Postnatal Growth of the Ventral Prostate in Wistar Rats: A Stereological and Morphometrical Study

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ABSTRACT

Morphological and stereological analyses were used to characterize the growth kinetics of the Wistar rat ventral prostate (VP). Volume density and absolute volume of the epithelium, lumen, smooth muscle cells (SMCs), and nonmuscular stroma were determined by stereology and paired with plasma testosterone levels and different morphometric measurements. The VP shows an initial growth within the first 3 weeks, a resting phase, and the puberal growth. The puberal growth was coincident with the raise in plasma testosterone. Lumen formation occurred within the 3 postnatal weeks. After an expected increase during puberty, the lumen showed a further increase at the 12th week. The volume density of the nonmuscular stroma and of the SMCs decreased slowly postnatally. Absolute volume of the luminal compartment showed three phases of growth (weeks 1–3, 6–9, and 11–12). On the other hand, the increase in the absolute volume of the epithelium was steady up to the 8th week and then showed a marked increase up the 10th week. The increase in epithelial volume was characterized morphologically by the presence of epithelial infoldings and sprouts. The growth of the epithelium showed a 2-week delay as compared to the lumen and occurred only until the 10th week. The epithelial height was variable but could be related to the synthetic activity of the epithelium. In conclusion, the postnatal growth of the VP results from a combination of epithelial proliferation/differentiation and synthesis/accumulation of the secretory products in the lumen. Anat Rec Part A, 288A:885–892, 2006.

Key words: epithelial growth; prostate development; prostate secretory activity; rat ventral prostate; stereology

Prostatic morphogenesis is not determined by the genetic sex, but by exposure to androgens. It is dependent on the androgen production by the testis in the fetus (Pointis et al., 1980; Cunha et al., 1987) and androgen insensitivity hinders prostatic development. It has also been demonstrated that the urogenital sinus of female and male may form functional prostatic tissue if it is properly stimulated by androgen at the correct embryonic stage (Takeda et al., 1986).

Epithelial budding of the rodent prostate is initiated at about the 17.5 day postcoitum (Timms et al., 1994). Even though testosterone is the androgen produced by the fetal testis, dihydrotestosterone (DHT) is responsible for the prostatic morphogenesis (Taplin and Ho, 2001). DHT is produced by the urogenital sinus by the enzyme 5-α reductase. This enzyme has been detected in the urogenital

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sinus and external genitalia of rats, rabbits, and humans (Wilson et al., 1983) and its deficiency results in the anomalous growth of the external genitalia and complete absence of the prostate.

The epithelial differentiation in the prostate takes place in parallel to the development of the stroma. Androgens act on androgen receptors in the urogenital mesenchyme to induce epithelial proliferation, ductal branching, and differentiation of the epithelial cells (Cunha et al., 1987, 1992; Marker et al., 2003). In turn, the developing epithelium directs the differentiation of the smooth muscle (Hayward et al., 1998). Neither epithelium nor smooth muscle is capable to develop in the absence of each other (Hayward and Cunha, 2000). During organogenesis, one of the functions of the androgens is to maintain the smooth muscle that surrounds the urethra as a thin layer (Thomson et al., 2002). If the smooth muscle layer is thick, as it occurs in the female, the initial budding from the urethra cannot reach the urogenital sinus mesenchyme and there is no development of the prostate gland (Thomson et al., 2002).

The subsequent ductal morphogenesis, canalization, and epithelial differentiation also depend on androgens and take place in association with a transient perinatal increase in the serum testosterone concentration (Donjacour and Cunha, 1988).

The neonatal prostate is sensible to androgens. Testosterone administration accelerates prostatic growth, and adult size may be reached much earlier (Berry and Issacs, 1984). On the other hand, neonatal castration inhibits pubertal prostatic growth and development, and this effect may be reversed by testosterone (Cunha et al., 1987; Corbier et al., 1995). During puberty, there is an additional increment of the prostatic weight and a slight increase in the number of ductal branches (Sugimura et al., 1986). This suggests that the prostate is sensitive to the low levels of androgens for the ductal branching and that the response to the increasing levels of testosterone during puberty is different from the initial response (Hayward and Cunha, 2000).

A detailed study of the postnatal growth considering the different tissue compartments is still lacking for the rat ventral prostate, while it has been extensively studied in the mouse (Singh et al., 1999). Furthermore, the rat ventral prostate has also been demonstrated to be adequate for the testing of endocrine disruptors, given its extreme dependency on androgen stimulation and high susceptibility to estrogenic stimulation (Prins et al., 2001; Putz et al., 2001a, 2001b).

Given the usefulness of rat ventral prostate in environmental risk assessment, the demonstration of strain-specific variations, the sensibility of prostatic development and function to androgen stimulation, and disturbance by steroid compounds, we have found it reasonable to undertake a detailed analysis of the prostatic development in Wistar rats, trying to define morphological and stereological parameters for the main tissue compartments from the first postnatal week to adulthood.

Pursuing this task, we have employed a series of analyses, which demonstrated the differential kinetics of the prostatic compartments in postnatal life and an alternation between proliferative and secretory states of the epithelium that culminates in prostatic growth.

MATERIALS AND METHODS

Animal Protocol

Sixty male Wistar rats were obtained from Centro Multidisciplinar para Investigação Biológica (CEMIB)/State University of Campinas. Groups of five animals were used for each time point and for the different analyses. They were maintained in a controlled environment with free access to food and water. Experiments were performed according to the *Guide for Care and Use of Laboratory Animals*. Rats were weighed and sacrificed by cervical dislocation. The ventral prostate was carefully dissected out, weighed, and fixed.

Serum Testosterone Quantification

Blood samples were collected either after decapitation or cardiac punction. Serum testosterone was quantified using a modular chemiluminescence immunoassay analyzer ECi (Johnson and Johnson) according to Weeks and Woodhead (1984). Triplicate measurements were performed for each sample and serum samples from three animals were used for each time point and serum samples were assayed in duplicate. The sensitivity of the test was 0.94 ng/dL. The intra-assay and interassay variations were 5.36% and 5.10%, respectively.

Histology

The ventral prostate was immediately fixed by immersion in 4% formaldehyde in phosphate-buffered saline (PBS) for 24 hr. Samples were then washed, partially dehydrated, and embedded in Leica historesin. Two micrometer sections were obtained and stained with hematoxylin and eosin (Behmer et al., 1976).

Stereological Analyses and Morphometry

Six microscopical fields from the hematoxylin and eosin-stained sections from three animals for each group were photographed and subjected to stereology using Weibel's system and a 168-point grid as applied to the ventral prostate by Huttunen et al. (1981) and employed previously (Garcia-Florez et al., 2005). The volume density (Vv) of the epithelium, lumen, smooth muscle, and nonmuscular stroma was determined. The nonmuscular stroma corresponded to everything besides the epithelial structures but the smooth muscle cells (Garcia-Florez et al., 2005). The total stroma was the sum of the smooth muscle and nonmuscular stroma. The volume (or absolute volume) of each of these compartments was determined by multiplying the volume density by the mean prostatic weight based on the determination that 1 mg of fresh rat ventral tissue had a volume of approximately 1 mm^3 (DeKlerk and Coffey, 1978).

The epithelial cell height was measured on hematoxylin and eosin-stained sections using the Image Pro Plus software (Media Cybernetics, Silver Springs, MD) after digitalization of the microscopical images. Calibration was done using an Olympus graded microscopical slide. Seventy-five measurements were done for each experimental point.

Mitotic Cell Frequency

Historesin sections were subjected to the Feulgen's reaction. They were hydrolyzed with 4 N HCl for 1 hr 15 min and then reacted with Schiff's reagent for 40 min, followed

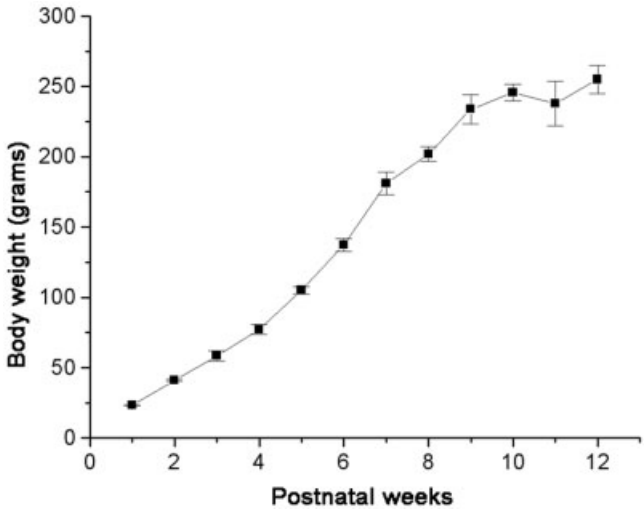

Fig. 1. Body weight of normal rats during the first 12 postnatal weeks. Values are in grams (mean ± SEM; n = 5).

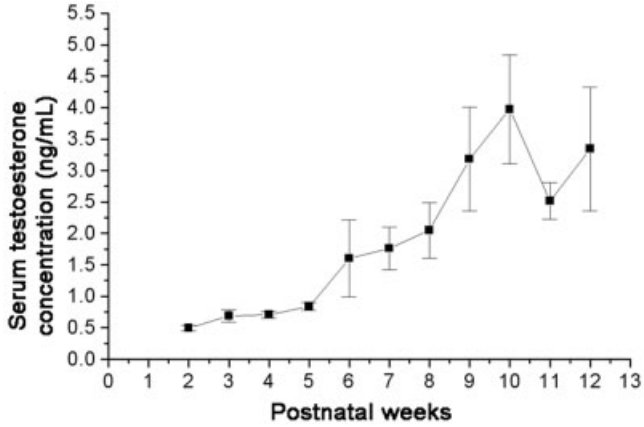

Fig. 2. Plasma testosterone concentration (ng/mL) along the 12 postnatal weeks (mean ± SEM; n = 5).

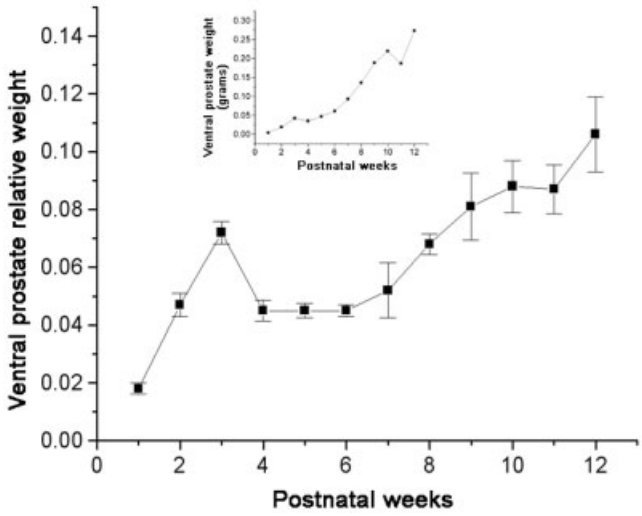

Fig. 3. Weekly variation in absolute and relative weight of rat ventral prostate from the 1st to the 12th week of postnatal development. Organ absolute weights are represented as grams (inset) and relative weights are represented as the ratio of prostatic to total body weight (mean ± SEM; n = 5).

by extensive washing, dehydration in ethanol, and mounting in Canada's balsam (Márquez et al., 2001). Forty fields were taken at random and the mitotic figures were counted (15 fields per animal; three animals).

RESULTS

Body Weight and Testosterone Levels

There was a continuous increment of body weight in the rats employed in this work (Fig. 1). This growth continued from the first to the ninth week and plateaued at about 250 g. The serum testosterone concentration was low (~ 0.75 ng/mL) until the fifth week, raised to 1.5–2.0 ng/mL from the sixth to the eighth week, then leveled at ~ 3.5 ng/mL by the ninth week (Fig. 2). A greater variation in the serum testosterone level was observed in the early adult life, as demonstrated by oscillation of the mean concentration, as well as by the larger SEM.

Ventral Prostate Growth and Histological Modifications

The ventral prostate showed an initial postnatal growth, a resting period from the fourth to the sixth week, and a pubertal growth up to the adult size (Fig. 3). The resting period observed in the relative weight was not so evident when the absolute weight of the gland was taken into account (Fig. 3, inset), indicating the presence of a somatotrophic growth before puberty.

From the first to the third week, there was a definition of the lumen (Fig. 4a–c), which was filled with secretory material. The formation of the lumen was coincident with the differentiation of the epithelial cells, which formed mostly solid cords by the first week (Figs. 4a and 5a) and became progressively polarized and organized in a single layer (Figs. 4b and c and 5b). The stroma was reduced and exhibited decreased cell density. From the 3rd to the 6th weeks, no change in histological organization was evidenced (Fig. 4d–f). However, by the sixth week, we observed the presence of some images of epithelial infoldings and sprouts. These latter became more evident later on (Figs. 4f–i and 5c and d) and consisted of projections of groups of epithelial cells toward the stroma. Smooth muscle cells (SMCs) were excluded from their surface but fibroblasts were commonly observed. From the 10th to the 12th weeks, there was an engorgement of the lumen, with corresponding distension of the epithelium, i.e., disappearance of foldings and sprouts (Figs. 4j–l and 5e and f).

Variations of the epithelial height were noticed and quantified. The measurements of epithelial height are shown in Figure 8. After a progressive increase up to the sixth week, there was a marked drop in the mean epithelial height, followed by another increase to the maximum epithelial height. The polarized cell with a large, faintly stained supranuclear area (corresponding to the Golgi complex) was observed throughout (Fig. 5b, e, and f).

The volume densities of the different prostatic compartments (Fig. 6) confirmed some of the histological observa-

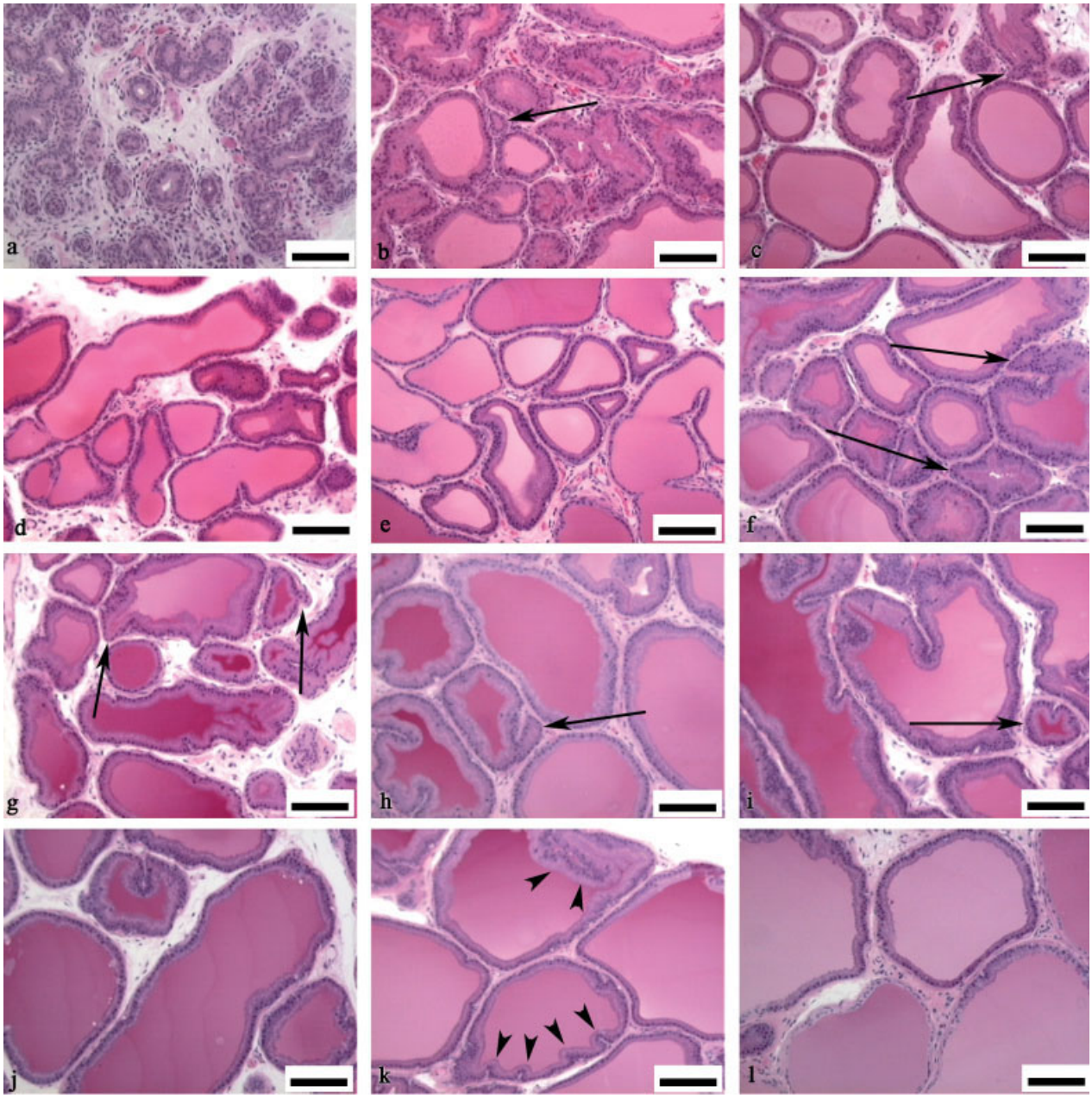

Fig. 4. Histological organization of rat ventral prostate from the 1st to the 12th week of postnatal development (**a–l**, respectively). The arrows indicate small alveoli or sites of epithelial sprouting. The arrowheads point to regions of epithelial infolding. Scale bars = 100 μm.

tions. The formation of the lumen took place within the first 3 weeks. The lumen then became the predominant compartment in the prostate. The proportion between the volume of the lumen to that of other compartments was maintained up to the eighth week, when it showed a further increase, reaching as much as 70% of the prostatic volume density. The volume density of the stroma decreased from the first to the second week, remained constant up to the eighth week, then decreased again from the eighth to the ninth week. The volume density of the smooth muscle cells showed a similar behavior. The second drop in the volume density of the stroma, which took place after the eighth week, counterbalanced with an increase in the volume density of the epithelium.

The estimation of the absolute volume for the different compartments clearly showed the contribution of both epithelium and lumen to the growth of the prostatic gland (Fig. 7). After the formation of the lumen within the first 3 weeks, there was a prominent growth of this compartment after the sixth week. This was then followed by an increase in the epithelial compartment, which was observed after the eighth week. The absolute volume of both

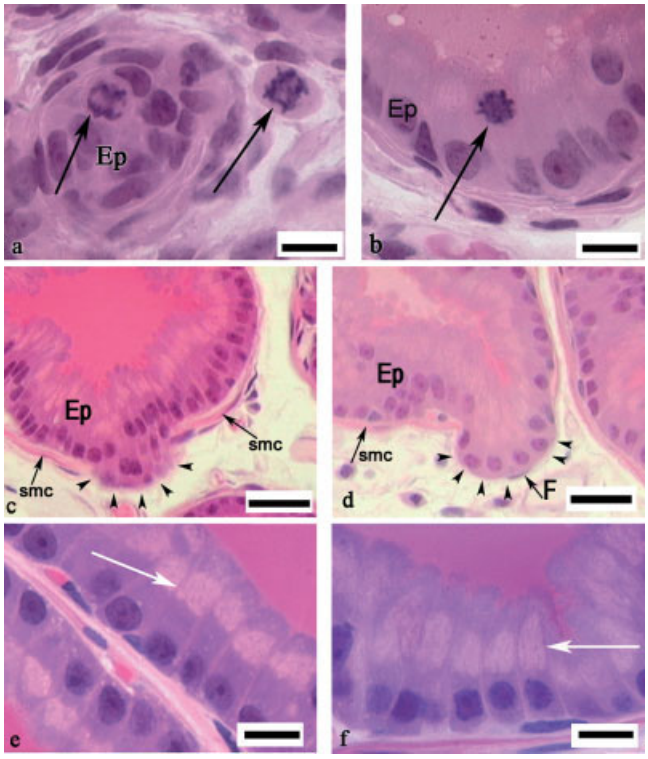

Fig. 5. Details of the prostatic epithelium during the postnatal development. **a:** The epithelium in the 1-week-old rat is composed mainly of solid cord. The arrows point to mitotic cells in the epithelium and stroma. **b:** By the second week, the proximal areas of the epithelium present differentiated cells and a completely formed lumen. The arrow points to a mitotic epithelial cell. **c** and **d** are aspects of epithelial sprouting observed at the sixth and seventh weeks, respectively. The arrowheads delineated the sproutings, which are characterized by epithelial projections toward the stroma. The SMCs are excluded from the sprouting area, while fibroblasts (F) were commonly seen in this region. **e:** The epithelial cells in the prostate of a 10-week-old rat are tall and organized in a single layer. They present a prominent supranuclear chromophobic area (arrow). **f:** The epithelial cells in the prostate of a 12-week-old rat preserve the well-developed supranuclear chromophobic area (arrow). Ep, epithelium. Scale bars = 10 μm (a, b, e, and f); 25 μm (c and d).

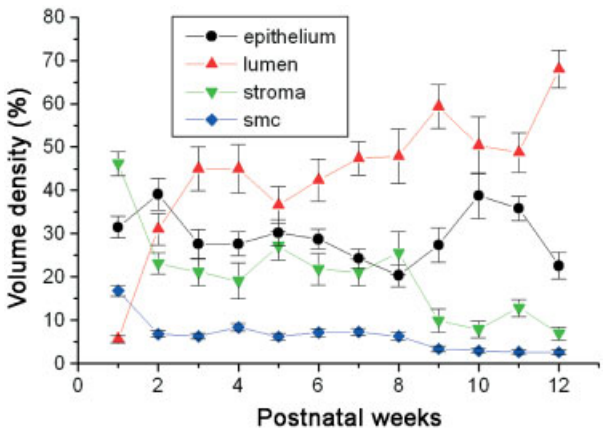

Fig. 6. Volume density variation for the different prostatic compartments (epithelium, lumen, stroma, and smooth muscle cells) along the postnatal development as determined by stereology (mean ± SEM; the number of animals employed was three).

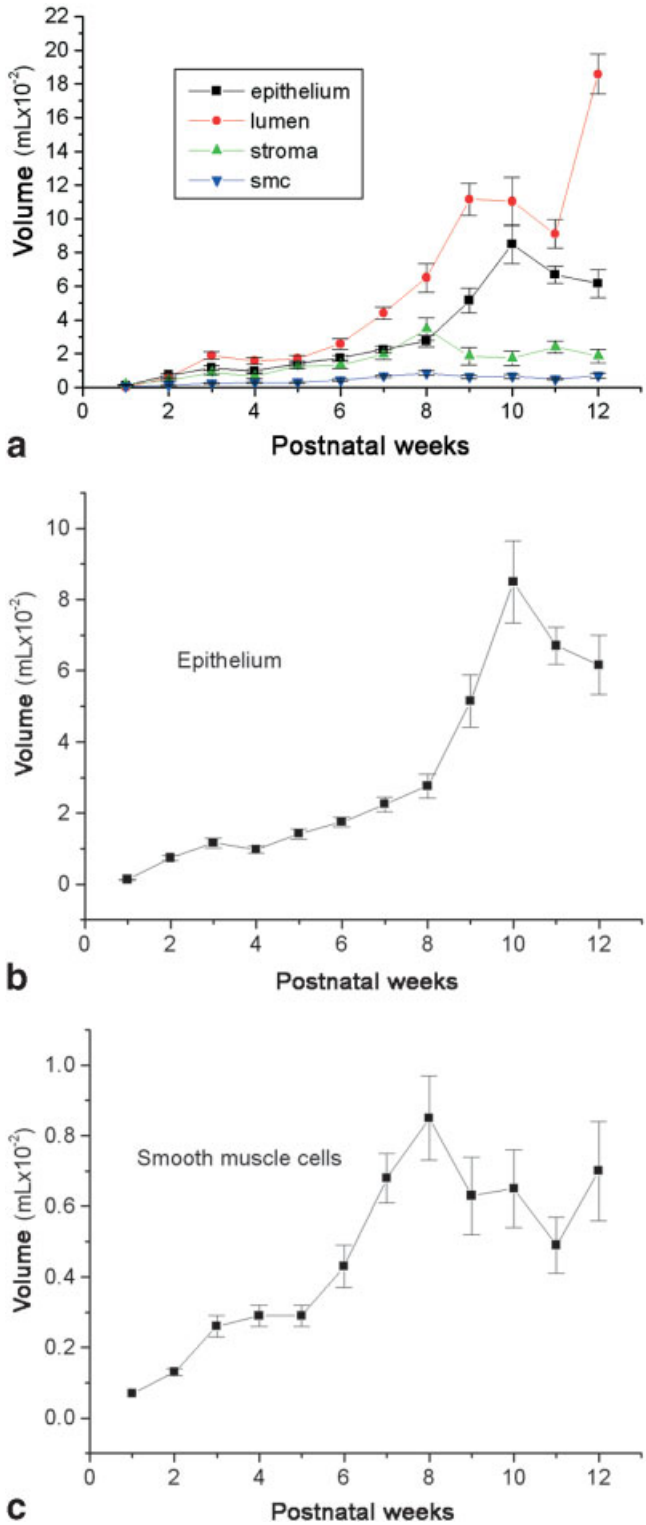

Fig. 7. Absolute volume variation analysis for the different prostatic compartments (epithelium, lumen, stroma, and smooth muscle cells) along the postnatal development as determined by stereology (**a**). **b** and **c** show expended views of the graph sections with data obtained for epithelium and smooth muscle cells (mean ± SEM; the number of animals employed was 3).

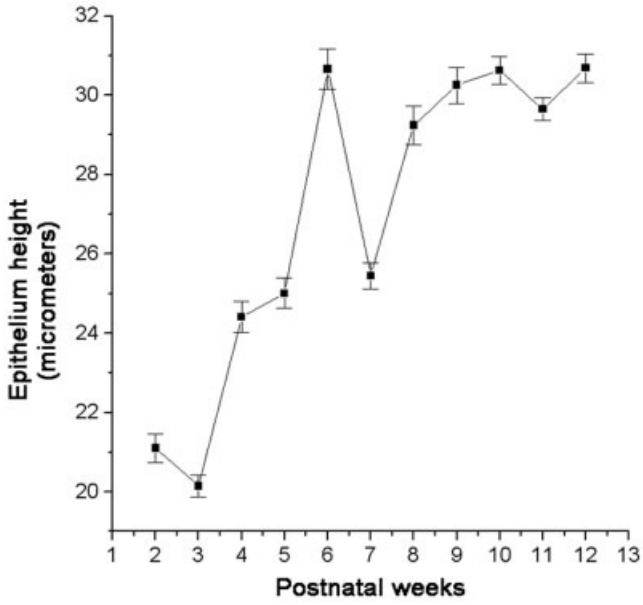

Fig. 8. Weekly variation in epithelial cell height (mean ± SEM values) of rat ventral prostate from 1st to 12th week of postnatal development. Values are in micrometers.

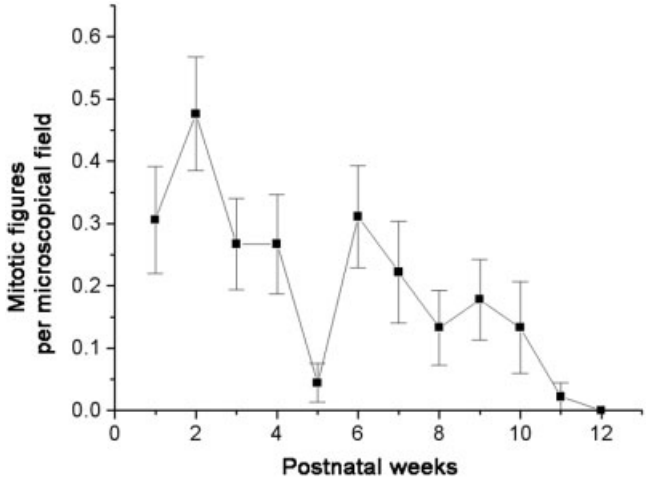

Fig. 9. Frequency of mitotic figures in rat ventral prostate sections along the postnatal development. Values correspond to the number of mitotic figures per microscopic field. (mean ± SEM) counted on 45 fields taken from three animals.

compartments plateaued at the 9th and 10th weeks, respectively, but the lumen showed a further increase at the 12th week. The smooth muscle cells also showed a contribution to the overall increase in prostatic volume, with a pattern very similar to the epithelium, but anticipating it, with the pubertal growth beginning at the sixth week.

Figure 8 shows the variation in epithelial cell height along the 12 postnatal weeks. Increases in epithelial heights started at the fourth postnatal week, preceding the increase in testosterone concentration in the plasma.

Cell Proliferation

The frequency of mitotic cells was also evaluated. The number of mitotic cells per microscopical field showed an initial peak at the second postnatal week and a second one at the sixth week. This second peak showed a slow decline up to the 10th week. Mitotic cells were rare at the 5th, 11th, and 12th weeks (Fig. 9).

DISCUSSION

This article presents a detailed analysis of the postnatal growth of the rat ventral prostate. It was demonstrated that the ventral prostate (VP) grows progressively after birth. Since most of the prostatic growth in rodents occurs after birth, the prostate is adequate for studies of occupational exposition to a series of steroidal compounds. In this sense, the adequate characterization of prostatic development seems necessary, specially to avoid strain-specific variations (Putz et al., 2001a). Furthermore, the postnatal growth is complex, and we thought that the characterization of growth kinetics of the different tissue compartments could contribute to the knowledge of prostatic physiology.

After the early postnatal increase in prostatic weight, a resting period was observed between the fourth and sixth week, in which the growth follows the general body growth and precedes the pubertal growth initiated at the seventh week. As expected (Banerjee et al., 1994), testosterone sets the growth response of the organ. In addition, it was observed that prostatic size increases in parallel to the overall body weight, indicating a somatotrophic regulation.

A remarkable phenomenon taking place within the first 3 weeks is the formation of the lumen, which then becomes the predominant tissue compartment of the organ. This aspect, as well as the proliferative response and branching of the epithelium, results from testosterone surge, taking place immediately after birth (Corbier et al., 1995). It is interesting to note that the peak of prostate growth at the third week occurs 1 week after the observed peak of cell proliferation, demonstrating that cell growth and the accumulation of secretory material in the lumen contribute to the observed increase in weight. The epithelial proliferation observed within the first 3 postnatal weeks reproduced a similar event reported before for the mouse (Weihua et al., 2002).

The volume density of the epithelium was kept approximately the same until the eighth week. Thereafter, there was an increase in the epithelium, which is compensated by an equivalent reduction in the volume density of the stroma. These two events revealed a progressive involvement of the gland with secretory activity. They also illustrates that the VP shows a two-phase response to the increasingly testosterone levels during puberty. First, there is a clear activation of the secretory activity, manifested by the increase in the absolute volume of the lumen, then a delayed increase in the volume of the epithelium, which takes place 2 weeks later. The variation in the absolute volume confirmed these observations.

To our understanding, this growth pattern is due to two rather distinct responses. The raising testosterone levels stimulate the secretory activity of the existing epithelial cells. The growth of the lumen is immediate, given the accumulation of secretory material produced by the epithelium. Then, the same rise in plasma testosterone stim-

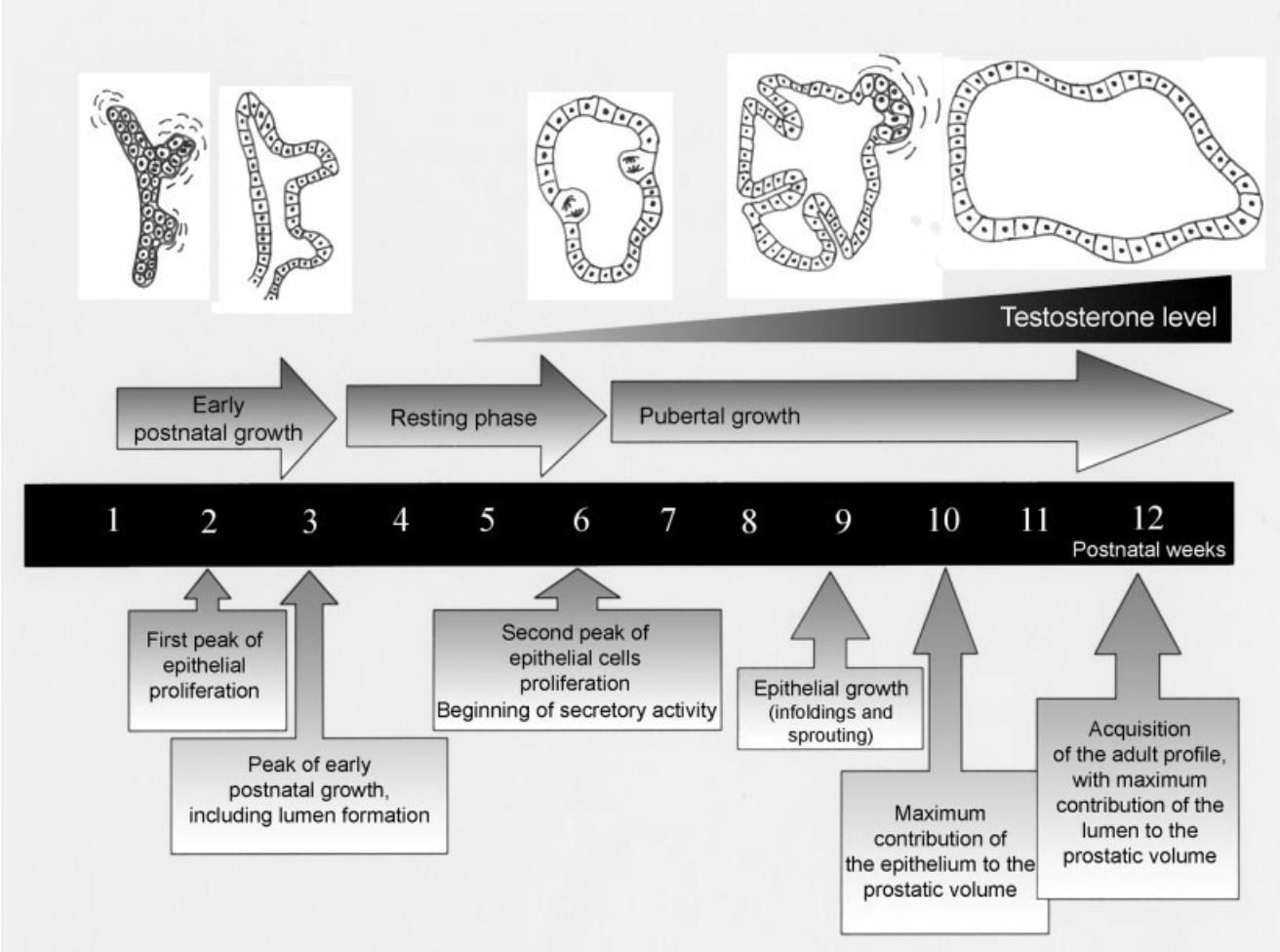

Fig. 10. Schematic diagram of the selected events detected during the 12 weeks of rat ventral prostate development.

ulates a second set of (basal?) epithelial cells to proliferate and then to differentiate. Proliferation and differentiation of the epithelium occurs within the 2-week period between the increase in the lumen and the manifested increase in epithelial volume.

It may also be considered that the increase in epithelial volume is initiated at the sixth week by the proliferative response of the epithelial cells and only observed 2 weeks later, after their growth and differentiation. This is suggested by the peak of mitotic activity in the epithelium at the sixth week, coincident with the rise in testosterone concentration.

This pubertal peak of cell proliferation is secondary to a higher peak observed at the second week. The pattern of mitotic activity demonstrates that the two main phases of prostatic growth results at least in part from epithelial cell proliferation. The occurrence of mitotic cells correlates well with previous findings on the distribution of 3H-thymidine incorporation in the ventral prostate of Sprague-Dawley rats (Banerjee et al., 1991), which showed an increase in proliferating cells at about the sixth week (45 days) in the intermediate region, but not in the distal region. Even though we have not distinguished between the ductal regions, these findings allow the conclusion that the two main phases of prostatic growth results at least in part from epithelial cell proliferation.

Another important observation of this work was the remodeling of the epithelium in response to the increased cell number. It seems that the new cells are organized as both epithelial infoldings and sprouts. Both structures appear as intermediated states of epithelial organization, since they virtually disappeared by the eighth week. We suggest that these structures are resolved by distension of the epithelial acini, likely in response to the accumulation of secretory material in the lumen. However, sprouts seem to require localized stromal reorganization, as they project toward the stroma. The exclusion of SMCs from the sprout region seems to correlate with this stromal remodeling. Further studies are necessary to evaluate these changes and to determined their similarity to the budding taking place much earlier.

It seems that the accumulation of secretion in the lumen results in a marked predominance of this compartment at the 12th week. This in turn results in a decrease of the epithelial compartment, perhaps by a negative effect of the accumulated secretion on the synthetic activity. How-

ever, there was not any variation in the epithelial height from the 9th to the 12th week. As mentioned above, this increase in the lumenal volume is associated with the disappearance of the epithelial infoldings and sprouts observed at the seventh week.

The present results then suggest that prostatic growth is characterized by a two-phase phenomenon. First, there is a proliferative response with the formation of epithelial infolding and/or sprouting. Second, there is a marked increase in the accumulation of secretion in the lumen due to the stimulation of the synthetic activity. This biphasic growth pattern was also observed for the female Mongolian gerbil prostate after experimental testosterone administration (Santos et al., 2006).

However, the present results suggest that the pubertal growth of the ventral prostate of male rats is characterized by a secretory-proliferative-secretory response of the epithelium to the rising serum testosterone concentration.

It is worth mentioning that the absolute volume of the smooth muscle cells also increases during puberty and decreases again after the eighth week. We cannot ascertain at the moment whether this variation reflects a variation in the number of the smooth muscle cells, the hyperthrophy of preexisting cell, or a combination of both. The definition of the factors involved in this increase in the smooth muscle cell absolute volume is certainly important for the characterization of the VP physiology and will require further examination. Figure 10 summarizes the main events of the postnatal development of the rat ventral prostate.

Finally, this work will certainly be useful for the investigation of factors affecting prostatic growth as they define morphological and stereological parameters that might be considered for future studies at the cellular and molecular levels, as well as during testing of endocrine disruptors.

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