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Dynamics of the Epithelium During Canalization of the Rat Ventral Prostate

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ABSTRACT

Outgrowth and branching of solid cords are the initial events in postnatal prostatic morphogenesis. These processes involve cell proliferation and their projection into the stroma and precede epithelial canalization. The purpose of the present study was to examine the dynamics of the prostate epithelium during canalization of the rat ventral prostate in the first week of postnatal development using histological, stereological, and ultrastructural analyses. The terminal deoxynucleotidyltransferase [TdT]-mediated deoxy-UTP nick end labeling assay was used to investigate the occurrence of DNA fragmentation. Our results demonstrate that canalization of the prostate epithelium starts as early as on day 1 (24 hr after birth) and progresses thereafter. By the end of the first week (day 6), luminal volume density reached ~3% ($P < 0.05$) of the organ. Canalization was the result of epithelial cell differentiation and apoptosis. The former involved organization of the epithelial cells into a single layer sitting on the basement membrane, polarization, enlargement of secretory organelles and accumulation of secretory vesicles, microvilli formation, and establishment of the adult pattern of cell junctions. The latter was observed to occur mostly to epithelial cells not in contact with the basement membrane. Structures of variable electron density were observed in the developing lumen. In conclusion, different phenomena seem to be involved in the canalization of the rat ventral prostate. However, it was evident from the present results that complex epithelial cell fate decisions take place during this process. Anat Rec, 290:1223–1232, 2007.

Key words: apoptosis; canalization; epithelial cell differentiation; prostate development

The rodent prostate gland is characterized by a postnatal developmental step that takes place within the first 3 weeks after birth (Hayward and Cunha, 2000) in response to a testosterone surge occurring on the day of delivery (Corbier et al., 1995). The initial event in prostatic morphogenesis is the outgrowth of solid epithelial cords into the surrounding mesenchyme/stroma (Timms et al., 1994). At birth, as these solid buds elongate within the stroma, they begin to bifurcate and send out side branches and also to canalize (Sugimura et al., 1986a). Most of these events are dependent on androgens, because castration and/or anti-androgens block wet weight gain, cell proliferation (as measured by DNA accumulation), and branching morphogenesis (as measured by the total number of ductal tips; Donjacour and Cunha, 1988).

Cavitation and canalization are important events during morphogenesis. The development of body cavities and the formation of canals in tubular organs all result from the coordinated activity of cells. Apoptosis is involved in cell elimination during blastocyst cavitation

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and isolation of the inner cell mass (Coucouvanis and Martin, 1995) and in the canalization of hollow organs (Jaskoll et al., 2001; Sunil et al., 2002).

Different growth factors, morphogens (Sonic Hedgehog, fibroblast growth factor-10 and -7, and bone morphogenetic protein-4), and signaling pathways are shared between prostatic development and morphogenesis of other organs such as lung, kidney, salivary gland, and mammary gland (Thomson, 2001). It seemed to us that these organs could also share some aspects related to canalization and lumen formation.

We have previously shown that lumen formation in the Wistar rat ventral prostate takes place within the first 3 postnatal weeks (Vilamaior et al., 2006). However, no specific study has defined the dynamics of epithelial cells during canalization. We therefore decided to investigate the canalization process within the first postnatal week using structural, stereological, and ultrastructural analyses. In addition, to determine whether some cells undergo apoptosis, 4′,6-diamidine-2-phenylidole-dihydrochloride (DAPI) staining, and the terminal deoxynucleotidyltransferase [TdT]-mediated deoxy-UTP nick end labeling (TUNEL) assay were used to determine the occurrence of nuclear changes and DNA fragmentation, respectively.

MATERIALS AND METHODS

Animals

Wistar rats were purchased from CEMIB-UNICAMP. Animals at day 0 (the day of birth) to day 6 of age were used. All rats were killed by decapitation and dissected under a stereoscopic microscope. The ventral prostates were collected and frozen or properly fixed for the following experiments.

Histological Processing

The ventral prostates were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 24 hr, dehydrated in a graded ethanol series and embedded in historesin (Leica, Heidelberg, Germany). Sections (2 μm) were cut with glass knives and stained with hematoxylin–eosin, toluidine blue, or DAPI. Observations and photomicrographs were made with a Zeiss Axioskop microscope or an Olympus microscope both equipped for fluorescence microscopy.

Morphological and Stereological Analyses

Hematoxylin-eosin–stained sections were submitted to morphological and stereological analysis. The structure of the epithelial cords and its differentiation in a lumen-bearing structure were studied. Apoptotic cells were identified by the typical morphology of the cell nucleus and by the presence of a clear halo around the cells, which results from the loss of adhesion to the neighbor cells.

Volume densities of the epithelium, lumen, and stroma were determined by the method of Weibel. A total of 144 dots/72 grid lanes were analyzed as described previously for the ventral prostate (Huttunen et al., 1981; Antonioli et al., 2004; Garcia-Florez et al., 2005). Four or five microscopic fields taken at random were analyzed per animal (n = 3), resulting in 13–15 fields per group. Volume density was calculated by considering the number of points falling on a given compartment after conversion to percentages (144 points equal 100%). Because the ventral prostates of the newborn rats were not amenable to weighing, it was not possible to calculate the (absolute) volumes of the compartments, as it is usually done for the adult prostate.

Transmission Electron Microscopy

The material was processed by routine procedures (Carvalho and Line, 1996). In brief, the ventral prostates were fixed for 24 hr in 3% glutaraldehyde and 0.25% tannic acid in Millonig's buffer, postfixed in 1% osmium tetroxide for 2 hr, and dehydrated in a graded acetone series before embedding in Araldite (Electron Microscopy Sciences, Hatfield, PA). Ultrathin sections (50–70 nm) were cut with diamond knives and contrasted with uranyl acetate and lead citrate. The specimens were observed and documented under a Leo 906 transmission electron microscope.

TUNEL Assay

Dissected ventral prostates from 6-day-old animals were embedded in Tissue Tek OCT and cut frozen into 8-μm sections. The sections were air-dried for 20 min, fixed in 4% paraformaldehyde in PBS for 20 min at 4°C, and assayed for DNA fragmentation using the TUNEL assay (Apoptosis Detection System, Promega, Madison, WI) according to the manufacturer's instructions. After extension of the fluorescein-labeled deoxy-UTP tail with the TdT enzyme, a peroxidase-labeled anti-fluorescein antibody was used and peroxidase activity was revealed with 3,3′-diaminobenzidine. The sections were counterstained with methyl green.

Mitotic and Apoptotic Cell Counting

The number of mitotic cells was determined by counting them with respect to the total number of epithelial cells in 15 microscopic fields taken at random in a medial section through the prostate taken from three animals. The number of morphologically recognizable apoptotic cells was determined and presented as percentage of total epithelial cells on the randomly taken microscopic fields. To avoid counting the same cell twice, only one section per gland was used. The total number of cells counted for each time point ranged from 363 to 925. The number of cells in contact with the basement membrane was also determined and presented as the percentage of total cells per time point. Likewise, we also determined the proportion of epithelial structures that were devoid of apoptotic cells in the microscopic fields used for the counts.

Statistical Analysis

Statistical differences were determined by analysis of variance followed by Tukey's test. Differences were considered to be significant when $P \leq 0.05$.

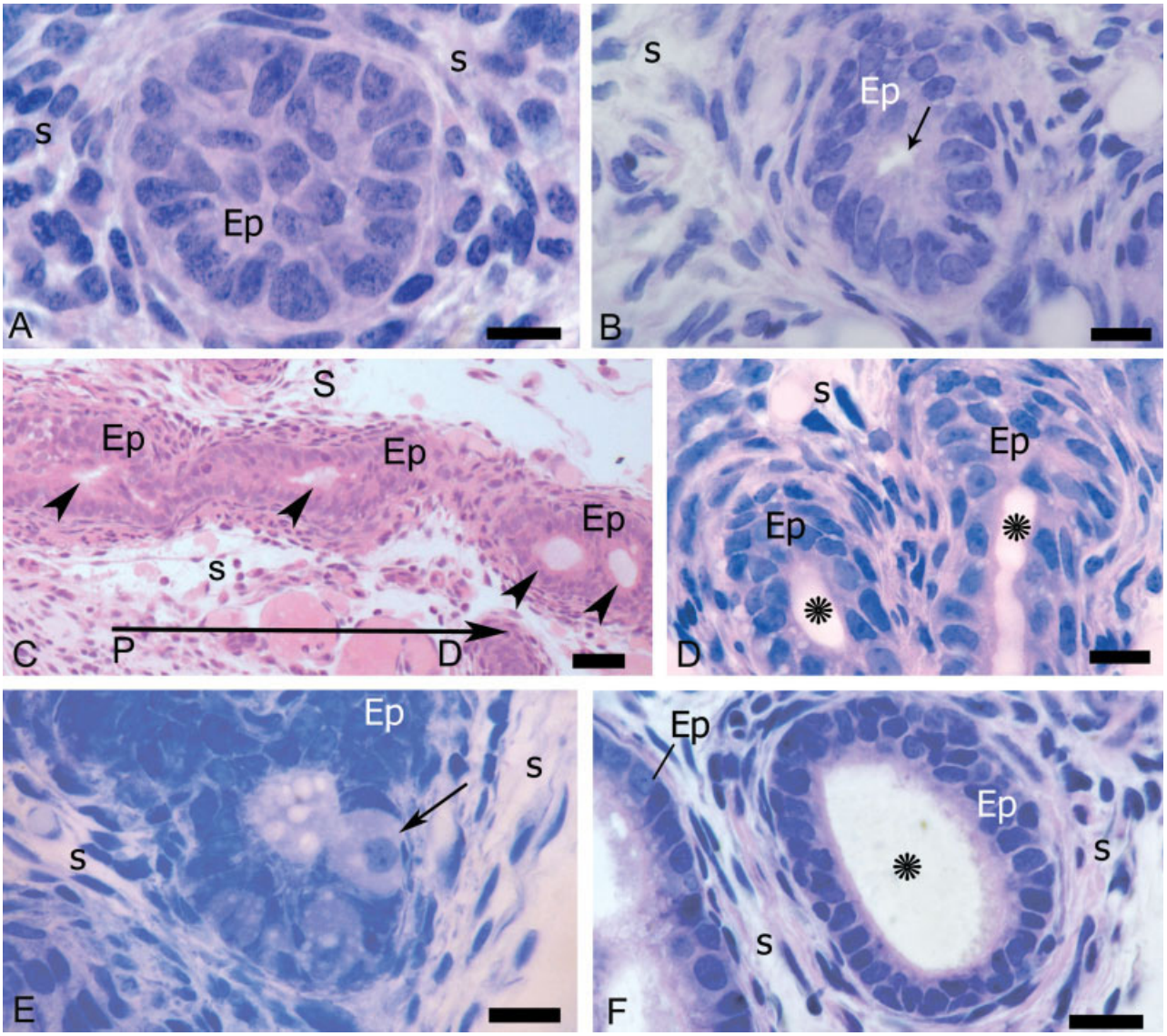

Fig. 1. Histological sections of the rat ventral prostate during the first postnatal week. **A:** On day 0, note that the epithelial structure consists of a compact cord. **B:** By day 1, the epithelial cells started to differentiate and the first signs of a lumen (arrow) could be seen. **C:** Aspect of epithelial canalization (arrows), on day 2, occurring simultaneously at different distances from the urethra (P→D indicates the proximodistal axis with respect to the urethra); **D:** Detail of the epithelial distal tips on day 3. Note that the lumen appears very close to the tips. **E:** Detail of one cell on day 4 presenting a rather distinct phenotype. This cell seemed to phagocytose entire adjacent cells. **F:** On day 6, the lumen (asterisk) had progressed distally and was enlarged compared with the previous days. Ep, epithelium; s, stroma. A–D,F: Hematoxylin–eosin. E: toluidine blue. Scale bars = 10 μm in A,B,D–F; 100 μm in C.

RESULTS

Structural Aspects of Prostate Development and Detection of Apoptotic Cells

On day 0, the epithelium of the rat ventral prostate consisted of compact cords (Fig. 1A). The epithelial cells contained large nuclei and showed no polarization with respect to the basal lamina. Canalization was observed at some points but was very limited in extension. By postnatal day (PND) 1, many of the epithelial structures showed signs of canalization, which was associated with the polarization of the epithelial cells with respect to the basal lamina (Fig. 1B). It was observed that epithelial canalization occurred simultaneously at different distances from the urethra (Fig. 1C). During the following days, the epithelium differentiated progressively, occupying a single cell layer and acquiring a phenotype more similar to that observed in adult animals. The epithelial cells became smaller and polarized, showing a basal cell nucleus and a weakly stained supranuclear region (Fig. 1B,F). This phenotype was relatively uniform, except in the distal tips where cell proliferation and differentia-

tion and epithelial canalization were still in progress (Fig. 1D). Mitotic cells were frequent in the epithelium and showed no preferred position within the cords in relation to the basal lamina or the tips of the cords.

In the region where this process was initiated, cells containing spherical and large nuclei were observed and were stained lighter by basic dyes than the usual epithelial cells (Fig. 1E). The cytoplasm of these cells appeared only faintly stained and extended from the basement membrane and the establishing lumen. These cells contained abundant vesicles (Fig. 1E).

Apoptotic cells were identified in the nascent lumen based on their characteristic morphology (Fig. 2A,B), nuclear compaction (Fig. 2C), and DNA fragmentation as assessed by the TUNEL assay (Fig. 2D). At least part of the apoptotic cells were phagocytosed by neighboring cells. Mass deletion of epithelial cells not in contact with the basement membrane was observed at given points and appeared as cellular agglomerates and/or groups of spherical nuclei within a single cytoplasm mass in the forming lumen (Fig. 2E,F).

Ultrastructure of the Developing Prostate

During the earlier stages, the epithelial cells were mainly undifferentiated (i.e., lacking the morphological and ultrastructural characteristics of luminal secretory cells as described below), contained large nuclei and numerous mitochondria, exhibited an irregular outline, and were characterized by abundant cell processes and few points of cell-to-cell adhesion (Fig. 3A). Secretory organelles such as rough endoplasmic reticulum, Golgi apparatus, and secretory vesicles were relatively rare. With time, the intercellular spaces diminished as the cells established more contacts with each other and organized themselves into a single cell layer (Fig. 3B,G,H).

The differentiated cells showed many aspects of the luminal secretory cells, with rough endoplasmic reticulum and Golgi complex (Fig. 3B,D). The number and size of secretory vesicles, however, were modest. The apical surface presented well-developed microvilli (Fig. 3D).

Mitotic cells were observed in the epithelium both in contact with the basal lamina and in contact with nascent lumen (Fig. 3E). Elements with variable substructure and electron density were found inside the developing lumen (Fig. 3E,G,H). Some nuclei with no separating plasma membrane were observed in a cytoplasm filled with amorphous substance (Fig. 3F). Some cells, corresponding to the faintly stained and vesicle bearing cells at the light microscopic level, were identified and displayed a morphology quite distinct from that of the epithelial cells. The most prominent characteristics of these cells under electron microscopy were the high content of vesicles and the apparent phagocytosis of entire cells (Fig. 3I).

Quantitative Results

Proliferating epithelial cells were relatively frequent in the first postnatal week. Quantification of the mitotic index (Fig. 4) showed a linear decrease ($R = -0.924$; $P = 0.003$) from day 0 (1.8% ± 1.0) to day 6 (1.1% ± 0.5).

Quantitative assessment of apoptotic cells revealed a significant decrease in their frequency after PND 3 (Fig. 5). It was also possible to note that apoptosis occurred mostly in the central region of the epithelial structures and that cells in contact with the basement membrane that underwent apoptosis corresponded to a very limited fraction of the epithelial cells. Additionally, it was possible to detect that, within the first postnatal week, the number of epithelial structures missing any evidence of apoptosis increased progressively. By PND 5 and 6, apoptotic cells were found in only 50% of the developing epithelial structures (Fig. 5).

There was little variation in the volume density of the epithelial and stromal compartments (Fig. 6A,C). However, the volume density of the lumen showed a significant change, increasing from 0.1% ± 0.07 of the ventral prostate volume on day 2 to 2.7% ± 0.8 on day 6 ($P < 0.05$; Fig. 5B).

DISCUSSION

In rodents, unlike in humans, a series of important events takes place in the development of the prostate gland in the early postnatal period (Hayward and Cunha, 2000; Vilamaior et al. 2006). This developmental stage seems to result from a testosterone peak that occurs on the day of birth (Corbier et al., 1995) and includes epithelial growth, branching, and canalization (Sugimura et al., 1986a).

The present study focused on the dynamics of the epithelium during the first postnatal week to determine which processes are involved in the canalization of the epithelium. The results suggest that epithelial cell differentiation and deletion are the leading events. In addition, the secretion of glycoproteins by some distinct cells might contribute at different steps to canalization.

The lumen (canalization process) starts to appear on day 0; however, this compartment was detectable by stereological analysis only on day 2, showing a marked increase thereafter and reaching ~3% of the organ volume by day 6. Even though this variation in the volume density of the lumen was statistically significant, it had no effect on the volume density of the other compartments (i.e., epithelium and stroma), which were undergoing marked reorganization. The increase in the volume density of the lumen will affect the contribution of the other compartments by the end of the second postnatal week, when it is approximately 30% of the organ. This increase continues up to the third week, when the volume density of the lumen reaches 45% of the organ before becoming quiescent and resuming growth at puberty (Vilamaior et al., 2006).

On the day of birth, epithelial cells formed a compact cord, with no features of differentiation or lumen formation (canalization). The differentiation of PC-3 prostatic cancer cells induced by mycophenolic acid, an inhibitor of 5′-monophosphate dehydrogenase, has been shown to involve the formation of cytoplasmic vesicles (Floryk and Huberman, 2005), and this kind of vesicle has been associated with the formation of an intracellular canal resembling early differentiation of the epithelium (Floryk et al., 2004). Indeed, we observed relatively large vesicles in the cytoplasm of nondifferentiated epi-

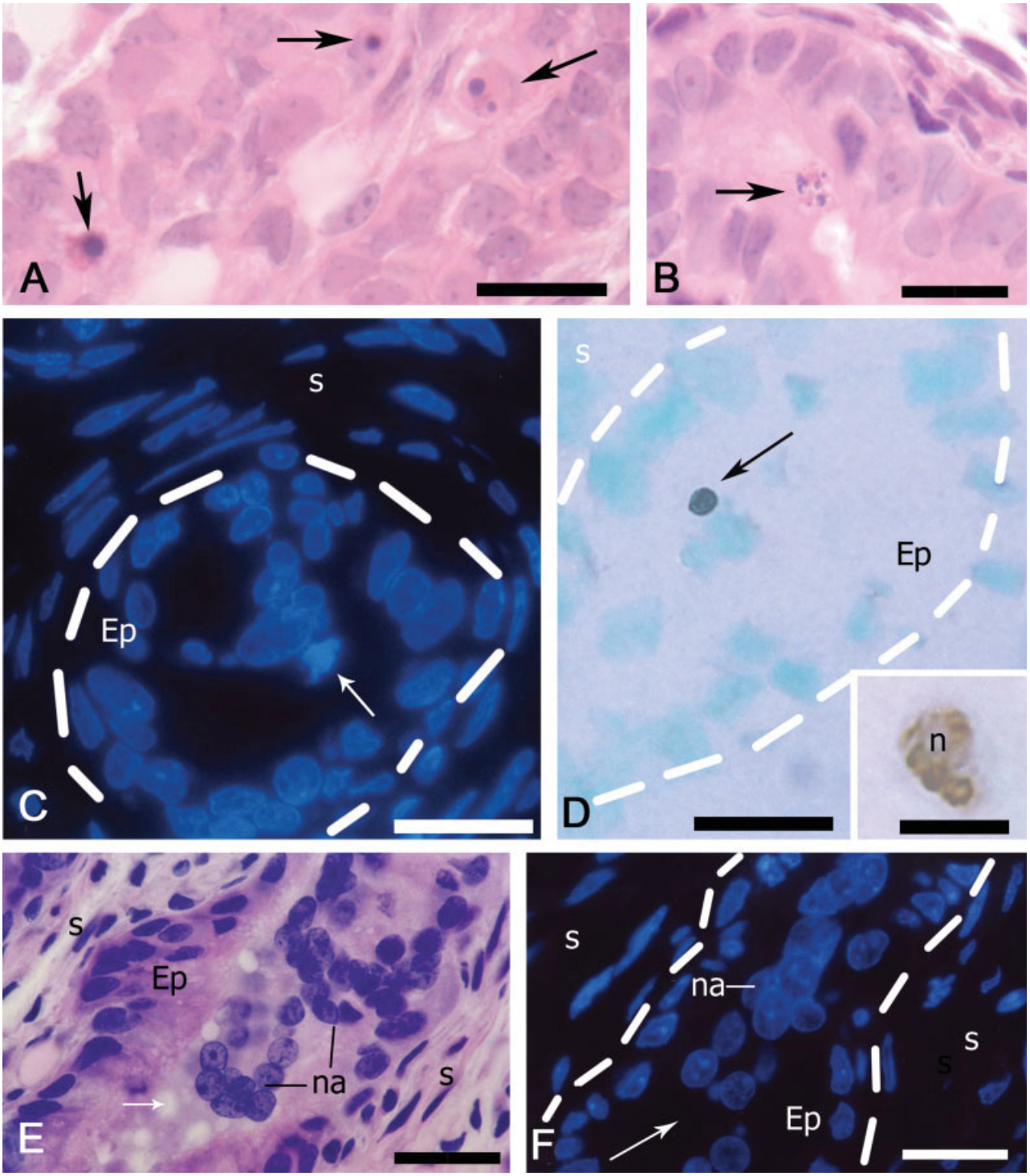

Fig. 2. **A,B:** Hematoxylin-eosin–stained sections of the ventral prostate on days 1 and 2, respectively. The arrows indicate cells with morphological aspects of apoptosis. **C:** The 4′,6-diamidine-2-phenylidole-dihydrochloride (DAPI) staining was used to identify nuclear compaction and fragmentation in the epithelium during lumen formation. The arrow points to a cell nucleus with both characteristics. **D:** The terminal deoxynucleotidyltransferase [TdT]-mediated deoxy-UTP nick end labeling (TUNEL) staining revealed the presence of DNA fragmentation in cells of the forming lumen (arrow), here shown on day 6. The inset shows another TUNEL-positive cell nucleus, which also displayed nuclear fragmentation **E:** Hematoxylin–eosin staining also showed some aspects of cell deletion during canalization. In this case, agglomerates of cell nuclei (na) were observed in the lumen. **F:** The DAPI staining revealed the absence of signs of chromatin compaction in a group of nuclei like those observed in the previous figures, although some of them exhibited an irregular outline compatible with disintegration of the nuclear lamina. The dashed lines in A, B, and D show the margins of the epithelium. Ep, epithelium; S, stroma. Scale bars = 20 μm in A,B,E,F, 25 μm in C,D, 3 μm in inset of D.

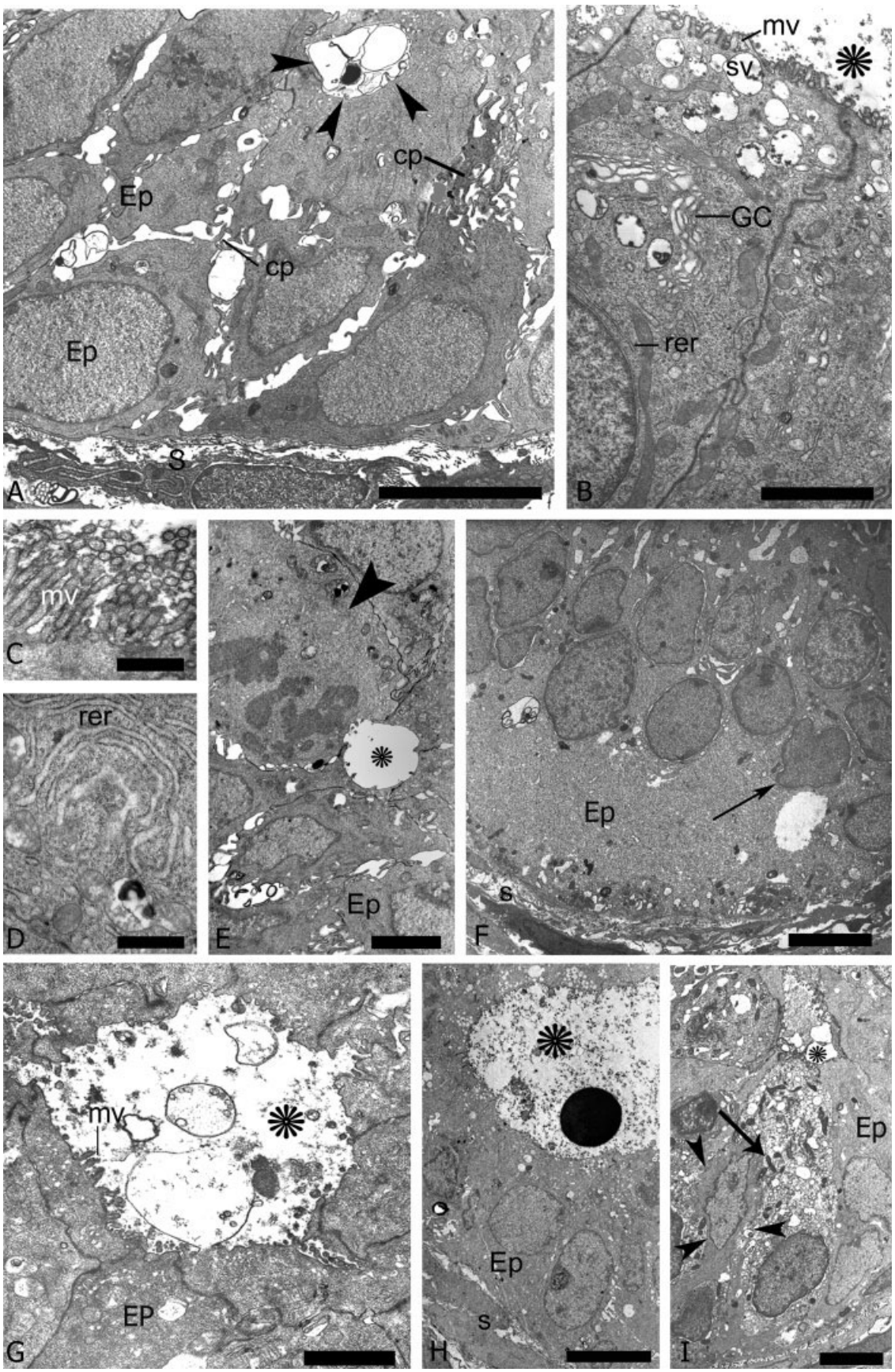

Figure 3.

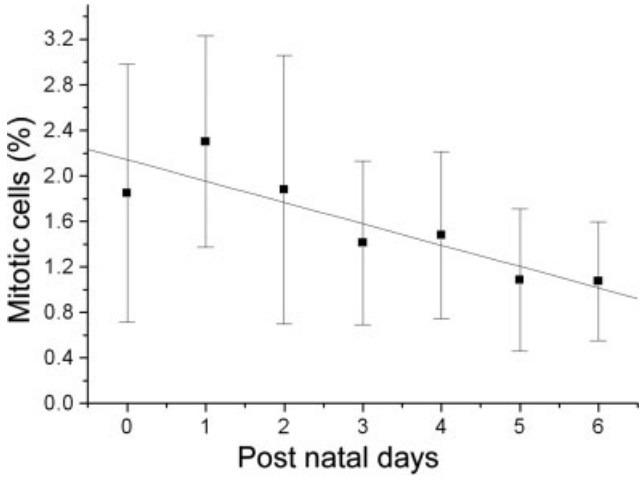

Fig. 4. Mitotic index of the rat ventral prostate epithelial cells during the first postnatal week estimated based on the percentage of mitotic cells. Values are expressed as mean ± standard deviation. A linear fit of the data is included in the figure. The calculated correlation coefficient was −0.924.

thelial cells in the areas devoid of a defined lumen by transmission electron microscopy. However, a detailed morphological analysis involving three-dimensional reconstruction and/or the use extracellular space tracers is necessary to confirm that the in vitro process is also occurring in vivo.

Canalization seemed to result from or occur simultaneously with the differentiation of the epithelium. Secretory organelles were common in the apical region of the differentiating cell. The presence of rough endoplasmic reticulum and Golgi complex suggests the occurrence of active secretion. Other remarkable characteristics of the differentiated cells were the presence of microvilli on the apical surface and the contact with the basal lamina. Whereas Price (1936) described that the differentiation of the luminal cells, as evidenced mostly by a supranuclear clear zone, takes place by day 12, one could observe differentiation characteristics by day 5.

Some of the secretory products might be directly involved in the canalization process and lumen consolidation, in addition to primary components of the prostatic secretion. However, it remains to be determined whether the secretory material corresponds to authentic prostatic secretion. Actually, it was shown for the anterior and dorsolateral prostate lobes that components of the adult prostatic secretion (i.e., DP-1 and probasin) are not produced before day 12 (Lopes et al., 1996).

In addition to the differentiation of the main luminal cells and their possible secretory activity (they produced and secreted glycoproteins into the forming lumen), some cells were also distinguishable at the ultrastructural level as they were especially rich in membrane-bound vesicles. It was noticed that these appeared to phagocytose neighbor cells. We were unable to determine the nature of these cells, but it is possible that they are intraepithelial macrophages and/or plasmacytoid dendritic cells.

In contrast to previous assumptions that canalization would result from the segregation of basal and luminal epithelial cells (Hayward et al., 1996a), it could be believed that the morphological (and hence physiological) differentiation of the luminal cells are responsible for canalization, especially because clear segregation of basal cells preserving the expression of cytokeratins 5 and 14 took place but without evidence of canalization (see Figs. 2 and 3 in their article).

Simultaneous to the differentiation of the epithelial cells, we observed the deletion of cells in the central region of the cords. The elimination of these cells appeared to be due to and regulated by cell death. The characteristic morphology, nuclear compaction, and fragmentation, as well as the positive reaction to the TUNEL assay, which reveals DNA fragmentation, permit us to conclude that these cells underwent apoptosis. It was also clear from the present results that cell death occurred to cells not in contact with the basement membrane, that the frequency of apoptotic cells decreased with time (mostly after PND 2), and that, accordingly, the number of epithelial structures presenting no epithelial cell death increased within the first week. In the present study on the rat prostate gland, it was apparent that apoptosis occurred in cells not in contact with the basement membrane, as shown for the cavitation of embryoid bodies (Coucouvanis and Martin, 1995).

These results allow the suggestion that apoptotic cell death is related to the early canalization. The drop in

Fig. 3. Ultrastructural aspects of the rat ventral prostate during the first postnatal week. **A:** Ultrastructure of the epithelium on day 2. Cells appeared mainly undifferentiated. Points of cell adhesion were scarce and the intercellular spaces were wide. Most of the cells showed prominent cell processes (cp). The arrowhead points to a large vesicle in the cytoplasm of an epithelial cell, which apparently resulted from the fusion of smaller membrane-bound vesicles. **B:** The ultrastructure of a differentiated epithelial cell of the ventral prostate on day 6. The cell is polarized and contains a basal nucleus and supranuclear organelles such as rough endoplasmic reticulum (rer) and Golgi complex (GC). Some secretory vesicles were observed in the apical region of the cytoplasm. The cell is tightly connected to neighboring cells through cell junctions. On the apical surface, the cell exhibits microvilli (mv) in contact with the lumen (asterisk). **C:** Detail of the apical portion of an epithelial cell on day 6 showing the presence of long microvilli (mv). **D:** Detail of an epithelial cell on day 6 showing a well-developed rough endoplasmic reticulum (rer). **E:** Ultrastructural aspects of the epithelium on day 3 showing a mitotic cell (arrowhead) close to the forming lumen. Note that lumen formation anticipates epithelial cell differentiation. **F:** The ultrastructural aspect of an agglomerate of cell nuclei within a single cytoplasm filled with an amorphous and compact substance. At least one of the nuclei showed an irregular outline (arrow). **G:** The ultrastructural aspect of the forming lumen on day 4. Note the presence of some membrane-bound vesicular structures, some of them continuous to the apical cell membrane, and other material in the lumen (asterisk). The presence of microvilli (mv) suggests that the epithelial cells in this region are at least partially differentiated. **H:** On day 5, the epithelium in the proximal ductal regions was composed of a single layer. The cells showed most of the differentiation aspects. The lumen (asterisk) contained different structures. **I:** An aspect of a distinct cell (arrow) in the epithelium of a prostate of a 4-day-old rat. Note the presence of abundant vesicles and polarization of the cell that extended from the basement membrane to the lumen (asterisk). Note also that this cell is apparently phagocytosing another cell (arrowheads). Ep, epithelium; S, stroma. Scale bars = 2 μm in A,B,H, 0.5 μm in C, 1 μm in D, 5 μm in E,F, 2.5 μm in G.

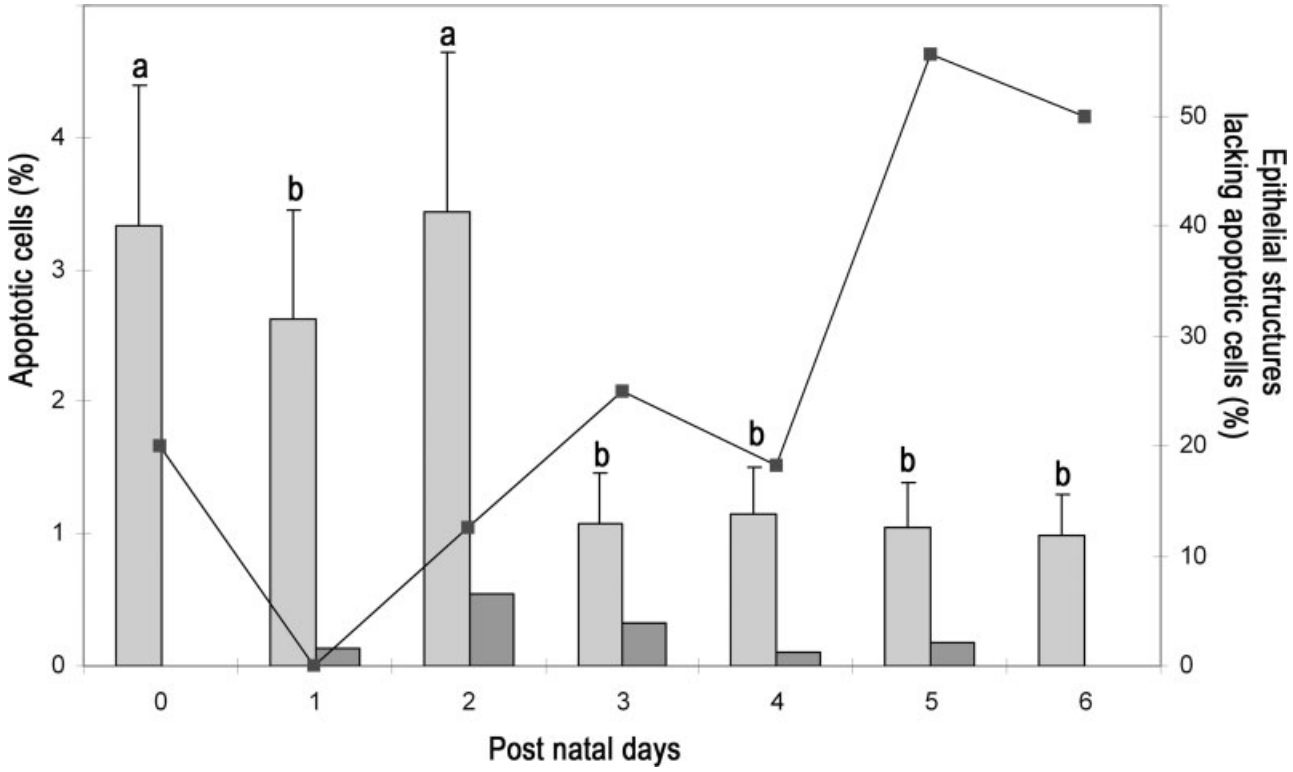

Fig. 5. Quantitative aspects of apoptotic cells in the early postnatal development of the rat ventral prostate. Apoptotic cells were identified by the characteristic morphological aspects shown in Figure 2. They were counted with respect to the total epithelial cells (light gray bars). The number of apoptotic cells in contact with the basal lamina was also determined and shown to contribute very little to the total amount of apoptotic cells (dark gray bars). The figure also shows the percentage of epithelial structures appearing in the histological sections that were devoid of apoptotic cells (black line), which amounted to approximately 50% by postnatal days 5 and 6. Different letters indicated differences between the groups after analysis of variance and Tukey's multiple comparison test.

cell death detection is likely associated with the fact that canalization is concentrated on PND 0 to 2 and/or that the dying cells are quickly lost in the lumen and eliminated from the organ later on.

Apoptosis has also been shown to be involved in the canalization of the mammary (Sunil et al., 2002) and submandibular salivary glands (Jaskoll et al., 2001), in which the deletion of some epithelial cells gives rise to the space occupied by the lumen. Apoptosis of interstitial cells plays a role in the remodeling mechanisms of the developing fetal lung to achieve the mature alveolar structure (Scavo et al., 1998), and in the proposed mechanism of ureter lumen formation and maturation (Kakuchi et al., 1995). Similarly, lumen formation by mammary acinar cells in culture has been shown to depend on apoptosis (Debnath et al., 2002). In this system, the apoptotic cells appear in the lumen unattached to the extracellular matrix, while the surrounding surviving cells produced and were attached to extracellular matrix (Debnath et al., 2002). The basic mechanisms underlying the control of cell death during development include the direct initiation of apoptosis by an external signal, the absence of trophic factors, and/or the response of a cell to conflicting signals (Coucouvanis and Martin, 1995). Despite this proposition and although they occur in different models, the molecular events regulating the elimination of cells by apoptosis to create a lumen are poorly understood, and certainly result from complex cell fate decisions.

Another important aspect of the early postnatal development in the rat ventral prostate is that the epithelial canalization occurred at different distances from the urethra, in contrast to previous suggestions that this would happen in a proximal-to-distal manner (Marker et al., 2003). However, this is the case after PND 3, when the lumen was consolidated proximally, and growth consists only in elongation of previous canalized structures.

The importance of cell deletion in the canalization process was also demonstrated by figures of mass deletion of nondifferentiated cells in the forming canal. Although these cells showed no features of apoptosis, they seemed to correspond to dying cells, especially because they were not preserved during prostate development.

The relatively high mitotic index as observed in the present study is in accordance with results of a previous report (Weihua et al., 2002). Moreover, mitotic cells in direct contact with the canalization regions were observed, indicating that cell division might be related to luminal growth and consolidation. These mitotic cells may only represent epithelial cells in a late process of cell division. Redistribution of vital cells has been suggested to be responsible for the opening of spaces in the

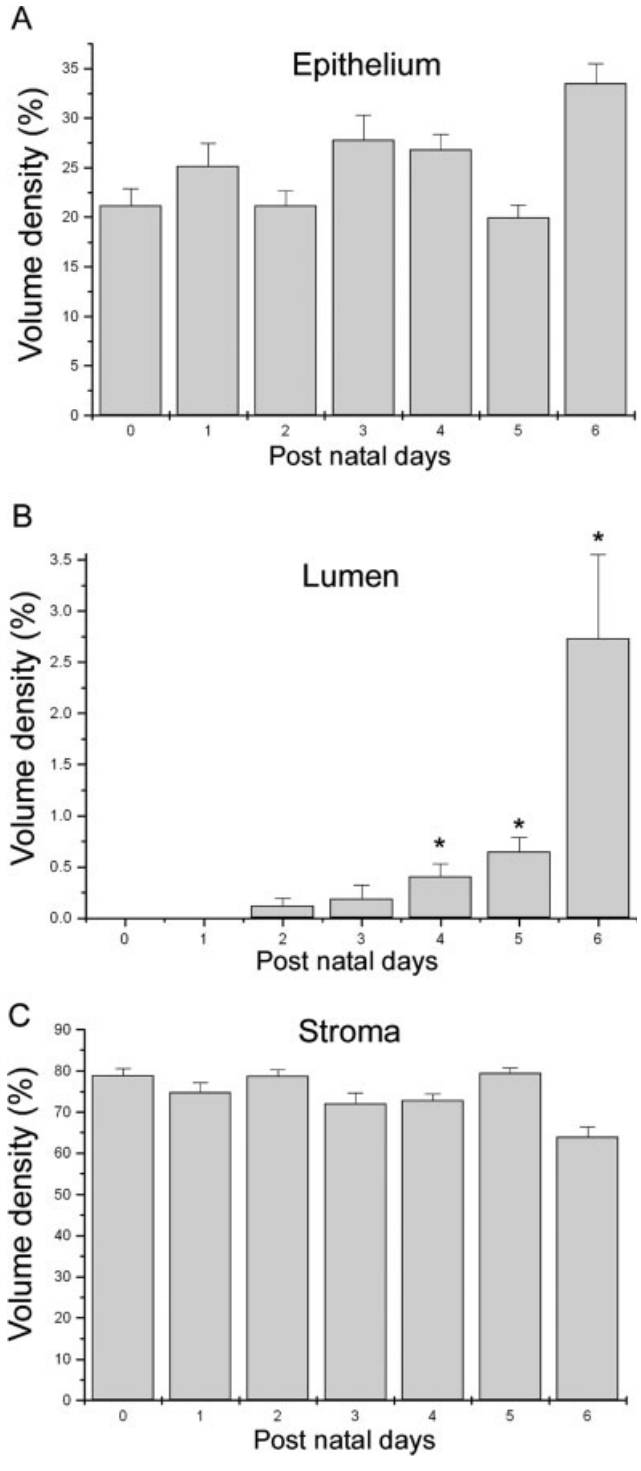

Fig. 6. Stereological analysis of the rat ventral prostate during the first postnatal week. **A:** Volume density of the epithelium. **B:** Volume density of the lumen. **C:** Volume density of the stroma. Values are expressed as mean ± SEM. No significant difference was observed in epithelial or stromal volume density. The asterisks in B point to significant differences in the volume density of the lumen compared with day 2 (Tukey's multiple comparison test).

developing middle ear, since mitotic figures were detected in the mouse middle ear as cavitation occurs (Van de Water et al., 1980). It is interesting that proliferation during the first postnatal week was not restricted to the epithelial tips, as it was shown to occur at later stages of postnatal development of the prostate (Sugimura et al., 1986b).

Different structures were observed in the forming lumen and may also be involved in the canalization process. Of interest, the cavitation of encephalic structures involves cell polarization and the production of negatively charged molecules (glycosaminoglycans), which, once in the cavity, attract water and help in expanding the cavity (Gato et al., 1993).

The lumen appeared simultaneously with the process of differentiation of the epithelium (cell polarization, appearance of secretory organelles, and microvilli), suggesting that both processes are directly related. It is important to realize that the major structural modification during the first postnatal week is canalization, as stereology showed no significant changes in the volumes of epithelium and stroma, which appears to take place later on (Donjacour and Cunha, 1988; Hayward et al., 1996b; Vilamaior et al., 2006).

In conclusion, the canalization process of the rat ventral prostate is the result of complex cell fate decisions involving epithelial cell proliferation, differentiation, and apoptosis. Although these seem to be the main events during canalization, other mechanisms such as the production and fusion of membrane-bound vesicles inside the epithelial cells and the secretion of molecules into the forming lumen might contribute to this process.

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