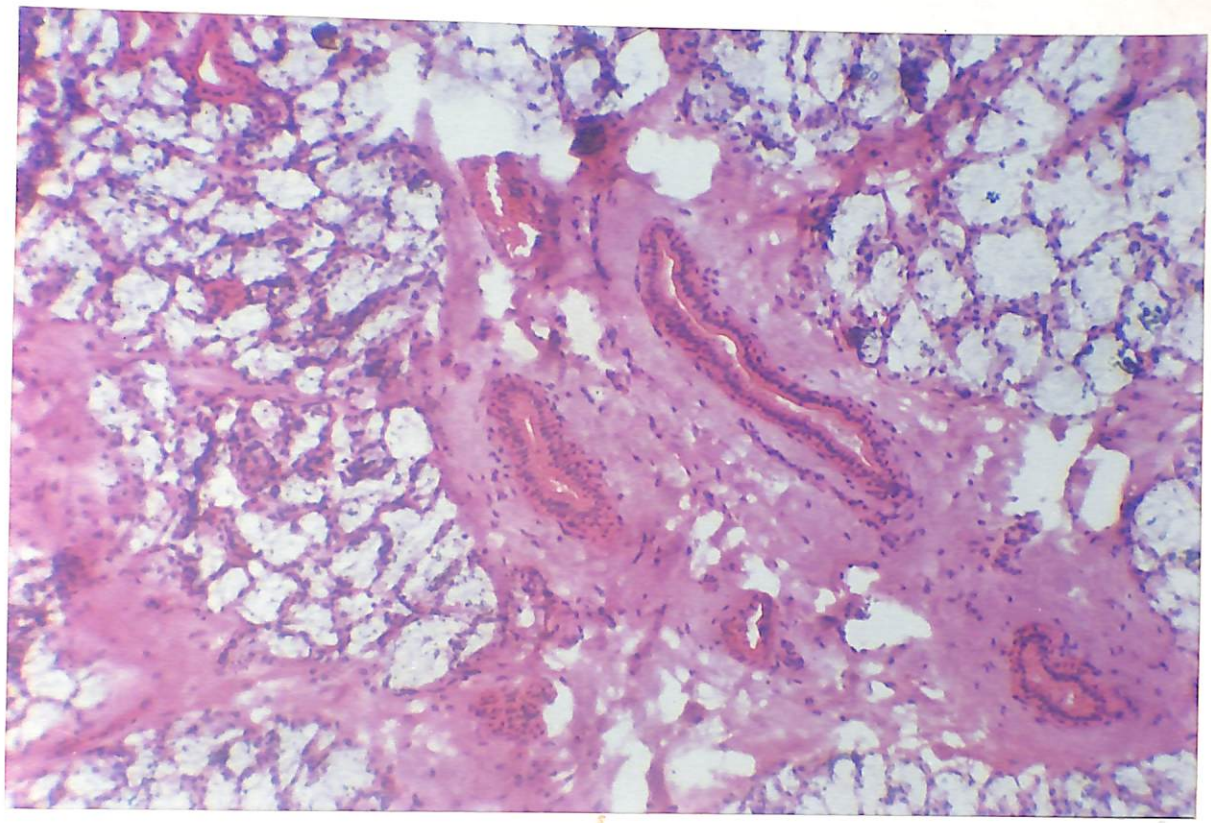

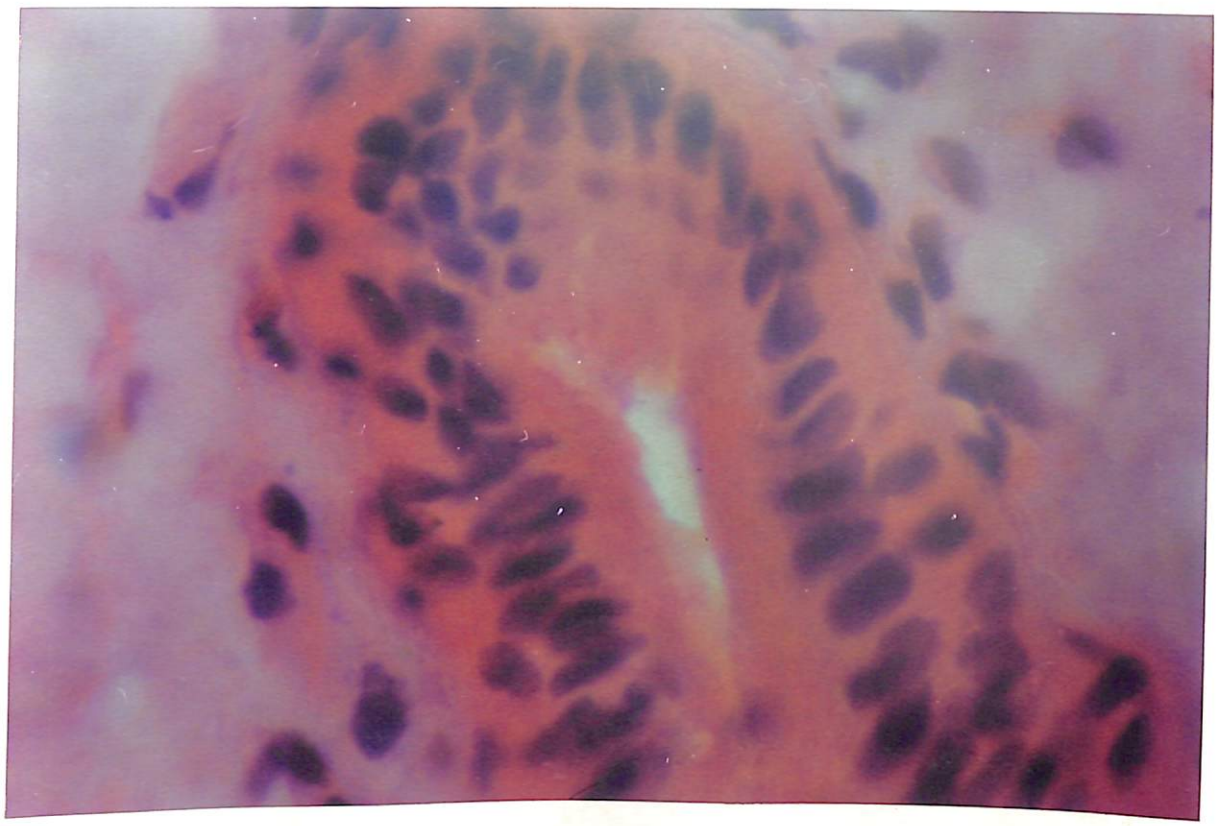

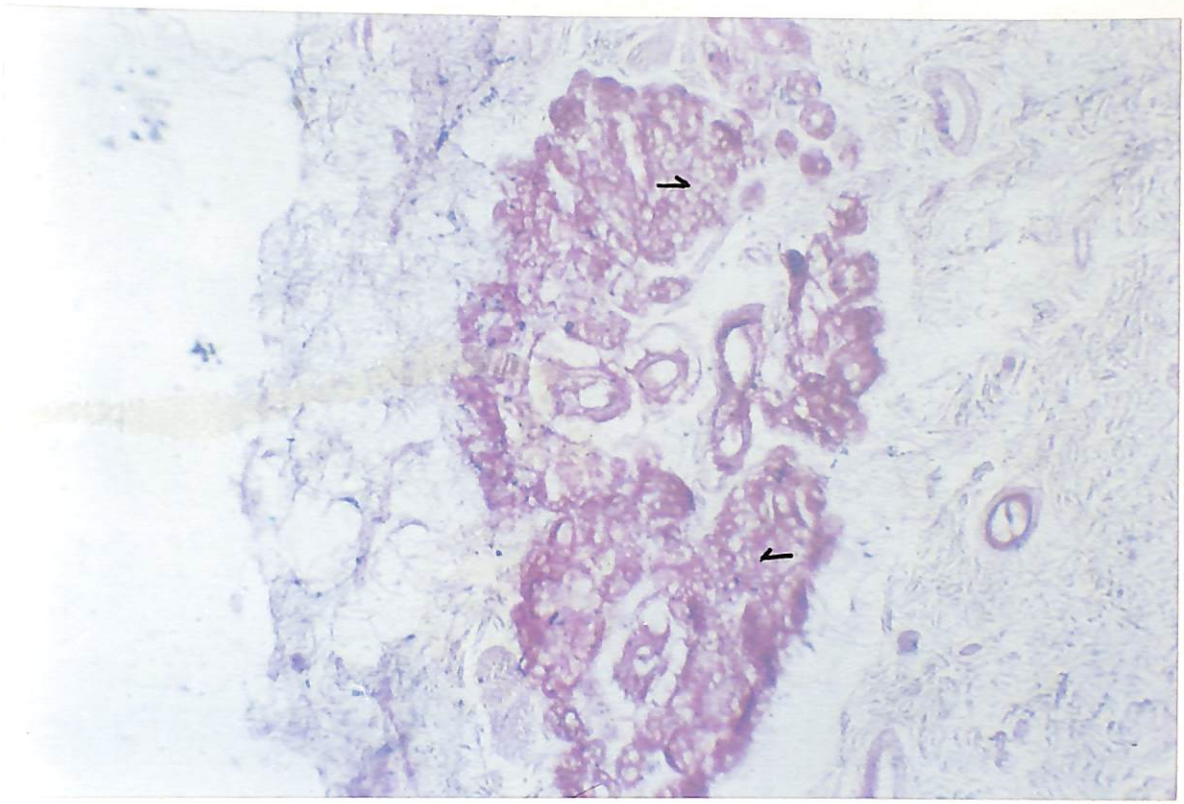

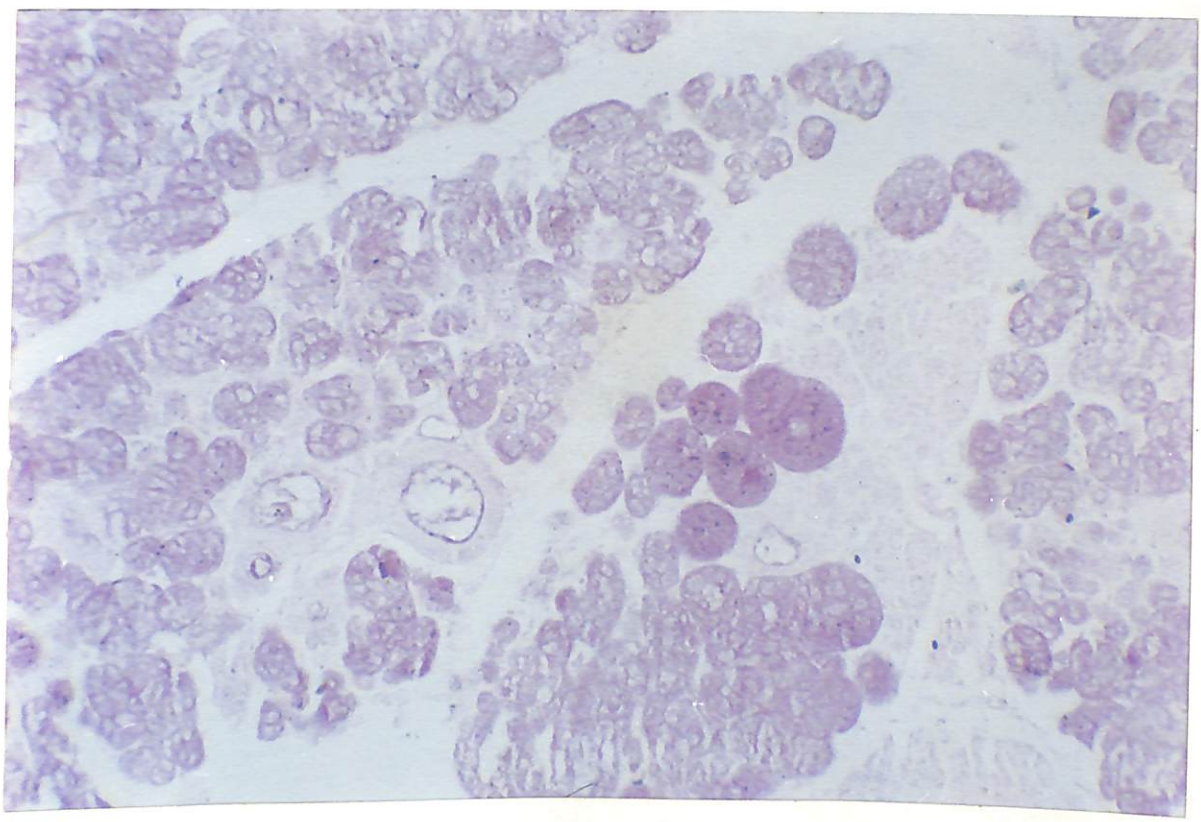

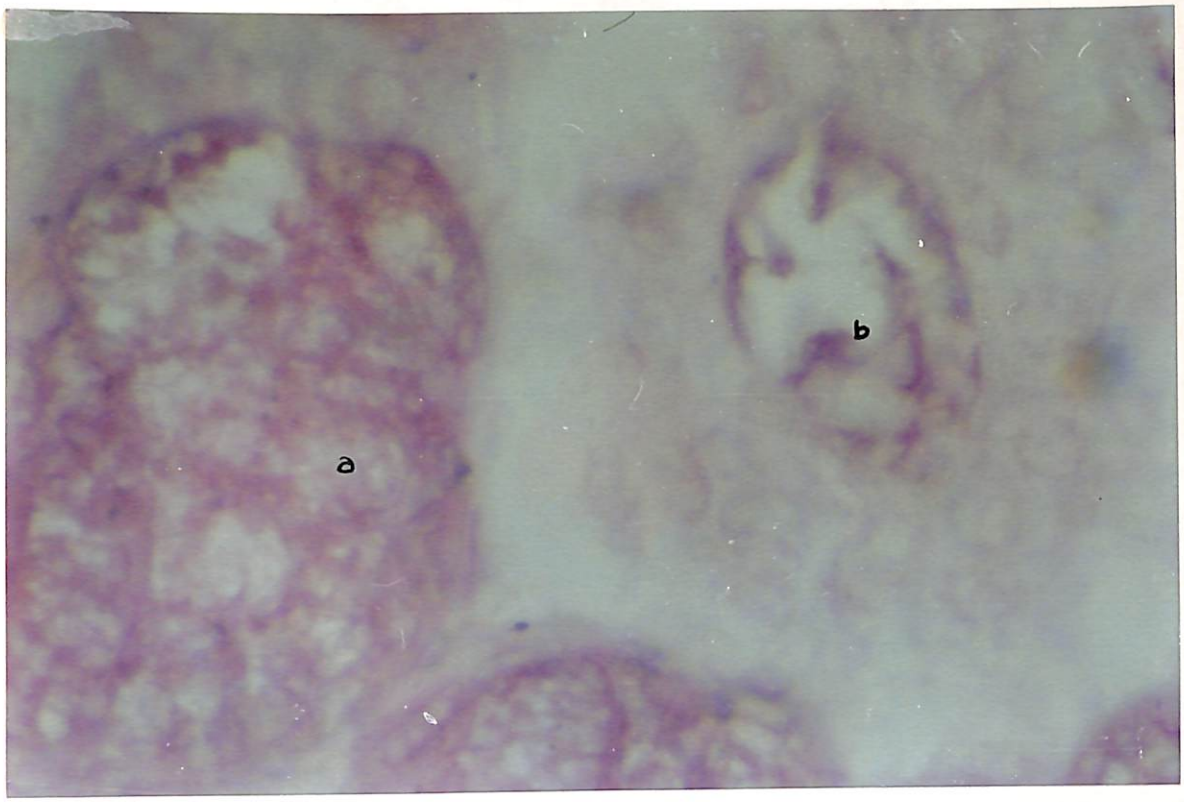
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