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POSTNATAL DEVELOPMENT OF THE CAT'S EPIDIDYMIS (LIGHT MICROSCOPIC STUDY)

(With 20 Figures)

By

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تطور ما بعد الولادة للبربخ في القط (دراسة بالميكروسكوب الضوئي)

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أجريت هذه الدراسة لمتابعة تطور ما بعد الولادة للبربخ في القطط. استخدم في هذا البحث عدد عشرون من القطط عند أعمار أسبوع، شهر، ثلاثة شهور وستة شهور بعد الولادة وكذلك القط البالغ وقد جهزت بعض العينات وصبغت بصبغة الهيميا توكسلين والإيوسين بينما جهزت أخرى لعمل قطاعات شبه رقيقة. وقد أظهرت النتائج أن الخلايا الطلائية في جميع مناطق البربخ كانت غير متميزة عند عمر أسبوع. وقد حدث التميز عند عمر شهر بظهور خلايا "هالة" ونوعين من الخلايا الداكنة الصبغة بينما ظهرت الخلايا القاعدية عند عمر ثلاثة شهور. أما عند عمر ستة شهور فقد حدث توسع وامتداد في النيبات الطلائية مع ظهور الخلايا الضيقة، الخلايا القمية والخلايا الصافية وكذلك حدوث تميز للخلايا الأساسية. فوق ذلك لوحظ عدم وجود حيوانات منوية عند هذا العمر بينما ظهرت الحيوانات المنوية في تجاويف بربخ القط البالغ مع تطور أكثر في الخلايا الأساسية واختفاء الخلايا الضيقة من منطقتي الجسم والذيل. كما أظهرت النتائج حدوث اختلافات في ارتفاع الجدار الطلائي للنيبات البربخية، قطر الجدار، طول الأهداب المجسمة وكذلك الخلايا العضلية الملساء حول النيبات وذلك في المناطق الثلاثة للبربخ.

SUMMARY

This study was carried out to follow up the postnatal development of the epididymis in cats. A total number of twenty cats was used at the ages of one week, one, three and six months as well as adult cats. Some specimens were prepared for routine histological examination and stained with haematoxylin and eosin while others were prepared for making semithin sections. The results showed that the epithelial cells of all regions of the epididymis were undifferentiated at the age of one week. At the age of one month differentiation occurred by the

appearance of "halo" cells and 2 types of dark cells. While at the three month of age basal cells appeared. At the age of 6 month, expansion of the epididymal tubules occurred with the appearance of narrow cells, apical cells and clear cells and also the principal cells became differentiated. No sperms were observed at this age. In adult cat, sperms appeared in the epididymal lumen with more development of principal cells and disappearance of narrow cells from corpus and caudal regions. The results also revealed differences in the epithelial height of the epididymal tubules, diameter of the lumen, length of stereocilia and peritubular smooth muscle cells of the three regions of the epididymis.

Key words: *Epididymis, Development, Cats*

INTRODUCTION

The epithelium lining the epididymis, contains a variety of cell types (Reid & Cleland, 1957) in which the principal cells are the predominant epithelial cell type (Herms *et al.*, 1992).

The mammalian epididymis has several important functions. Along the epididymal duct the sperms become fertile and motile (Bedford, 1966; Hamilton, 1975), the attainment of which is closely related to the secretory and absorptive properties of its epithelial lining (Bedford, 1975 & 1979; Courrot, 1981; Kessel, 1998). The cauda epididymis also acts as a sperm storage organ (Peirce & Breed, 1989). While the structural features of these cell types are well described in the adult cats (Arrighi *et al.*, 1986, Sánchez *et al.*, 1998; Axner *et al.*, 1999) there is little information about these cells during postnatal development. So the present work aims to study the cellular differentiation and the postnatal development of regional differences in the epithelium of the cat's epididymis.

MATERIALS and METHODS

Twenty domestic cats (*Felis domestica*) were included in this study. The animals were sacrificed at the postnatal ages, one week and one and three months. Six-month old and adult cats were submitted for surgical castration. Each epididymis was sectioned into 3 regions; caput, corpus and cauda. The right epididymes were fixed in Bouin's fluid for 8 - 24 hours according to the age. Samples were dehydrated in ascending grades of alcohol, cleared, embedded in paraffin and serially

sectioned at 8µm. Some sections from each region were stained with haematoxylin and eosin for histological examination.

The left epididymes were fixed in 5% coccodylate buffered gluteraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in araldite mixture. Semithin sections of one-micron thickness were cut with a glass knife in KLB ultramicrotome and stained with toluidine blue.

RESULTS

One-week old cat:

The tubules of the three different regions of the epididymis (caput, corpus and cauda) show the same features (Plate I, Figs. 1a, b & 2 a,b). They are small in size with empty appearance of the lumen. Stereocilia are not evident. The epithelium shows a single layer of cuboidal or low columnar undifferentiated cells. The nuclei of the cells are large relative to their size. Mitotic figures are common. Several widely spaced concentric layers of smooth muscle cells encircle the tubules.

One-month old cat:

The tubules of the epididymis show slight increase in size. The epithelial cells become simple columnar with large oval to rounded vesicular nuclei. In the caput epididymis, the cytoplasm of the columnar cells contains vacuoles (Fig. 4a,b), while in the cauda epididymis the cytoplasm exhibits fine dense granules (Fig. 6a,b). Different cell types appear between the columnar cells as dark cells and migratory cells (lymphocytes and macrophages). The latter are seen invading the epididymal epithelium. The lymphocyte is termed halo cell (Figs. 3b, 4a, 5) as it has more or less rounded and dark nucleus with a rim of light cytoplasm. It doesn't reach the surface of the epithelium. Macrophages show large irregular eccentric nucleus with pale cytoplasm (Fig. 4c).

Dark cells are either columnar with oval nuclei and lightly vacuolated cytoplasm (Fig. 4b) or flask shaped (Fig. 6b) with a slender base and expanded apical growth which contains the nucleus. Another type of dark cells in the cauda epididymis shows dark nucleus with more or less highly vacuolated cytoplasm (Fig. 6a). Mitotic figures are observed in the epithelial lining (Fig. 4c). The tubules become surrounded by more packed smooth muscle cells with restriction in the intertubular tissues (Fig. 3a).

Three-month old cat:

In addition to the previous findings, another cells, basal cells, appear. They are small triangularly shaped cells with oval to triangular or kidney shaped nuclei lying adjacent to the basal lamina (Figs. 7,8 & 9). Some dark columnar and flask shaped cells become more slender (narrow cells) (Figs. 7c & 9b). The intracytoplasmic granules become more obvious. Some cells show cytoplasmic apical protrusions (Fig. 9a). The intertubular tissue shows halo cells and macrophages (Fig. 8a,b).

Six-month old cat:

At this age, there is a marked increase in the size of the tubules of the epididymis (expansion) with an increase in the epithelial height at all levels. Stereocilia are evident at all levels. Stereocilia of the tubules in the caput region are longer (Figs. 10a & 15) than those of caudal epididymis (Figs. 11 & 17). No sperms are observed in the lumen at all levels. There are homogenous materials in the lumen of the cauda epididymis (Fig. 11) while some macrophages are present in the lumen of the caput epididymis (Fig. 15d).

The epithelium of the caput epididymis exhibits many types of cells similar to pseudostratified columnar epithelium (Figs. 10a & 15). These cells are principal cells, narrow cells, basal cells, apical cells and migratory cells (lymphocytes and macrophages). *The principal cells* are the predominant type in the epithelial wall. They are tall columnar cells with their oval to rounded vesicular nuclei located at the basal third of the cells. Their cytoplasm is highly vacuolated with presence of mitochondria (Fig. 15 a & b) which have sinuous thread like appearance. *The narrow cells* are slender tall columnar cells with dark oval and basal nuclei and dark cytoplasm. The most characteristic feature of the principal and narrow cells is the presence of a well developed supranuclear negatively stained Golgi region (Fig. 15) with a related well developed dense granules. There are some junctions between the columnar cells (principal and narrow cells) at their apical parts of cell membranes (Fig. 15).

The basal cells appear smaller in size than the columnar cells. They are resting on the basal lamina and don't reach the epithelial surface. They contain irregular oval or triangular nuclei with some of them have vacuolated cytoplasm (Figs. 10a & 15). *The apical cells* (Fig. 15f) appear goblet in shape (wide apical part and narrow basal part) extending from the basal lamina to the lumen. Their nuclei are irregular oval in shape, apical in position and deeply stained. Their cytoplasm contains numerous large vacuoles along the whole cell, some of them

contain homogeneous materials (Fig. 15c) with fine granules at the apical parts. *Lymphocytes* are more developed with darkly stained rounded nuclei and pale cytoplasm. They are present in intimate relation with the macrophages (Fig. 15e).

Another type of cells is present. These cells are tall columnar, extending from basement membrane to the lumen, with basally situated nuclei and highly vacuolated supranuclear and apical cytoplasm. Also, the apical regions show well developed large dense granules, some of them are observed in the lumen (Fig. 15a,b). Mitosis is still observed at this age (Figs. 10a & 15e). Two to three smooth muscle cells (Fig. 15e) surround the tubules.

In the corpus epididymis, the epithelial thickness is lesser than that of the caput (Fig. 16). The same types of cells are observed but the apical cells show well developed apical granules (Fig. 16d). Also, intraepithelial cavitations (crypts) are observed (Fig. 16a). They contain homogenous materials and their walls show stereocilia. Three to five smooth muscle cells (Fig. 16d) surround the tubules.

In the cauda epididymis, the epithelial thickness is lesser than those of the corpus and caput regions. The epithelial lining exhibits principal cells, dark cells, basal cells and migratory cells. The principal cells have light nuclei and light vacuolated cytoplasm with dense granules. The dark cells show dark basally situated nuclei with dark vacuolated cytoplasm. Both cells have well developed supranuclear negatively stained Golgi region (Fig. 17a). There are junctions between the cells at their apical regions. Another type of cells extending from the basement membrane to the lumen, with basally situated nuclei and highly vacuolated cytoplasm is observed. Their cytoplasm contains large dense bodies, some of which are semilunar in shape (Fig. 17). Their luminal border lacks stereocilia. Five to six smooth muscle cells (Fig. 17a) surround the tubules.

Adult cat:

The most significant feature of this age is the presence of sperms in the lumen. The tubules of the epididymis at all levels show more increase in their sizes with more increase in the height of the epithelial lining than those of the previous age (Figs. 12 & 18). There are regional differences in the height of the epithelial lining. Those of the caput epididymis show the highest thickness while those of the cauda show the lowest thickness. Also, the lumen in the caudal region is wider than that of the caput (Fig. 12a & 14). The lining epithelium of the tubules of the caput region attains the typical pattern of the pseudostratified epithelium

consisting of many cell types as principal, basal, apical, narrow and migratory cells (Figs. 12 & 18).

It was observed that the changes in the epithelial cells are minor and consist mainly of further development of the cytoplasmic contents. In the caput epididymis, *the principal cells* are tall columnar cells with well-developed long stereocilia at their luminal border and constitute the main part of the epithelium. They extend from the basal lamina to the lumen. Their nuclei are spherical to oval with prominent nucleoli and situated at different levels below the middle of the cells. Their cytoplasm shows more developed dense granules than those of the previous age (Fig. 18). *The basal cells* display larger oval nucleus, which lies parallel to the basement membrane (Figs. 12b & 18b). *The apical cells* are characterized by a wide apical part, a narrow body and an apical location of the nucleus. The distinctive feature of these cells is the vacuolation of all cytoplasm with the presence of granules in the wide portion (Fig. 18b). *The narrow cells*, scattered between the principal cells, are long slender cells with elongated oval dark more or less pyknotic nuclei (Fig. 18c). Their cytoplasm displays developed supranuclear negatively stained Golgi region and related dense granules.

Some epithelial cells show highly vacuolated apical cytoplasm with apical protrusions (into the lumen), as in the previous age, containing dense granules. Two or three concentric layers of myocytes constitute the peritubular muscle wall (Fig. 18).

In the corpus epididymis, the epithelial lining exhibits principal cells, basal cells and migratory cells (Fig. 19). Narrow cells are not observed. The principal cells are as those of the caput region but they are slightly shorter with shorter and more regular stereocilia. Some cells show also vacuolated cytoplasm with supranuclear dense granules, some of them are semilunar. Some of these cells show apical nucleus (Fig. 19b) while others show apical protrusions (Fig. 19a). Intraepithelial crypts are still observed (Fig. 19c). The cells lining them rest on the basal lamina and their main axis runs around the lumen of the crypt. The peritubular muscle wall is formed of three to five smooth muscle cells.

In the caudal region, the epithelial thickness is shorter than those of the caput and corpus regions (Fig. 20). The principal cells are columnar with oval vesicular nuclei and prominent nucleoli. Some cells show apical cytoplasmic protrusions (Apocrine mode of secretion) (Fig. 20b). Stereocilia are shorter and more regular. Basal cells are also observed lying on the basement membrane with triangular to oval nucleus and pale cytoplasm. No narrow dark cells are observed. Cells

with basal oval nucleus are observed. Their cytoplasm exhibits large vacuoles and large dense particles in the apical cytoplasm. Their apical surface show no stereocilia (Fig. 20c). In the lumen there are spherical bodies. The peritubular muscular wall is thicker than that of the caput and corpus epididymis (Fig. 20a,b), with inner smaller and outer larger muscle cells.

LEGENDS FOR FIGURES

Plate I:

Fig. (1 a,b): a) A photomicrograph of the epididymal epithelium (caput region) of one-week old cat showing undifferentiated cuboidal cells, intertubular tissue (it), peritubular smooth muscle cells (thick arrow), mitosis (thin arrow). (Hx. & E. X 400)

b) A higher magnification of one of the epididymal tubules showing undifferentiated epithelium (e), smooth muscle cell (arrow). (Hx. & E. X 1000)

Fig. (2 a,b): a) A photomicrograph of the epididymal tubules (caudal region) of one-week old cat showing undifferentiated cells with empty lumen (1), mitosis (arrows). (Hx. & E. X 400)

b) A higher magnification of part of epididymal tubule showing cuboidal or simple columnar epithelium (e) with connective tissue cells encircling the tubule (arrow).

(Hx. & E. X 1000)

Fig. (3 a,b): a) A photomicrograph of the epididymal epithelium (e) (caput region) of one-month old cat with decrease in the intertubular tissue (it) and more concentric layers of connective tissue cells (arrow). (Hx. & E. X 400)

b) A photomicrograph of part of an epididymal tubule (caudal region) showing columnar epithelium (e) and halo cell (arrow).

(Hx. & E. X 1000)

Plate II:

Fig. (4 a-c): Semithin sections of epididymal tubules of one-month old cat showing; a) halo cell (thick arrow), invading migratory cell (intermediate arrows) and peritubular connective tissue cells (thin arrows), b) Dark columnar cell (arrow) and c) Mitosis (thin arrow) and macrophage (thick arrow).

(Toluidine blue X 1000)

Fig. 5: A semithin section of part of an epididymal tubule (corpus region) of one-month old cat showing columnar epithelium (e), invading migratory cells (arrows) and an empty lumen (1).

(Toluidine blue X 1000)

Fig. (6 a,b): Semithin sections of epididymal tubules (caudal region) of one-month old cat showing; **a)** Dark cells with vacuolated cytoplasm (intermediate arrows) and migratory cells (thick arrows) and peritubular smooth muscle cells (thin arrows). **b)** Flask shaped dark cell (arrow). (Toluidine blue X 1000)

Plate III:

Fig. (7 a-c): Semithin sections of epididymal tubules (caput region) of three-month old cat showing; **a)** Intracytoplasmic granules (thin arrows) and basal cell (thick arrow). **b)** Basal cells (arrows). **c)** Slender dark cells (thick arrows) and migratory cell i.e. macrophage (thin arrow). (Toluidine blue X 1000)

Fig. (8 a,b): Semithin sections of epididymal tubules (corpus region) of 3 month-old cat showing; **a)** Intraepithelial macrophage (thick arrow), macrophage in the intertubular tissue (intermediate arrow) and basal cell (thin arrow). **b)** Intraepithelial lymphocyte (thick arrow), migratory cell in the intertubular tissue (intermediate arrow) and basal cell (thin arrow).

(Toluidine blue X 1000)

Fig. (9 a,b): Semithin sections of epididymal tubules (caudal region) of three-month old cat showing; **a)** Basal cells (thick arrows), dark cells with more vacuolated (frothy) cytoplasm (intermediate arrows) and apical cytoplasmic protrusions (thin arrows). **b)** Dark slender cells (thin arrow) and basal cell (thick arrow).

(Toluidine blue X 1000)

Plate IV:

Fig. (10 a,b): Photomicrographs of epididymal tubules (caput region) of six-month old cat showing; **a)** Epididymal epithelium (e), principal cells (p), mitosis (m), narrow cells (thin arrows), basal cells (thick arrows), stereocilia (st) and lumen (1).

(Hx. & E. X 400)

b) Apical cells (thin arrows), principal cells (p), narrow cells (n) and stereocilia (st). (Hx. & E. X 1000)

Fig. (11): A photomicrograph of epididymal tubules (caudal region) of six-month old cat showing epithelium, cell with highly vacuolated cytoplasm and basal nucleus (thick arrow),

homogenous material (asterisk) in the lumen (1) and intertubular tissue (it). (Hx. & E. X 400)

Fig. (12 a-c): Photomicrographs of epididymal tubules (caput region) of adult cat showing; **a)** pseudostratified columnar epithelium, apical cells (thick arrows), narrow cells (thin arrows), stereocilia (st) and sperms (sp). (Hx. & E. X 400)
b) Principal cells (p), basal cells, (b) apical cells (thick arrows), narrow cell (thin arrow), stereocilia (st), sperms (sp) and lumen (1). **c)** Lymphocyte (thick arrow), macrophage (thin arrow), epithelium (e), stereocilia (st) and sperms (sp).

(Hx. & E. X 1000)

Fig. (13): A photomicrograph of epididymal tubule (corpus region) of adult cat showing epithelium (e), stereocilia (st) and sperms (sp). (Hx. & E. X 400)

Fig. (14): A photomicrograph of epididymal tubule (caudal region) showing pseudostratified epithelium (e), principal cells (p), basal cells (arrows), stereocilia (st) and sperms (sp).

(Hx. & E. X 400)

Plate V:

Fig. (15 a-f): Semithin sections of epididymal tubules (caput region) of six-month old cat showing; **a&b)** Principal cells with well developed supranuclear negatively stained Golgi regions (thin arrows), tall columnar cells with highly vacuolated cytoplasm and apical large dense granules (thick arrows) some of them seen in the lumen (1) and basal cells (b). **c)** Supranuclear Golgi area (thin arrows) and lumen (1). **d)** Narrow cells (arrows) and intraluminal macrophages (m). **e)** Principal cells (p) with supranuclear negatively stained Golgi regions (thin arrows), narrow cells (n), mitosis (m), macrophage and lymphocyte (intermediate arrow), stereocilia (st) and peritubular myocytes (thick arrows). **f)** Principal cells (p) and apical cell (arrow).

(Toluidine blue X 1000)

Plate VI:

Fig. 16 (a-d): Semithin sections of epididymal tubules (corpus region) of six-month old cat showing; **a)** Intraepithelial cavitation (arrow) and lumen (1) (Toluidine blue X 400)

b) Principal cells (p) with supranuclear Golgi zones (thin arrows), basal cells (b), columnar cells with basal nucleus, highly vacuolated cytoplasm and apical dense granules (thick arrow) and lumen (1). **c)** Principal cells (p), basal cells (b), dark

narrow cells (arrows), stereocilia (st) and lumen (l). **d)** Principal cells (p), basal cells (b), apical cell (thick arrow) and peritubular smooth muscle cells (thin arrows).

(Toluidine blue X 1000)

Plate VII

Fig. (17 a-c): Semithin sections of epididymal tubules (caudal region) of six-month old cat showing dark cells (intermediate arrows) and cells with basal kidney shaped nucleus, vacuolated cytoplasm and dark large granules, some of them are semilunar (thick arrows), supranuclear Golgi regions (thin arrows) of the principal cells (p) and basal cells (b).

(Toluidine blue X 1000)

Plate VIII:

Fig. (18 a-c): Semithin sections of epididymal tubules (caput region) of adult cat showing; **a&b)** Principal cells (p) with supranuclear Golgi regions (thin arrows), basal cells (b), apical cell (thick arrow), cells with vacuolated cytoplasm with apical protrusions containing dense granules (intermediate arrows), stereocilia (st) and sperms (sp). **c)** Principal cells (p) and narrow cells (arrows).

(Toluidine blue X 1000)

Plate IX:

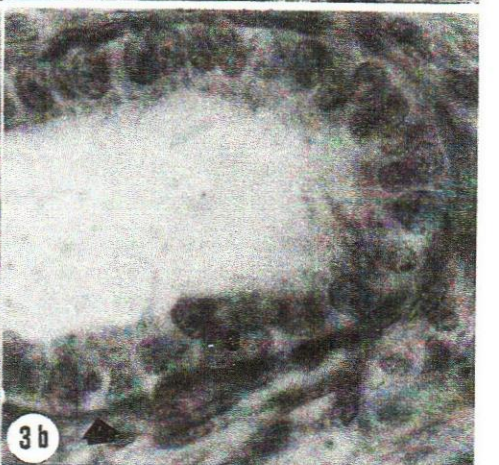
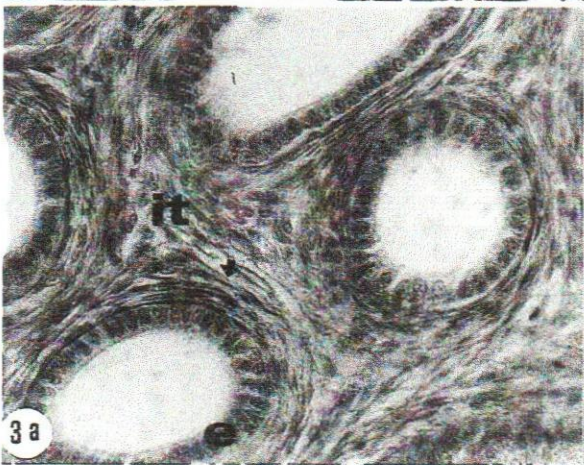
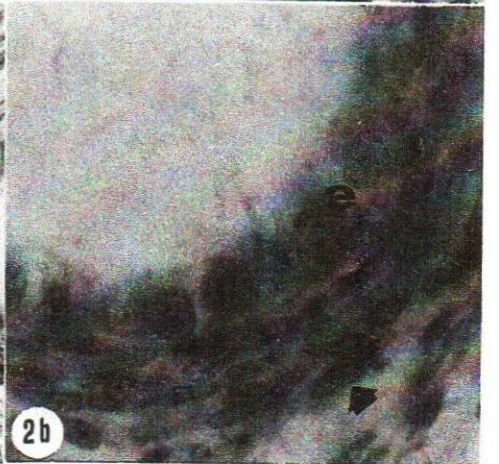
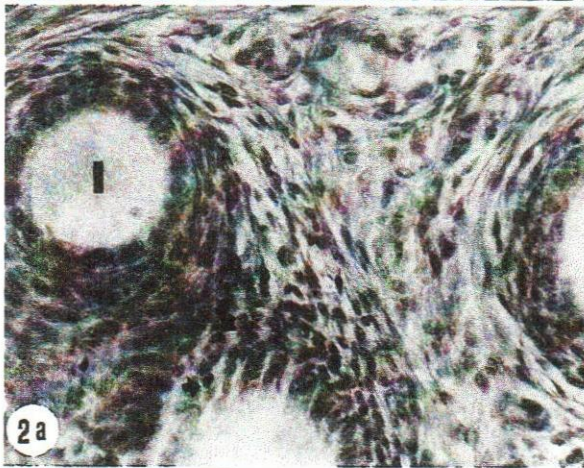
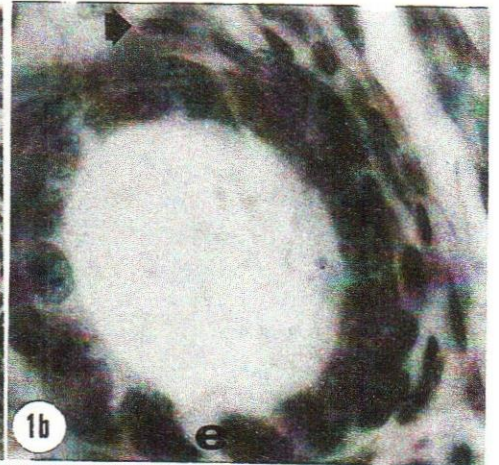
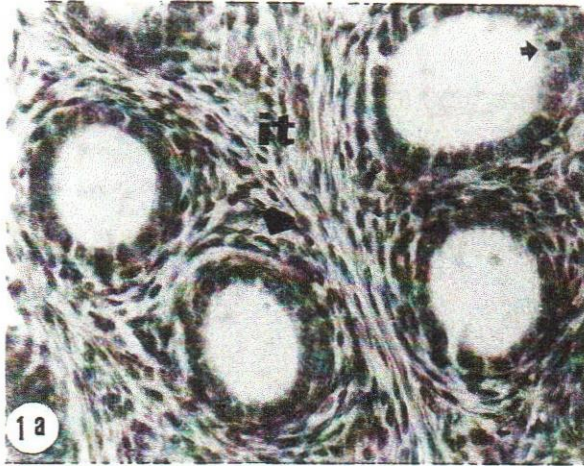
Fig. (19 a-c): Semithin sections of epididymal tubules (corpus region) of adult cat showing; **a)** Principal cells with supranuclear negatively stained Golgi regions (thin arrows), cytoplasmic protrusions (intermediate arrows) and peritubular smooth muscle cells (thick arrows). **b)** Cell with apical nucleus and dark granules (thick arrow). **c)** Intraepithelial cavitation (asterisk) with stereocilia (arrows) and lumen (l).

(Toluidine blue X 1000)

Plate X:

Fig. (20 a-c): Semithin sections of epididymal tubules (caudal region) of adult cat showing; **a)** Principal cells (p) with supranuclear Golgi region (thin arrow), basal cell (b) and inner smaller and outer larger peritubular smooth muscle cells (thick arrows). **b)** Principal cell (p), basal cell (b) and cytoplasmic apical protrusions i.e. apocrine mode of secretion (arrows). **c)** Cell with highly vacuolated cytoplasm and dense granules some of them are semilunar (arrow).

(Toluidine blue X 1000)



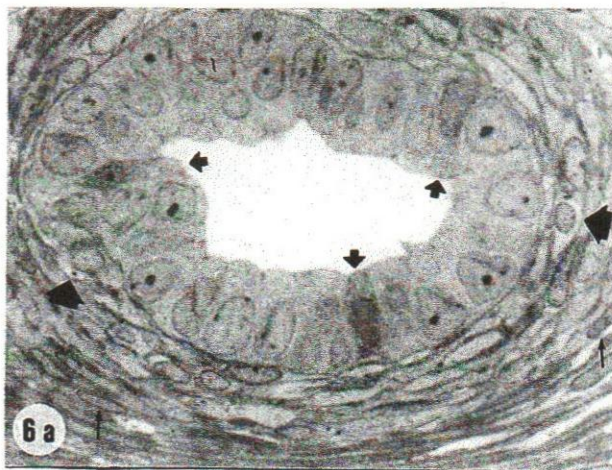
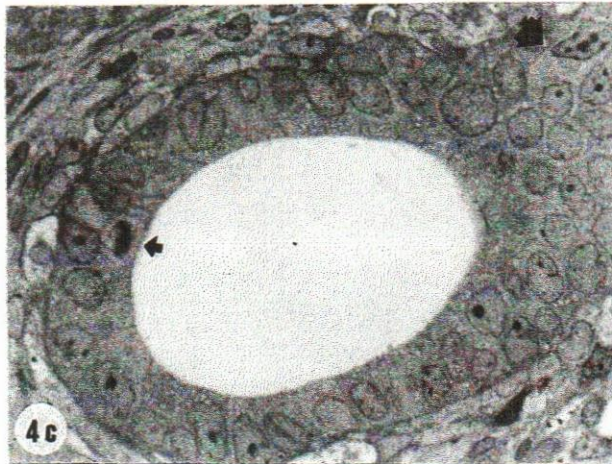
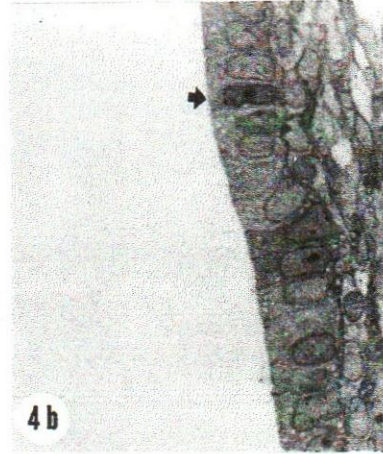
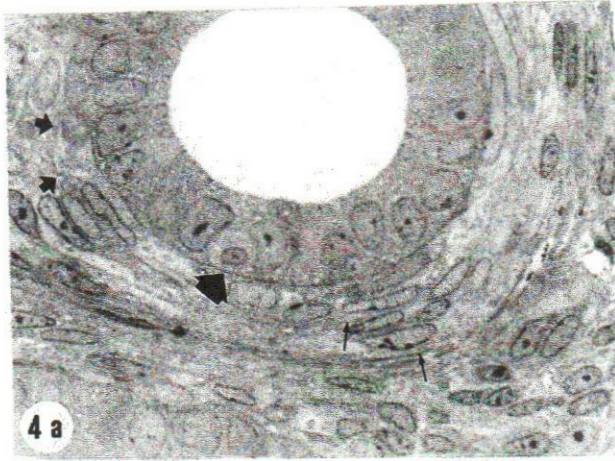


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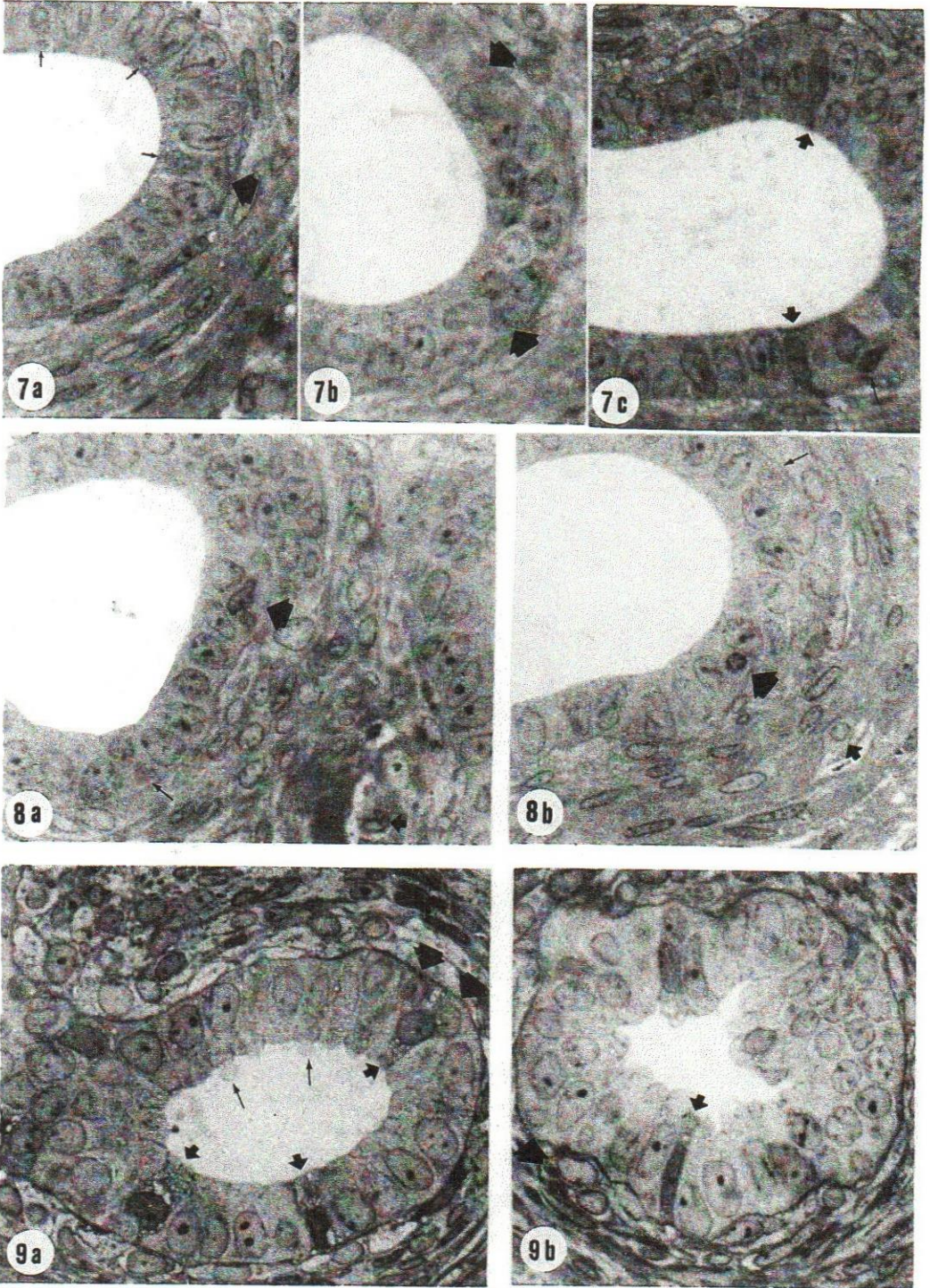


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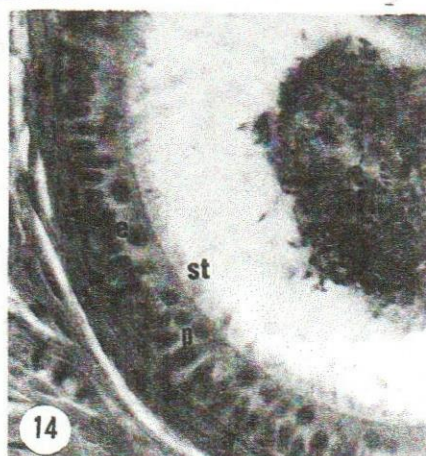
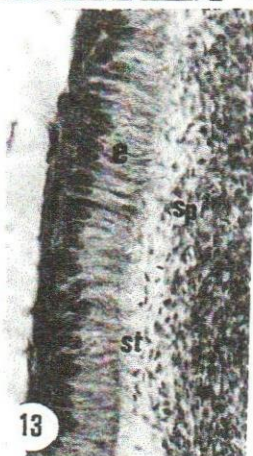
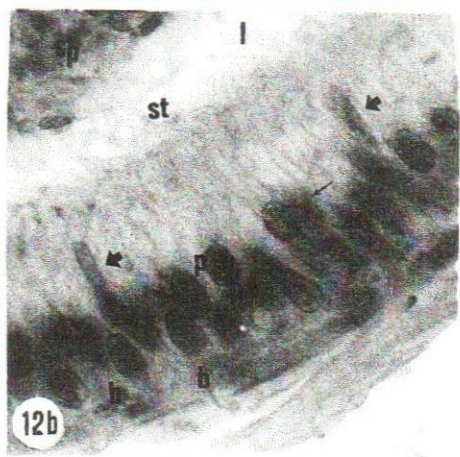
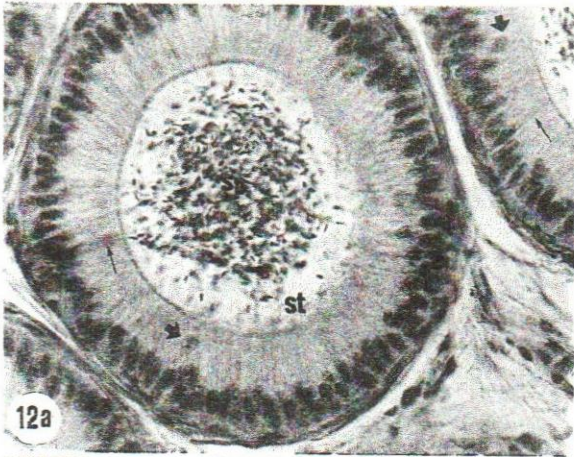
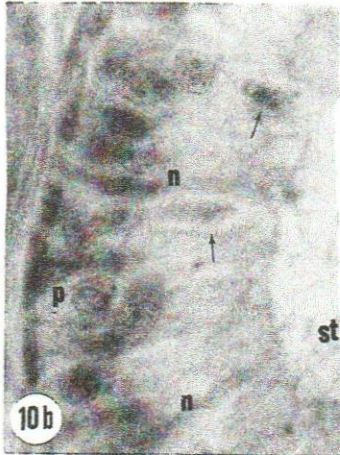
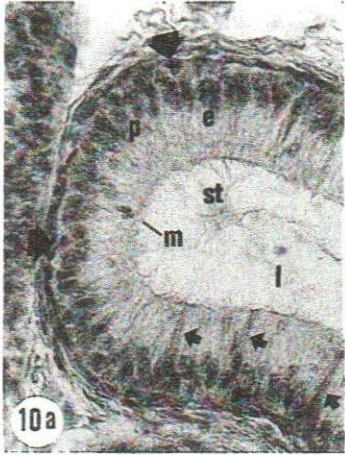


Plate (IV)

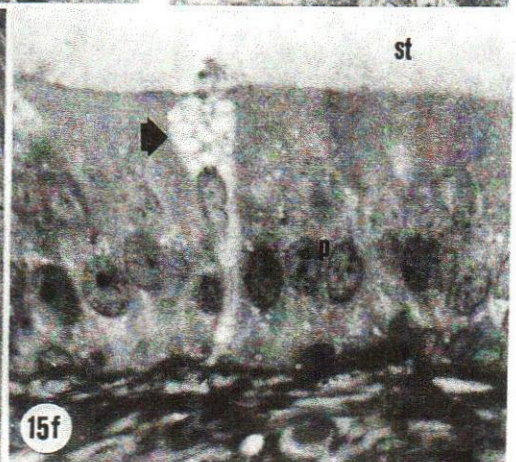
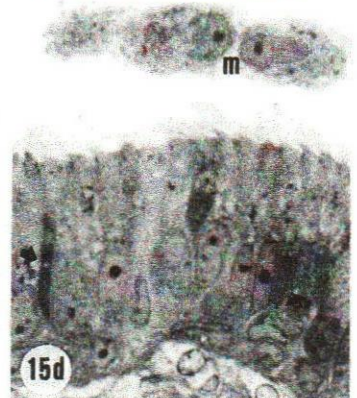
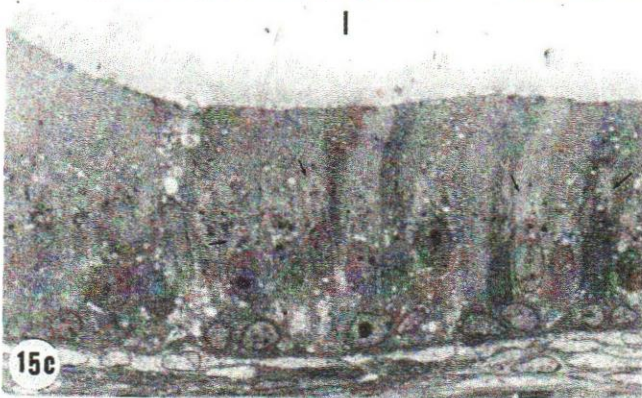


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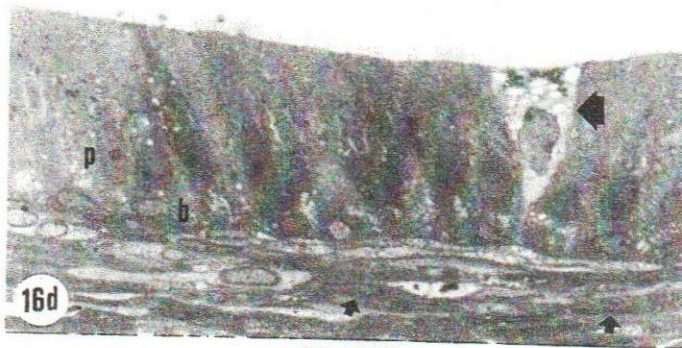
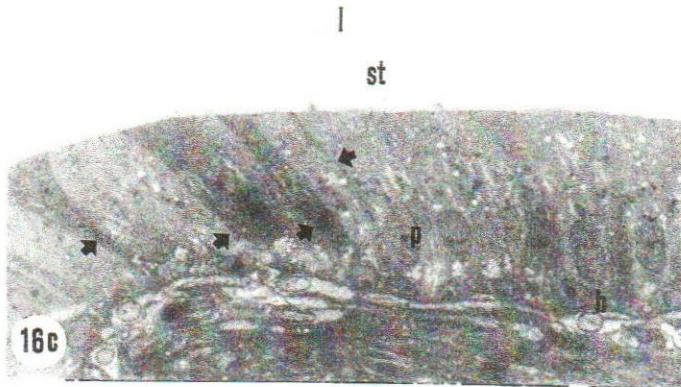
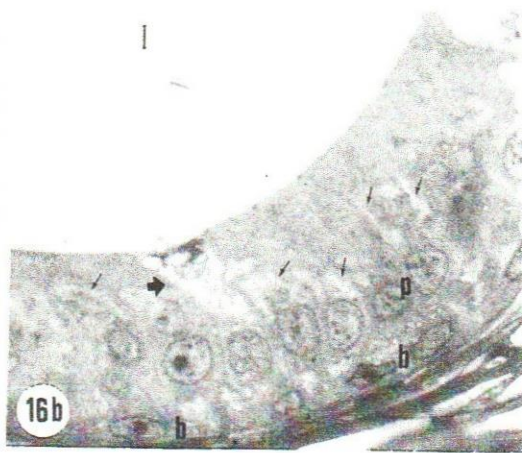
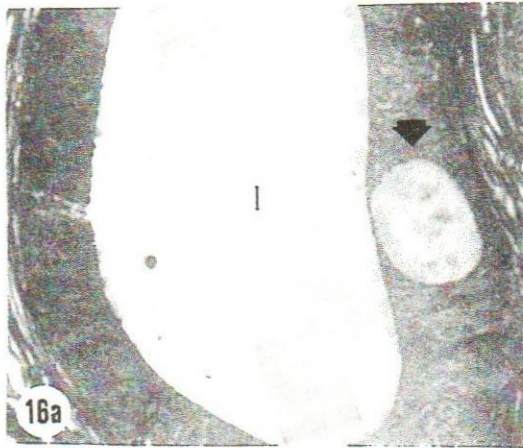


Plate (VI)

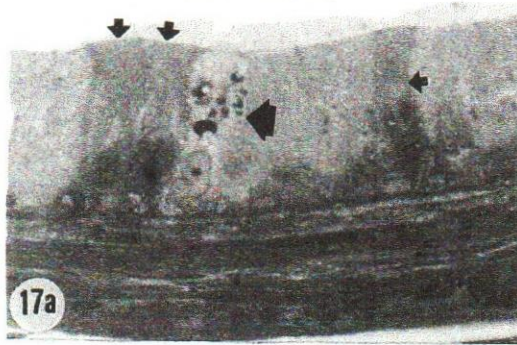


Plate (VII)

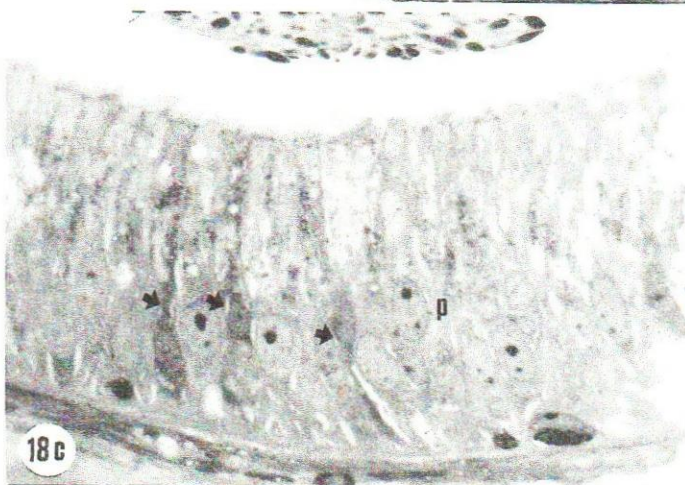
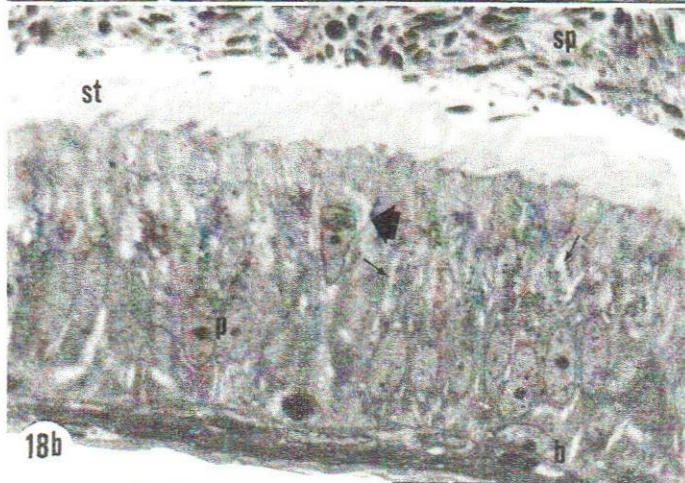


Plate (VIII)

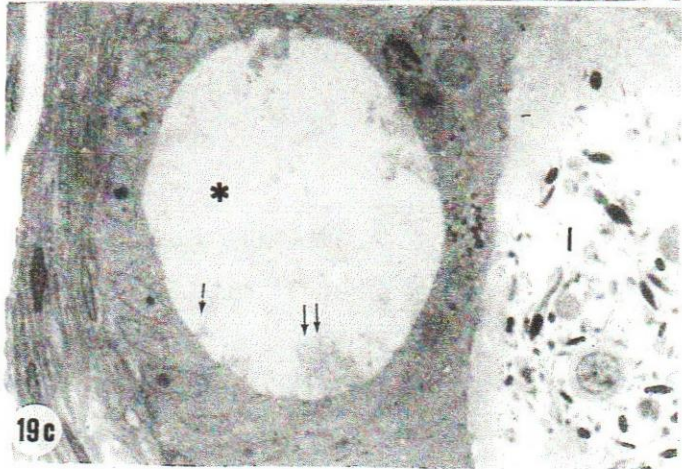
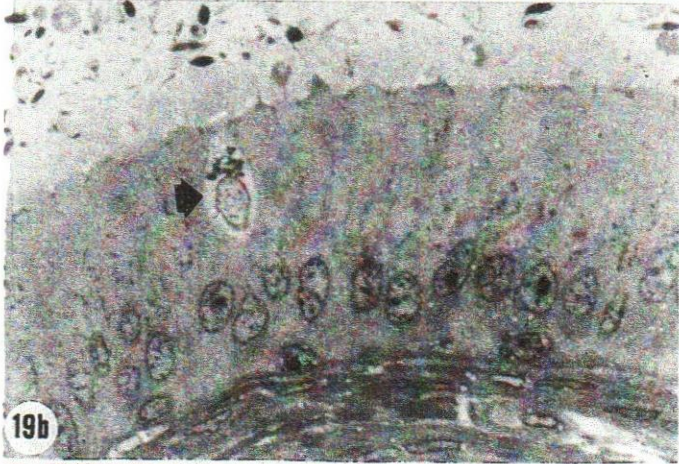
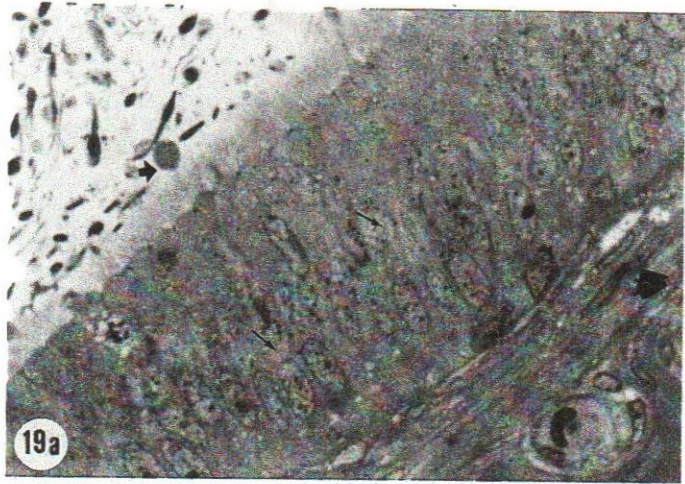


Plate (IX)

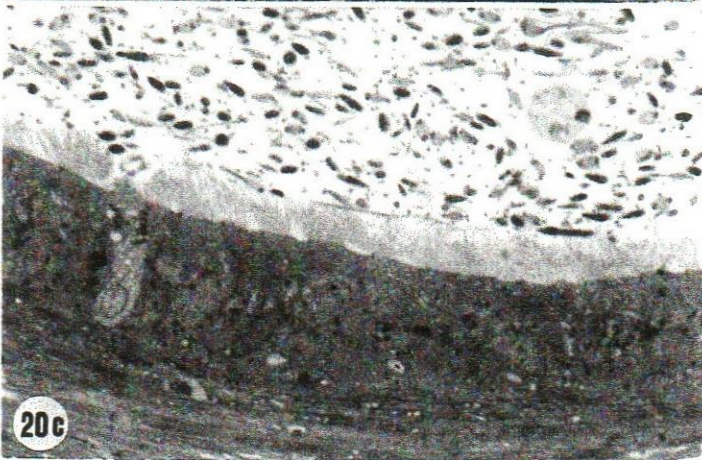
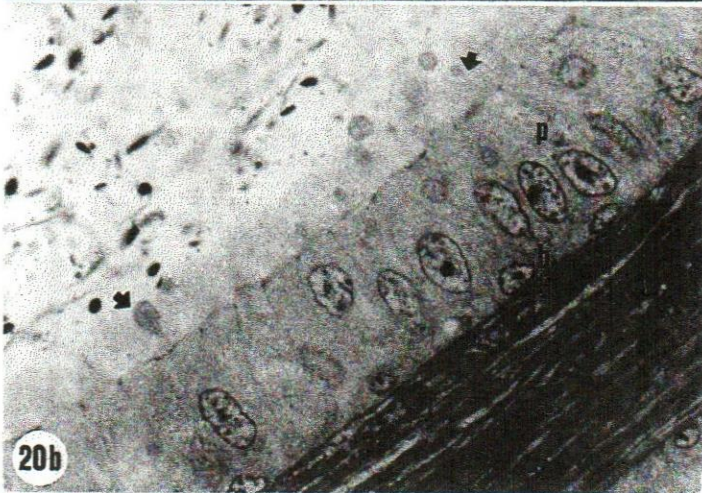
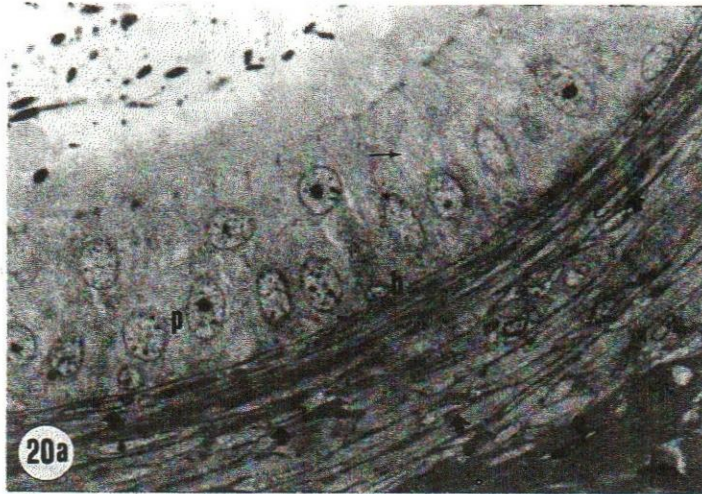


Plate (X)

DISCUSSION

The present study revealed the postnatal developmental changes of the cat's epididymis. At the age of one-week old cat, the epididymis appeared to be in a quiescent period in which there were no morphological indications of cellular differentiation. This result is in harmony with that of Sun & Flickinger (1979) in rat. The authors observed that these undifferentiated cells are inactive in secretion or absorption.

At the age of one-month old cat, the present study showed signs of differentiation of the epididymal epithelium in the form of appearance of two types of cells, migratory cells and dark cells in relation to the other cells of the lining epithelium.

These cells were either lymphocytes (halo cells) or macrophages. In rat Sun & Flickinger (1979) observed halo cells on the postnatal day 14 while Hermo *et al.* (1992) observed these cells on postnatal day 21. Studies comparing the fine structure of halo cells and leucocytes have suggested that halo cells in the adult are leucocytes that have infiltrated the epididymal epithelium (Hoffer *et al.*, 1973; Dym & Romrell, 1975). Furthermore, Sun & Flickinger (1979) observed cells similar in morphology to the halo cells in the connective tissue underlying the basal lamina in developing animals. Other features such as lack of junctional complexes with other cells and blunt pseudopod-like processes also indicate that the halo cell is a wandering leucocyte (Hoffer *et al.*, 1973). Moreover the migratory cells were observed invading the epididymal epithelium in the present study. Therefore, this cell type does not appear to be a true epithelial cell of the epididymis.

The results of the present study at the age of six-month old and adult cats revealed the presence of intraepithelial lymphocytes and macrophages in the basal area. The intraepithelial lymphocytes were characterized by heterochromatic nuclei and pale cytoplasm and the macrophages had euchromatic irregular and eccentric nuclei with their cytoplasm containing particles of different densities. In accord with these findings, similar cells have been recognized in epididymes of many adults as in man (Wang & Holstein, 1983), bull (Goyal, 1985), giant rat (Oke *et al.*, 1988, 1989), goat (Goyal & Williams, 1991) and cat (Morales & Cavicchia, 1991 and Sánchez *et al.*, 1998). Moreover, Hoffer & Greenberg (1978) in guinea pig, Sun & Flickinger (1979) in rat, Peirce & Breed (1989) in hopping mice, Tingari (1989) in camel as well as Arrighi *et al.*, (1986) and Axner *et al.* (1999) in cat, observed

intraepithelial lymphocytes but no intraepithelial macrophages as seen in the present study. As these cells are immuno-competent cells, Dym & Romrell (1975) have suggested their role in keeping the epithelium of the male genital tract free from sperm antigens, which are obviously foreign to the body. The close topographical contact between them, observed in the present study as well as in the epididymis of man (Wang & Holstein, 1983) and in the goat epididymis (Goyal & Williams, 1991), provides further support for their immunological role in the male reproductive tract.

In agreement with the present study, Morales & Cavicchia (1991) in cat and Goyal & Williams (1991) in goat observed luminal macrophages containing phagocytosed sperms. Whether these cells have an epididymal origin cannot be ascertained because, in the present study as well as in the study of Goyal & Williams (1991), intraepithelial macrophages are found only near the basal cells.

At the age of one-month old cats, the dark cells were observed in the three regions of the epididymis of the present study. These were recognizable on the basis of staining properties. Some of the presumptive narrow cells had a columnar shape similar to other components of the epithelium while others acquired a flask-shape. At the age of three-month old cat, these cells became more slender displaying the characteristic narrow configuration. The same was observed in the epididymis of rat by Sun & Flickinger (1979) who first observed these cells on day 16 postnatal and assigned the descriptive term "narrow" for these cells. While Hermo *et al.* (1992) in same animals observed undifferentiated narrow cells at postnatal day 21 which became differentiated at day 39 when they took on an adult appearance (Robaire & Hermo, 1988).

At the age of six-month old cat of the present work, with the expansion of the epididymal tubules, the dark narrow cells became long extending between the basal lamina and the lumen with apical stereocilia. They occurred singly or in pairs between the principal cells in all regions. Their nuclei were dark and elongated, occupying the basal cytoplasm with prominent nucleolus. Their cytoplasm was dark with apical vacuoles and well developed supranuclear negatively stained Golgi region. While in adult cat, these cells disappeared from the corpus and cauda epididymes. In the caput region, they became more slender and their cytoplasm developed dense granules.

Sun & Flickinger (1979) in rat observed the disappearance of narrow cells from the middle and terminal segments of the epididymes

about day 35. This coincided with the appearance of light cells. The authors suggested that the narrow cells might be precursors to the light cells in these segments. Their suggestion depended on some similarities between the two cell types as both having a small number of stereocilia on the luminal surface and some mitochondrial cristae with a tubular character. On contrary to the present study, the same authors considered their narrow cells corresponding to apical cells described by Cohen *et al.* (1976) in rat. The latter authors have demonstrated the presence of carbonic anhydrase in the light cells and cells with apical nuclei in the initial segment. However, Sun & Flickinger (1979) described apical cells similar to the principal cells except for the apical position of the nucleus and these cells were corresponded to apical cells of Reid & Cleland (1957) in the same animal.

In harmony with the present study, Sánchez *et al.*, (1998) described the narrow PAS positive cells which were seen mostly in the initial regions of the cat's epididymis. While on contrary to the present study Arrighi *et al.* (1986) and Axner *et al.* (1999) did not describe narrow cells in the cat's epididymis. Also, narrow cells were not described in many species as in guinea pig (Hoffer & Greenberg, 1978), bull (Goyal, 1985), giant rat (Oke *et al.*, 1988), australian rodents (Peirce & Breed, 1989), and goat (Goyal & Williams, 1991).

However, Tingari (1989) described rare tall and slender dark cells in the camel epididymis. These cells were seen singly or in pairs in all regions and exhibited ultrastructure similar to the adjacent principal cells. There were hypotheses with regard to the role of dark cells in epididymis. Namely, that these cells represent modifications of a single cell type at different phases of its metabolic cycle (Ladman & Young, 1958, Tingari, 1972). Another hypothesis that they are holocrine secretory cells which are characterized by lack of stereocilia, the presence of large clear vacuoles containing finely granular material and an overall denser cytoplasm than that of other principal cells (Martan & Allen, 1964; Martan *et al.*, 1964). The third one is that they are dead or dying columnar cells (Hoffer, 1976; Paniagua *et al.*, 1981, Tingari, 1989). The latter proposal, seems to be the most plausible for the cat's epididymis. In support of this is the similarity between the dark narrow cells and the principal cells at the age of 6 month old cat in having stereocilia, vacuoles and supranuclear negatively stained Golgi zone and also the disappearance of these cells from the corpus and cauda epididymes with their persistence in the caput region with a pyknotic nucleus and very slender cytoplasm (Sánchez *et al.*, 1998).

The present study revealed another type of dark cells in the cauda epididymis. These cells have dark elongated basal nuclei with apical vacuolated cytoplasm. This result coincides with that of Hermo *et al.* (1992) who observed these cells in the three regions of the epididymis (caput, corpus and cauda) at postnatal day 21 in rat. The authors classified these cells as undifferentiated clear cells since they lacked a frothy apical region and dense lysosomes. However, Sun & Flickinger (1979) in the same animal, by day 35, observed this type of cells but they termed them light cells. They noticed that their appearance in the middle and terminal segments is associated with the disappearance of narrow cells.

At the age of three-month old cat in the present study, the cytoplasm of the light (clear) cells attained a frothy appearance but still no dark granules were observed. While at the age of six-month old and adult cat, these cells became differentiated. They became highly vacuolated with development of large dense granules, some of which are c-shaped or semilunar. In agreement with these findings, at the age of 45-day old rat and in adult rat, Sun & Flickinger (1979) observed differentiated light cells in the terminal segment with many dense bodies. These cells resembled the "active clear cells" described by Reid & Cleland (1957). However, Hermo *et al.* (1992) observed that the clear cells of rat cauda epididymis differentiate before those of corpus and caput at postnatal day 39. Their possible explanation was the fact that the lumen of the cauda at day 39 was filled with degenerating cells, some of which were recognized as being spermatids. In addition, numerous small particles and membranous profiles of different size were found not only in clear cells but also free in the lumen. Thus Hermo *et al.* (1992) suggested that these cells may be triggered into differentiation by the need to remove this debris from the lumen. The present study is partly in accordance with the suggestion of those authors as the lumen of cauda epididymis was more or less clear in some tubules while it contained homogeneous material in others.

In harmony with the present findings, clear cells were described also in epididymal epithelium of rats (Reid & Cleland, 1957, Hamilton, 1975), hamster (Nicander & Glover, 1973; Flickinger *et al.*, 1978), rabbit (Jones *et al.*, 1979), giant rat (Oke *et al.*, 1988, 1989) and australian rodents (Peirce & Breed, 1989). On contrary to these findings, clear cells have not been observed in the epididymis of the rabbit (Nicander, 1957), stallion, ram, bull (Nicander, 1958), mole (Suzuki & Racey, 1976), camel (Tingari, 1989); Goat (Goyal & Williams, 1991) or

cat (Arrighi *et al.*, 1986, Morales & Cavicchia, 1991, Sánchez *et al.*, 1998, Axner *et al.*, 1999).

Hermo *et al.* (1988), using ultrastructural cytochemistry and immunocytochemistry; have demonstrated that the cytoplasmic droplets of the testicular sperms, first disintegrate into microparticles which are subsequently endocytosed and digested by the clear cells.

Apical cells observed in the present study at the age of six-month old cat in the caput and corpus regions were goblet in shape with highly vacuolated cytoplasm which contains fine granules. Some contained well-defined granules. Similarly, a few apical cells have been reported in certain regions of other species; the initial segment in the rat (Hamilton, 1975), hamster (Flickinger *et al.*, 1978) and mouse (Soranzo *et al.*, 1982), regions II to IV (corresponding to the head and the body) in guinea pig (Hoffer & Greenberg, 1978), in bull (Goyal, 1985) and in goat (Goyal & Williams, 1991). Moreover, apical cells were described in cat mostly in the initial regions of the epididymis (Arrighi *et al.*, 1986 and Sánchez *et al.*, 1998) or in all epididymal regions (Axner *et al.*, 1999). On contrary to these findings, apical cells were not observed in epididymis of rabbit (Jones *et al.*, 1979), giant rat (Oke *et al.*, 1988, 1989) and cat (Morales & Cavicchia, 1991). However Oke *et al.* (1988, 1989), observed scattered wedge-shaped cells in zone II of giant rat epididymis. The authors described them as 'apical absorbing cells' on account of their shape, location of the nucleus and organelle content.

Because of the carbonic anhydrase activity of the apical cells (Cohen *et al.*, 1976, Goyal *et al.*, 1980 and Goyal, 1985), it was postulated that these cells may have some role in the acidification of epididymal plasma (Levine & Kelly, 1978). Acidification of the epididymal lumen has been suggested to play an important role in sperm functions (Hermo *et al.*, 2000). Moreover, mitochondrial aggregation, observed in the apical cytoplasm of these cells (Arrighi *et al.*, 1986, Goyal & Williams, 1991), may be of importance in generating ATP required for the transport of H^+ and Cl^- ions across the cell membrane (Goyal & Williams, 1991). Some authors believed that the apical cells and narrow cells are the same cell type and called them apical mitochondria-rich cells (Brown & Montesano, 1980, Regadera *et al.*, 1993).

At the age of one-week old cat, the present study revealed undifferentiated cuboidal principal cells of the entire epididymis. These cells became columnar at the age of one-month old cat with cytoplasmic vacuoles and fine granules. The intracytoplasmic granules became more

obvious at the age of three-month old cat. With the expansion of epididymal tubules at the age of six-month old cat, the principle cells became differentiated. They were tall columnar cells, having rounded to oval nuclei with one or two nucleoli, and located in the lower half of the cell. The cytoplasm became highly vacuolated with well developed supranuclear negatively stained Golgi zone, scattered mitochondria, densely stained granules in relation to the Golgi region, and stereocilia projecting from the apical surface into the lumen. The mitochondria can usually be distinguished from cytoplasmic granules at the light microscopic level by their sinuous, thread-like appearance (Hoffer & Greenberg, 1978). In adult cat, the principal cells became taller with more development of the intracytoplasmic granules. These findings go in accord with Sun & Flickinger (1979) who observed undifferentiated principal cells until the age of 28 days old rat where the majority of the columnar cells in all three segments developed into principal cells displaying features characteristic of these cells in the adult animals. While Hermo et al. (1992) described differentiated principal cells in rat epididymis by 39 days postnatal, when the endocytic apparatus and rough endoplasmic reticulum became structurally differentiated (Sun & Flickinger, 1979; Francavilla *et al.*, 1987). In contrast, the Golgi apparatus appeared well developed on day 21 (Hermo *et al.*, 1992).

The endocytotic features (microvilli, canaliculi, pinocytotic vesicles, coated vesicles, subapical vacuoles) have provided unequivocal evidence for an absorptive function of the principal cells (Goyal, 1985, Arrighi *et al.*, 1986, Robaire & Hermo, 1988 and Goyal & Williams, 1991). Endocytosis is an important event in the epididymis as it contributes to a luminal environment conducive for sperm maturation. Principal and clear cells contain numerous lysosomes which degrade many substances internalized by endocytosis from the epididymal lumen (Andonian *et al.*, 2001). Also Crabo (1965) in the bull and Turner (1984) in the rat have indicated that more than 90% of the rete fluid is absorbed in the caput region of the epididymis.

The general morphology of the principal cell appears to be substantially similar in different species studied (Hamilton, 1975, Ramos & Dym, 1977; Nicander, 1979; Jones *et al.*, 1979; Goyal, 1985; Arrighi *et al.*, 1986; Tingari, 1989; Goyal & Williams, (1991). The presence of well developed endoplasmic reticulum and an extremely wide Golgi apparatus in principal cells is strongly indicative of an intense synthetic activity (Flickinger, 1979, 1985; Flickinger *et al.*, 1984). Several hypotheses about the possible vehicle moving secretory matter toward

the lumen have been suggested, based on morphological and/or experimental findings. Hoffer *et al.* (1973) postulated the existence of intermittent communications between apical endoplasmic reticulum and the luminal compartment. While Flickinger (1979), on the basis of autoradiographic studies, demonstrated that the coated vesicles carry the secretory products from Golgi apparatus to apical plasmalemma. However, Nicander & Malmqvist (1977) described granules apparently involved in merocrine secretory processes. Moreover, Holstein (1969) proposed a way of secretion said to be "microapocrine in nature". Arrighi *et al.* (1986) couldn't exclude the microapocrine mode of secretion in cat epididymis. However, Morales & Cavicchia (1991) have observed apocrine mode of secretion passing into several release mechanisms in different segments of the cat epididymis, one is extrusion of the apical cytoplasm followed by pinching off, and liberation of membrane bound portions into the lumen. In agreement with Morales & Cavicchia (1991), the present study showed the apocrine mode of secretion in corpus and cauda epididymis in adult cat.

The present study, at the age of six-month old cat and in adult cat, revealed a type of cells in the caput epididymis. These cells had basal nuclei with the upper two thirds of their cytoplasm were highly vacuolated. The apical cytoplasm showed markedly dense granules, some of them were seen exocytosed and some were seen in the lumen. Such type of cell may be actively secreting principal cells. Tingari & Lake (1972) and Bakst (1980) have described two types of principal cells in the avians epididymis, secretory and non-secretory. The varied appearance of the apical surfaces of the secretory cells suggests that they may go through phases of secretory activity and rest.

The junctions observed in this study at the apical ends of adjacent epithelial cells prevent materials from moving from the lumen between the cells (Kessel, 1998). In cat, Arrighi *et al.* (1986) described typical junctional complexes adjoining the epithelial cells at their luminal endings while more deeply typical dysmosomes and membrane interdigitations connect adjacent cells.

Basal cells were observed at the age of three-month old cat in the present study. They were triangular small cells in relation to the surrounding columnar cells. These cells increased in size in succeeding ages where they were insinuated between the bases of the principal cells throughout the epididymis. They were either triangular or semicircular with ovoid euchromatic nuclei, parallel to the basement membrane. In harmony with the present observation, Sun & Flickinger (1979)

described these cells in rat, on day 28 postnatal. Basal cells were also described in epididymes of almost all species studied. Holschbach & Cooper (2002) revealed results indicating that the basal cells may arise from extratubular sources. These cells showed no morphological evidence of absorption or secretion (Tingari, 1989, Goyal & Williams, 1991), but the scattered junctional areas found on their interdigitations with the principal cells (Tingari, 1989) assumed that they may play a role in stabilizing and giving rigidity to the epithelium (Hamilton, 1972; Ramos & Dym, 1977). Moreover, Grant (1958) and Suzuki & Glover (1973) demonstrated experimentally, in epididymes of albino rat and hamster respectively, the phagocytic activity of these cells.

Studies of Hamilton (1975), Goyal (1985); Robaire & Hermo (1988) and Goyal & Williams (1991) as well as of the present work showed rarely encountered mitotic figures of basal cells. Moreover autoradiographic studies showed very limited thymidine uptake by the basal nuclei (Clermont & Flannery, 1970; Sun & Flickinger 1982). These observations do not support the hypothesis that basal cells give rise to principal cells in the adult.

The present study showed intraepithelial cavitations in corpus region at the age of six-month old cat and in adult cat. These cysts or cavities have been described to be lined by cells closely similar to principal cells as seen in the present study but less complex (Arrighi *et al.*, 1986). In accordance with present findings, intraepithelial cysts have been described in cat epididymis (Arrighi *et al.*, 1986; Sánchez *et al.*, 1998; Axner *et al.*, 1999) and in other species such as the bull (Nicander, 1958), man (Hodges & Hanley, 1969 and Nistal *et al.*, 1990) and the camel (Singh & Bharadway, 1980). According to Arrighi *et al.* (1986) the intraepithelial cavities could constitute a secretory microenvironment while according to Sánchez *et al.* (1998) they have to do with an increase in the epididymal surface area thus favouring the metabolic exchange with the testicular fluid. A third hypothesis that they may be the site of epithelial cell renovation (Nistal *et al.*, 1990). The present study is in accordance with the suggestion of Arrighi *et al.*, (1986) as the intraepithelial cavities showed stereocilia and contained homogeneous materials. No sperms were observed in the cysts and no communications were observed between the cyst and the lumen in the present study. On the other hand, Arrighi *et al.* (1986) suggested that the communication is very small or peduncular so as to avoid the passage of sperms and is therefore visible only with difficulty.

In the present study, during the period of expansion at the age of six-month old cat, the different segments of the epididymis could be identified by the height of the epithelium and the different cell types comprising it. The epithelial height of the caput epididymis was the highest one while that of the cauda epididymis was the lowest one. Moreover, the duct of the caput region showed a relatively narrow lumen while that of the cauda revealed wide luminal diameter. These observations are in line with those of Hoffer & Greenberg (1978) in guinea pig, Sun & Flickinger (1979) in rat, Goyal (1985) in bull, Oke *et al.* (1988) in giant rat, Goyal & Williams (1991) in goat and Axner *et al.* (1999) in cat. The significance of height differences among the regions is unclear but it may be related to the different functions carried out by the various regions. The tall epithelium of the head region is involved in fluid absorption (Turner, 1984, Goyal, 1989), while the short epithelium of the caudal region may play a less active role, during sperm storage (Sun & Flickinger, 1979).

The present study showed gradual increase in the thickness of the surrounding muscular coat from the caput to caudal regions where it reaches its maximum thickness in the latter region. These findings go in accord with those of Hoffer & Greenberg (1978) in guinea pig, Sun & Flickinger (1979) in rat, Goyal & Williams (1991) in goat and Sánchez *et al.* (1998) in cat.

Regional differences in the motility of the duct system may be correlated with these differences in the cytology and organization of the musculature (Fawcett, 1994). In the caput region with slender muscle coat, the ductus epididymis exhibits rhythmic peristaltic contractions that slowly move the spermatozoa along the tract. Such movements are much reduced in the cauda, which is the principal site of sperm storage. The larger smooth muscle cells of this region has rich sympathetic nerve network (Fawcett, 1994). This is needed for powerful contractions-with the intrapelvic portion of ductus deferens-that expel spermatozoa during ejaculatory reflex (Fawcett, 1994).

CONCLUSIONS

Based on the results of the present study, the following could be concluded.

1. There are three stages in the postnatal development of cat's epididymis.
 - a) Undifferentiated stage from one week to one month old.
 - b) Differentiated stage from one month to six-month old.
 - c) Expansion stage from six month to adult.

2. There are regional differences in the epithelial height, luminal diameter and the peritubular smooth muscle coat.
3. The epithelial lining of cat's epididymis consists of predominantly columnar principal cells and less numerous basal, apical, narrow, clear and migratory cells.
4. The general appearance of this epithelium is similar to what is described in other species with some structural differences.
5. Principal cells of cat's epididymis show apocrine mode of secretion.
6. The intraepithelial crypts or cavities are lined by cells closely similar to principal cells.
7. More detailed studies are recommended to be done on the cat's epididymis to explain the different phenomena which occur throughout the different epididymal regions.

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