Postnatal Developmental Changes of the Prostatic Gland in Albino Rat

Ola Ali Abd El-Wahab Mustafa, Metwally Abd El-Bary Mansoor, Youssef Hussein Abd El-Atty and Amal Al Shaht Ibrahim

Department of Anatomy and Embryology, Faculty of Medicine, Zagazig Universty, Egypt
d.aiaahmad@yahoo.com

Abstract: The prostate is the main male accessory sex gland. There are post natal developmental changes in the histological structure of the prostate from neonate to senescence., **Aim of the work**: was performed to throw more light on the histological and ultra structural changes in the prostate of male albino rats during different ages of development. **Material and Methods**: Thirty healthy male albino rats were utilized in this study. Ten animals for each group. They were classified into three main groups according to their ages. Group 1, (prepubertal) ; Group 2 (adult) and Group 3 (senile).**Results**: Examination of prepubertal group showed that the prostate was composed of multiple simple acini lined with tall columnar epithelial cells and most of them are filled with acidophilic secretion and separated from each other by connective tissue stroma. Examination of the adult group showed that the prostatic lobes were composed of many loosely packed acini with multiple papillary projection. Examination of senile prostatic lobes showed stratification of lining epithelium of prostatic acini. Also, marked increase in papillary projections were noticed in some other focal areas. **Conclusion**: from the results of this study we found that there are age-related changes appeared in structure of the cells of the prostatic acini. Some areas of the prostatic acini revealed focal stratification of their lining epithelium and other areas showed increase of their papillary projections. So, the prostate is very liable to benign prostatic hypertrophy in men. These results are of great interest for those pathologists studying the development of the pathogenesis of benign and malignant growth of the prostate.

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Keywords: Prostate, Age changes, Rat.

1. Introduction

The prostate is the main male accessory sex gland. Its secretion together with that of seminal vesicles contributed to the volume of the ejaculate which provided nutrients for spermatozoa and had important role in retaining male fertility and reproduction and conditioning the urethral surface for sperm passage[(1)].

Men of advanced age have a propensity for the development of prostatic diseases such as benign prostatic hyperplasia and prostatic cancer[(2)]. Senescence-associated changes in the prostate are believed to be contributing factors to the pathogenesis of these diseases[(3)].

Many cellular changes, such as diminution of apoptotic potentials[(4)] and increased expression of survival factors[(5)] found in prostates of senescent animals or elderly men have been implicated as causative factors of prostatic diseases.

So, this current work aimed to through more light on the histological and ultra structural changes that occur in the prostate during post natal development from pre-pubertal life till aging

2.Material And Methods

This study carried out thirty healthy male albino rats of different ages. They were obtained from the laboratory animal unit, Faculty of Medicine, Zagazig University. They were housed in cages in controlled laboratory environment with a constant 12 hours light/ 12 hours dark cycle and at a temperature maintained at 25˚C, fed a standard balanced laboratory diet and had water *ad-libitum*[(6)]. These animals were classified into three groups according to their ages[(7)].

Group1: included ten animals aging three weeks, weighting 50-75 gm representing the pre-pubertal age.

Group2: included ten animals aging 3-4 months weighting 150-200gm representing the adult age.

Group 3: included ten animals aging 18-20 months weighting 250-300gm representing the aged (senile) group.

At the time of scarification all the animals were anaesthetized with ether inhalation. The urogenital complex was immediately dissected out of the abdominal cavity of each animal by midline incision and the lobes of the prostate were carefully removed; blotted into filter paper. Each lobe was subsequently divided into two portions for light and electron microscopes examination.

Processing of specimens

Light microscope study

Specimens were fixed in 10% buffered formalin overnight at a temperature of 4°C. Tissue samples were dehydrated in alcohol, cleared in xylol, and embedded in paraffin. Tissue sections (5 µm

thickness) were stained with H&E stain[8], and **Mallory's trichrome stain[9].**

Electron microscope study[10]

Specimens for electron microscopy were immediately fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4). Then, they were postfixed in 1% osmium tetroxide in the same buffer at 4°C, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined and photographed using a JEOL JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Electron Microscope Research Laboratory of the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University (Egypt),

3.Results

Group 1:

Examination of the haematoxylin and eosin stained sections of the prostate of this group revealed that the prostate was formed of simple acini which were lined with simple columnar epithelium with oval basal nuclei, the acini were separated from each other by few stroma. Their lumina contained acidophilic secretion.(Figs.1,2).

Examination of the Masson's Trichrome stained sections of the prostate revealed that there were few collagen fibers in the stroma (Fig. 3).

Electron microscopic examination of the prostate of this group revealed that the prostatic acini were lined with tall columnar epithelial cells containing basally located euchromatic nuclei and well developed rough endoplasmic reticulum, Golgi apparatus, secretory vesicles and apical microvilli. Basal cells were located between columnar cells (Figs. 4,5).

Group 2:

Examination of the haematoxylin and eosin stained sections of the prostate of this group revealed that the prostatic acini were lined with tall columnar epithelial cells with multiple papillary projections. The lumen of the acini contain acidophilic secretion. The stroma in between the acini contained blood vessels. (Figs. 6,7).

Examination of the Masson's Trichrome stained sections of the prostate revealed that the stroma in between the acini containing collagen fibers (Fig. 8).

Electron microscopic examination of the prostate of this group revealed that the prostatic acini were lined with tall columnar epithelial cells, containing numerous secretory vesicles, rough endoplasmic reticulum, Golgi apparatus and apical microvilli (Fig. 9,10). These cells were resting on well developed basal lamina (Fig. 11). Some acini were lined with pale cells with euchromatic nuclei and multiple nucleoli and dark cells with heterochromatic nuclei with prominent nucleolus (Fig. 12). There were two patterns of RER, may be in the form of parallel stacks (Fig. 10) or the more active form – concentric whorls – (Fig. 13).

Group 3:

Examination of the haematoxylin and eosin stained sections of the prostate of this group revealed that the lumen of acini had increased and some acini showed stratifications of their lining epithelial cells, other acini still showed some papillary projections and others were lined with flat epithelium (Fig. 14). Lymphocytic infiltration appeared in interstitial connective tissue. Some acini were containing calcified secretions (corpora amylacea) (Fig. 15). The cells of some acini showed loss of polarity and contained vacuoles (Fig. 16).

Examination of the Masson's Trichrome stained sections of this group revealed abundant collagen fibers inside the stroma between the acini (Fig. 17).

Electron microscopic examination of the prostate of this group revealed that the epithelium lining the acini was stratified with euchromatic nuclei. Epithelial cells were resting on slightly irregular basal lamina. (Fig. 18). Their cytoplasm contained rough endoplasmic reticulum, few secretory granules and some autophagic vacuoles. (Fig. 19).

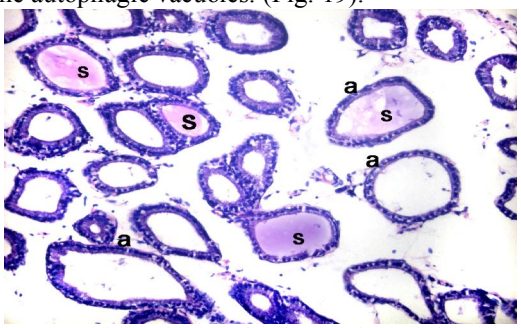


Fig. (1): A photomicrograph of a section of the prostatic lobe of a three weeks old albino rat showing thr gland formed of simple acini (a) with presence of secretion in some acini (s) (H&E X 200)

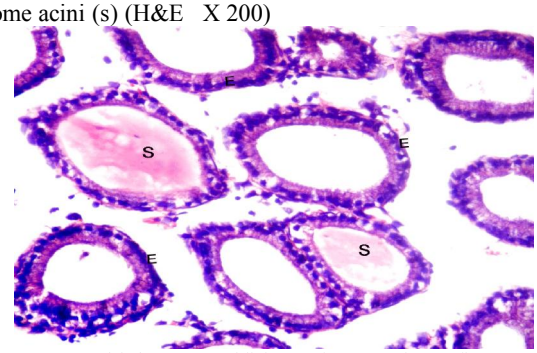


Fig. (2): A higher magnifition of a previous figure of a section of the prostatic lobe of a three weeks old albino rat showing the tall columnar epithelium (E) with oval basal nuclei linning the acini and some of them containing secretion (s). (H&E X 400)

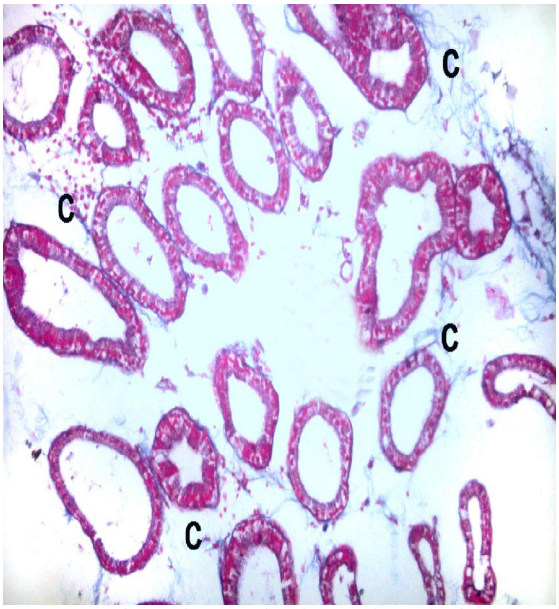


Fig. (3): A photomicrograph of a section of the prostatic lobe of a three weeks old albino rat showing the prostatic acini separated from each other with stroma containing few collagen fibers (**C**). (M&T X 200)

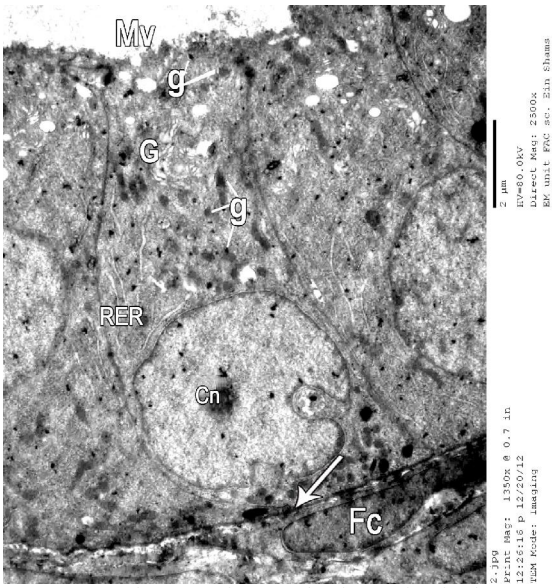


Fig. (4): An electron micrograph of a section in the prostate of the three weeks old albino rat showing tall columnar epithelial cells lining the acini with basal euchromatic nuclei (Cn), well developed rough endoplasmic reticulum (RER), Secretory granules(g), Golgi apparatus (G). Apical microvilli (Mv) are seen. Fibroblast cell (Fc) appears in the stroma. Notice the smooth and thin basement membrane (arrow). (X 2500)

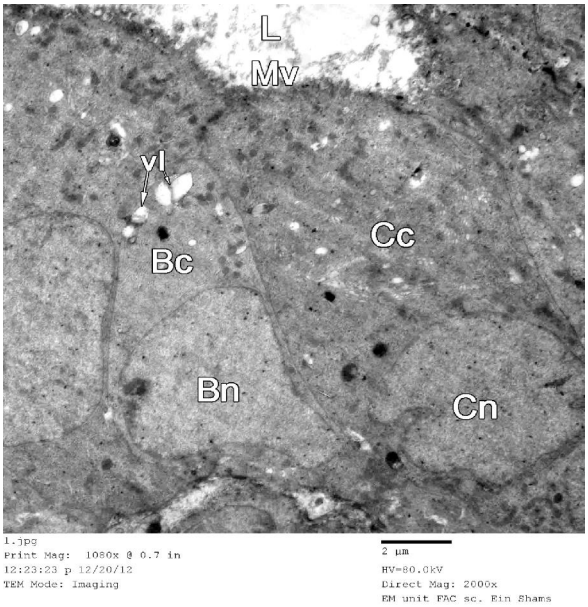


Fig. (5): An electron micrograph of a section of the prostate of three weeks old albino rat showing the tall columnar cell (Cc) with its basal nucleus (Cn) and the basal triangular cells (Bc) insinuated between the columnar cells with its nucleus (Bn) occupying most of the cytoplasm. Lumen of the acini (L) containing apical microvilli (Mv). Notice the presence of some vacuoles (Vl) in the basal cell.
(X 2000)

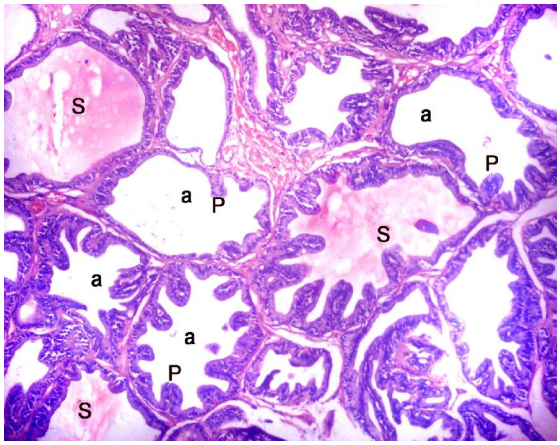


Fig. (6): A photomicrograph of a section of the prostate of three months old albino rat showing the prostatic acini (a) with multiple papillary projections (P) and acidophilic secretion (S) appear inside the acini. (H&E X 200)

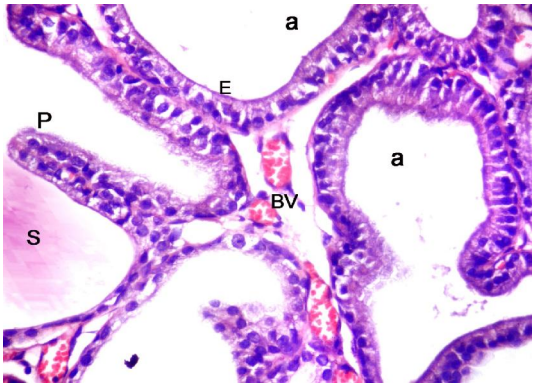


Fig. (7): A higher magnification of the previous section showing prostatic acini (a) lined with tall columnar epithelium (E) showing papillary projections (p) and acidophilic secretion inside the acini (S) . blood vessels (Bv) appear in between the acini (H&E X400)

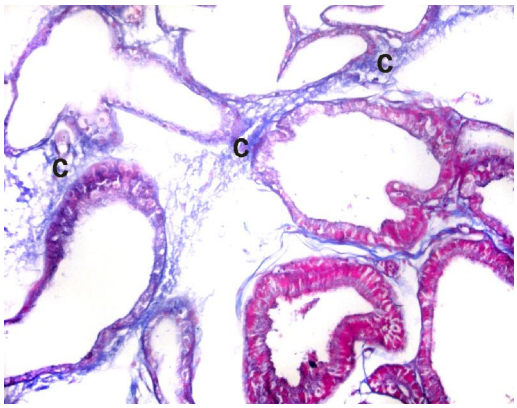


Fig. (8): A photo micrograph of a section of the prostate of three months old albino rat showing collagenous fibers (C) in the stroma.(M&T X 200)

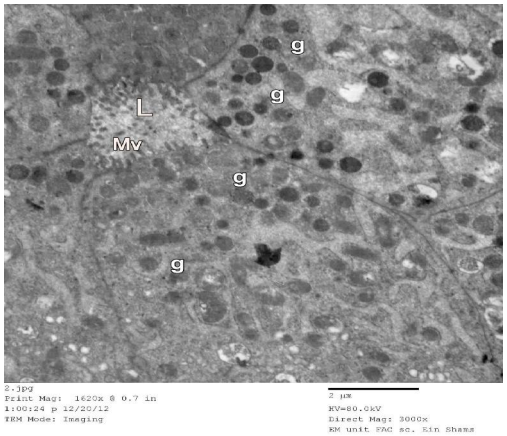


Fig. (9): An electron micrograph of a section of the prostate of three months old albino rat showing lumen of the acinus (L). The apical part of the cells containing microvilli (Mv). Notice the presence of numerous secretory granules (g). (X 3000)

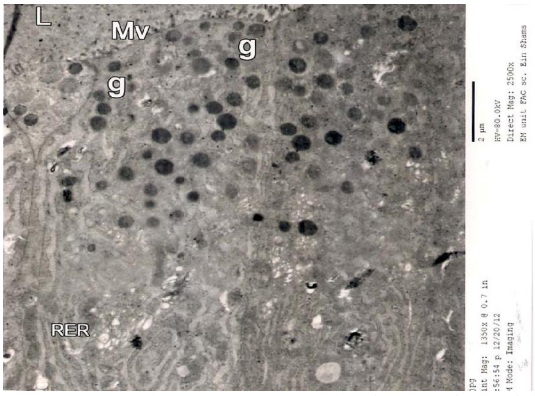


Fig. (10): *An electron micrograph* of a section of the prostate of three months old albino rat showing electron dense secretory granules (g) inside the cell with plenty of parallel stacks of rough endoplasmic reticulum (RER) and apical microvilli (Mv). Notice the presence of electron lucent secretory granules (gl). (X 2500)

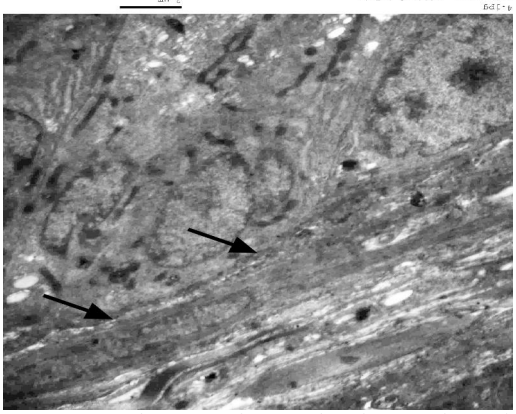


Fig. (11): An electron micrograph of a section of the prostate of three months old albino rat showing the basal part of the cells resting on well developed regular basal lamina (arrows). (X 2000)

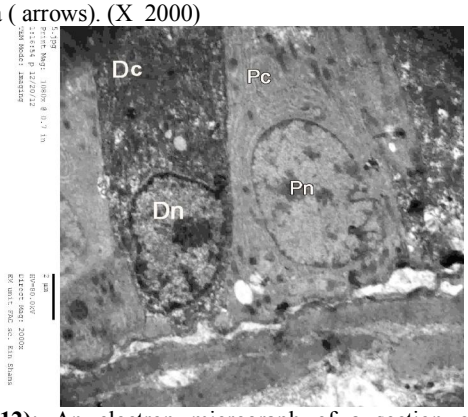


Fig. (12): An electron micrograph of a section of the prostate of three months old albino rat showing the presence of two types of tall columnar epithelial cells, the first is pale cell (Pc) with its euchromatic nucleus (Pn) and dark cell (Dc) with its euchromatic nucleus with peripheral clumping of chromatin (Dn) with prominent nucleolus. (X 2000)

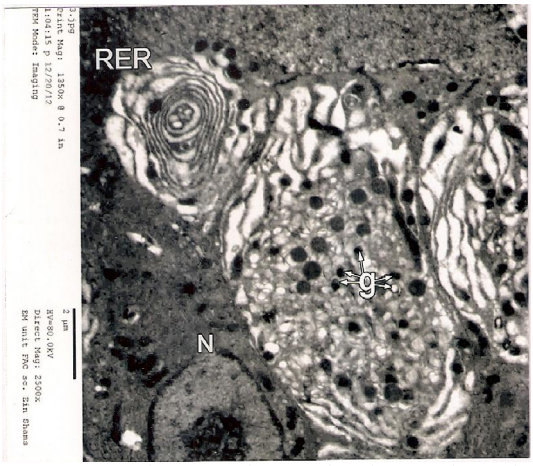


Fig. (13): An electron micrograph of a section of the prostate of three months old albino rat showing concentric whorls of rough endoplasmic reticulum (RER) containing multiple dark secretory granules (g). Notice the nucleus (N) of the cell and electron lucent secretory granules (gl). (X 2500)

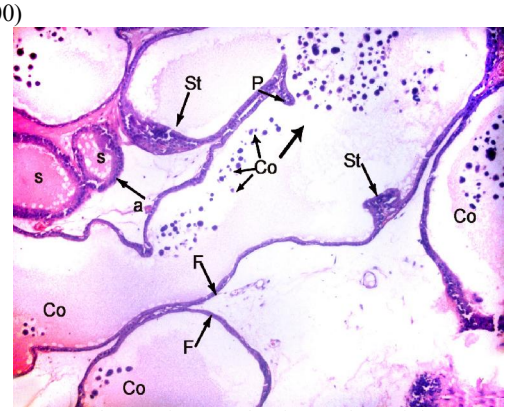


Fig. (14): A photo micrograph of a section of the prostate of senile albino rat showing most acini (a) with wide lumina and some showing stratification of their epithelial lining (St). Others containing acidophilic secretion (s). Corpora amylacea (Co) appear in the lumen of acini. Some areas showing papillary projections (P), Others lined with flat epithelium (F). (H&E X 100)

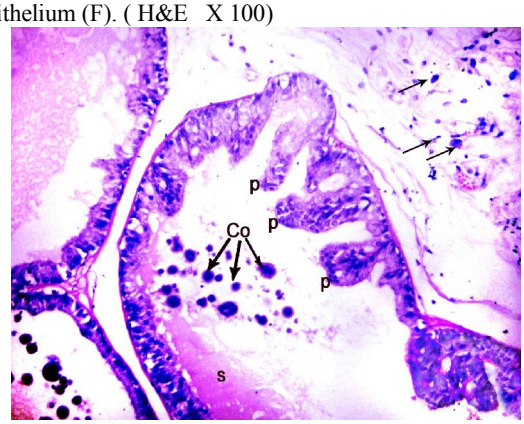


Fig. (15): A higher magnification of the previous section showing some papillary projections (p). Secretion (s) inside acini with corpora amylacea (Co). Lymphocytic infiltration (thin arrows) in the stroma appear. (H&E X 200)

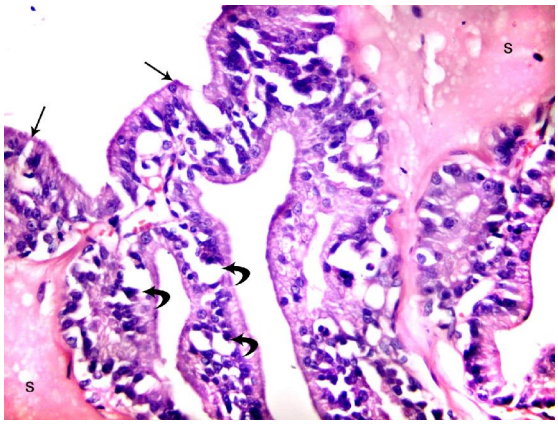
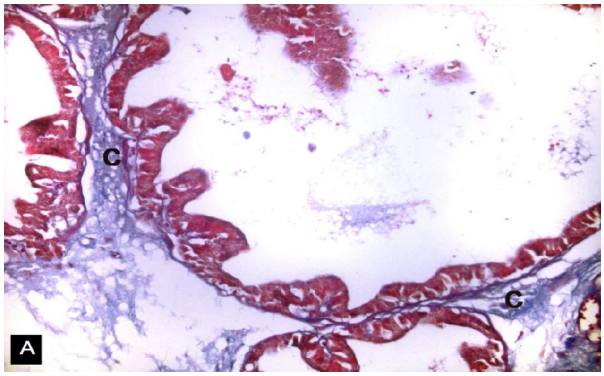


Fig. (16): A photo micrograph of a section of the prostate of senile albino rat showing loss of polarity of the cells (arrows) and appearance of vacuoles in epithelial cells (curved arrows). Notice the presence of secretion (S) inside the acini. (H&E X 400)



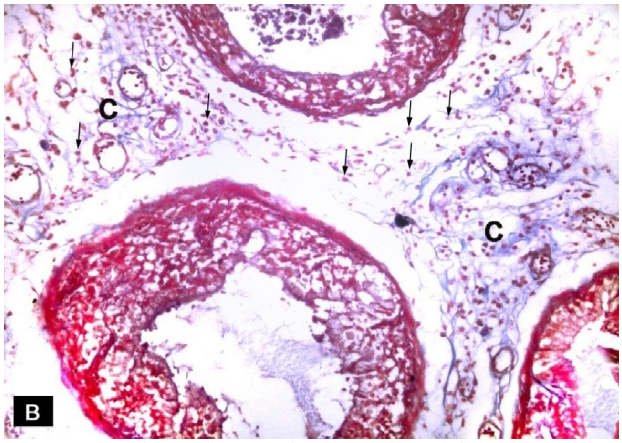


Fig. (17): A photo micrograph of a section of the prostate of senile albino rat showing abundant collagenous fibers (C) in the stroma with lymphocytic infiltration (arrows**).** (M&T X 200)

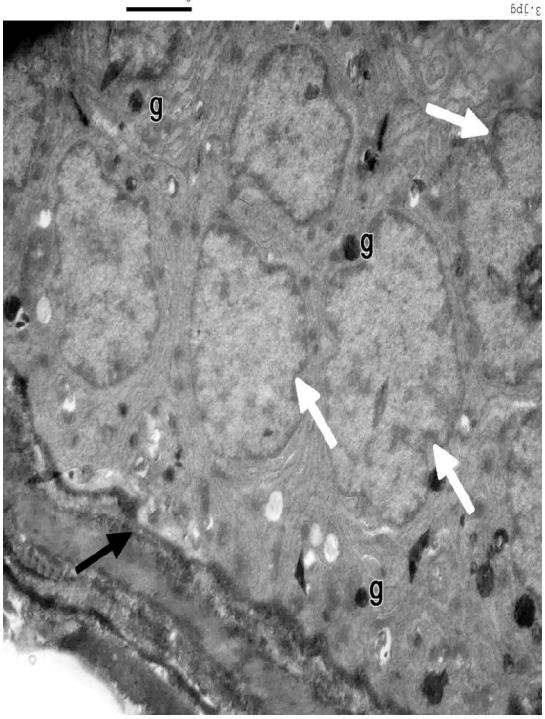


Fig. (18): An electron micrograph of a section of the prostate of senile albino rat showing stratification of the epithelial cells. The epithelial cells resting on irregular basal lamina (black arrow). Notice the indentation of their nuclei (white arrows)(X 2000)

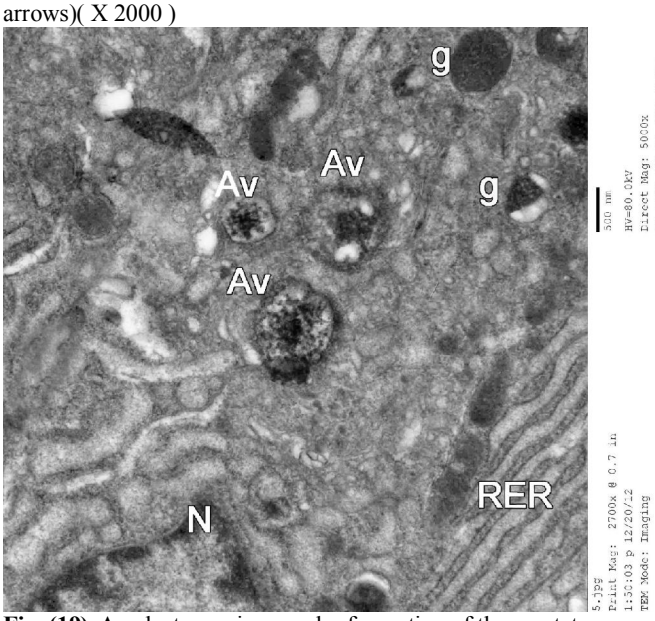


Fig. (19): An electron micrograph of a section of the prostate of senile albino rat showing some autophagic vacuoles (Av). part of nucleus (N), parallel cisternae rough endoplasmic reticulum (RER) are seen. (X 5000)

4. Discussion

The prostatic secretion is thin, milky, rich in proteolytic enzymes (acid phosphatase, prostate specific antigen and beta microsemen protein), citric acid and relatively high level of zinc inositol, transferring, phosphate ions and fibrinolysin, which liquefies the coagulated semen. In general, the primary function of the prostatic secretion is related to semen gelation, coagulation, and liquefaction[11].

Concerning the histological pictures of the prepubertal albino rat's prostate, this work revealed that the prostate is formed of acini lined with tall columnar epithelial cells with basal oval nuclei. Some of the acini were filled with acidophilic secretion. In between the acini, there were blood vessels and few collagen fibers. The above finding was explained by **Hayward et al.**[12] who mentioned that mesenchymal tissue plays an important role in development of the prostate via mesenchymal epithelial interaction and through its androgen receptors.

As regard the secretion which appeared in the lumen of some of acini of the prostate at this age. **Aumuller**[13] stated that secretion required the presence of epithelial androgen receptors in the ad luminal cells. He also mentioned that secretion started to appear on the 12 days postnatal in rats. But **Hayashi *et al.***[14] stated that secretory function was initiated at approximately 30 days postnatal in coagulating gland and dorsal prostate. They added that the rat prostate exhibited considerable heterogeneity both between and within lobes in functional expression.

Furthermore, **Donjacour and Cunha**[15] stated that the role of epithelial androgen receptors appears to be restricted to initiation and maintenance of secretory function. Growth and morphogenetic effect of androgens believed that is mediated by growth factors between the epithelial and mesenchymal/ stromal components of the glands[16].

Ultra structurally, this work revealed that the epithelium lining the acini was of the tall columnar type with few basal cells resting on relatively straight basal lamina. These results were coincided with **Jesik *et al.*** [17].

The present work revealed that columnar cells that lined the prostatic acini have basal euchromatic nuclei. The above finding is in contrast to hopping mouse whose cells were varying in height and have nuclei at their apical part[18].

The current work revealed the presence of abundant rough endoplasmic reticulum either in the form of small parallel stacks or forming concentric whorls. These results were in agreement with **Wong *et al.*** [18]. The concentric RER profile that was seen in the prostate might represent a change from less active to more active cells and vice versa. **Kochar and Pinto**

de Silva[19] revealed that endoplasmic reticulum which exist in all regions of the cells appeared to have a role in maintenance of cellular organization

Light microscope examination of the prostate of adult albino rats revealed that the prostatic acini were generally large and variable in size. The walls are infolded into lumen forming papillary projections-papillae-. The acini were lined with columnar epithelial cells with basal nuclei. Acidophilic secretion was found in the acinar lumen. These results was in accordance with **Alvarez *et al.*** [20] who mentioned that dorsolateral and ventral prostates of Wistar rats showed tall columnar epithelial cells lining the prostatic acini, which were filled with an homogeneous secretion product and there were prostatic invaginations.

It was found that the maximal differentiation and maturation of prostatic lining epithelium is observed in adult age. This may be attributed to the rise of circulating hormone secreted by mature gonads[21].

Ultra structurally, in this work, microvilli were observed in the apical surface of columnar cells of all adult animals examined in the study. These results in agreement with some authors [22,23]. Furthermore, **Dahi *et al.***[22] had observed microvilli in rats at birth and became densely packed in mature animals.

The density of microvilli on columnar cells differs according to the species and functional development of the gland. They vary in number and size in man[24] and in the dog[25].

Electron microscope examination of adult group in this work revealed the presence of basal cells appeared as short triangular or rounded cells resting on the basal lamina and insinuated between the columnar cells lining the acini. The basal cells possessed euchromatic nuclei and little cytoplasm which contained few organelles.

Merchant *et al.*[26] mentioned that the role of the basal cells remains controversial. The cells could not be mainly -secretory- as they contain few cytoplasmic organelles and do not show secretory activity. They suggested that they act as -stem cells- that continually renew the secretory layer. **Aumuller**[27] proposed that basal cells are (reserve cells) or so called –facultative stem cells- responsible for secretory cell renewal in times of need, such as with androgen stimulation.

Zaviacic *et al.* [28] mentioned that beside the two basic types of mature prostatic cells, intermediary cells were also seen located between the basal and secretory cells. The finding of intermediary cells in the lining of prostatic glands supported the role of the basal -reserve- cells in the renewal of cells in glands of the prostate.

This work revealed the presence of two types of the columnar secretory cells, pale cells and dark cells. These results are in agreement with **dos Santos, *et al.*** [29] who found two types of secretory cells in the gerbil's female prostate and named them typical merocrine cells and dark merocrine cells.

Light microscopic examination of senile albino rat's prostate revealed that there were some acini in which the columnar secretory cells had been replaced by a stratified epithelium in some areas and also revealed increase in papillary projection in some focal areas. Only a few intact acini were seen. These results in agreement with **Kiplesund *et al.*** [30]**.** The explain of these results is proved by some authers[31] who revealed that dihydrotestosterone accumulation within the gland serves as the hormonal mediator for the hyperplasia. The accumulation probably occurs in part because of enhanced intracellular binding of the molecule. The process is accelerated by estrogen, which enhances the level of the androgen receptor in the gland; increase their sensitivity to androgen. So, allows for androgen-mediated growth even in the face of declining androgen production with advanced age. They also, proved that the level of androgen receptors expression in the epithelial cells decreases with age in the prostatic lobe. However, **Kessler *et al.*** [32] explain these results by modulation of cytokine system. **Ghafar *et al.***[33] found that dorsolateral prostatic lobe was irrigated less than the ventral lobe. So the dorsolateral lobe was more liable to suffer from affection of their blood supply with age. The author mentioned also that the vascular system is the main site for androgen action.

The present work revealed also that the acinar lumens are filled with densely packed secretion –prostatic concretion or corpora amylacea– and some cellular debris were frequently found in the lumen. These results were in line with **Timms and Chandler**[34] who proved that cellular shedding is a peculiarity in dorsolateral prostatic lobe. The author explained that spontaneous cellular exfoliation is due to decreased androgen level, and dietary noxia.

Also, examination of aged albino rat's prostate revealed that most of the lumen was significantly larger than those seen in young adult rats and the majority of cells lining the acini were decreased in height and became cuboidal, only few regions containing columnar cells were seen. These results were coincided with **Lau *et al.***[35]**,** the author added that aged prostatic acini were characterized by increased accumulation of concretions in the glandular lumen.

Aumuller and Seitz[11] explained the presence of the inflammatory cells commonly seen in aged lobe of the prostate. The authors revealed that the prostate has one secretory protein, which kept in its

soluble form only in the presence of spermine and spermidine, it is also able to precipitate actin. It is conceivable that degraded prostatic cells which contain a considerable amount of actin form nuclei for precipitates in the presence of this protein. Accumulation aggregates will increase intra luminar viscosity. Moreover, they are targets for inflammatory cells especially phagocytic elements such as leukocytes and macrophages. Furthermore, **Aumuller *et al.*** [36] added that the weak muscular layer of the highly ramified tubules of the prostate, become ineffective in emptying the gland with increase viscosity of the glandular contents. The accumulating cellular bebris was then invaded by inflammatory cells, initiating a sterile inflammation.

In the present work, light microscopic examination revealed also that the epithelial cells lining the acini were irregularly hyperplastic with cellular atypia in addition to the presence of intra-epithelial vacuoles in between them. These results were in agreement with **Lau *et al.*** [35] whom added that these features began to appear with age of 9 months then diffuse atrophy and wide spread epithelial atypia and atypical hyperplasia became common features of the aged prostate in 16- to 19-months old rats.

Electron microscopic examination of the senile prostate demonstrated the stratification of the epithelial cells lining the acini and the cells are resting on a slightly irregular basal lamina. The apical part of the epithelial cells showed short microvilli. Secretory cells contained only a few secretory vesicles. Rough endoplasmic reticulum, mitochondria and other course structure of secretion were seen. Autophagic vacules were also found. These results coincided with **Martikainen and Isaacs**[37]**.** They mentioned that autophagic vacuoles were a part of mechanism involved in reduction of the volume of the cells.

In this work, both light and electron microscopic examinations revealed the presence of collagen fibers in the stroma of all groups. These results were in agreement with **DeCarvalho *et al.***[38]**.** The authors added that Collagen type VI was identified by the indirect immuno- peroxidase method in the rat ventral prostate, especially associated with the basement membrane of epithelial structures such as lobules and ducts, dispersed over the stroma, and concentrated at the blood vessel wall.

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