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Stromal Fibrosis Reaction in Rat Prostates Induced by Alpha 1 Adrenergic Stimulation

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ABSTRACT: Most of the publications dealing with the experimental induction of prostatic neoplasia have focused on the description of epithelial lesions, but little attention has been paid to the involvement of their stromal alterations. The present study is a first attempt to assess the stromal changes in both collagen and elastic fibrils as well as in its cellular constituents, which accompany prostatic intraepithelial neoplastic (PIN)–like lesions induced by phenylephrine (PE) in rats. Adolescent rats received subcutaneous injections of PE daily (10 mg/kg/d) for 1 month. At the end of the experimental period the rats were sacrificed; the dissected ventral prostates were fixed in Stieve solution and paraffin-embedded; and sections were cut and stained accordingly. Most of the stromal cells were identified by immunohistochemistry techniques using primary antibodies to ED2 (resident macrophages), actin (fibrocytes and vascular smooth muscle cells), vimentin (mesenchymal cells), and 5′-bromo-2′-deoxyuridine (S-phase proliferating cells). Collagen stromal mass was visualized by Gomori trichrome and individual collagen fibers by picrosirius red staining under polarized light, whereas the fine fibrils were stained according to the Pinkus method. The untreated rat prostates are characterized by a delicate interacinar stroma with scanty cells and fibrils. The PE-treated prostates showed a significant increase in both cellular and fibrillar elements as well as an increase in arteriolar density, in addition to the typical PIN lesions. The presence of such an interstitial fibrosis, which also includes inflammatory cells, neoangiogenesis, and synthesis de novo of collagen and fibers, might be regarded as a desmoplastic reaction. It is suggested that these changes could be related to a tissue repair process occurring subsequent to the inflammatory exudate that takes place during the incipient phases of the PE treatment.

Key words: Phenylephrine, prostatic intraepithelial neoplastic, neoangiogenesis, collagen, prostate.

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The rat ventral prostate is a ducto-acinar gland with a delicate interacinar stroma. The latter contains a vasculo-nervous network, a few smooth muscles and fibrocytes (Scolnik et al, 1994; Nemeth and Lee, 1996), as well as negligible amounts of mononuclear leukocytes (Rosenzweig et al, 2004).

The close relationship between epithelium and stroma enables the transition of many growth factors, neurotransmitters, neuromodulators, cytokines, hormones, and the like, producing paracrine or autocrine effects that lead to a mutual crosstalk (Chung, 1995; Chung and Davies, 1996). As can be expected, epithelial-stromal interaction plays an important role not only in the normal development of the prostate but also in its tumorogenicity (Cunha, 1994; Chung and Davies, 1996; Wong and Wang, 2000).

Human prostatic stroma smooth muscle cells and blood vessels express various subtypes of adrenergic receptors (Lepor et al, 1993; McVary et al, 1998). We have previously shown that daily administration for 1 month of phenylephrine (PE), an alpha-1 adrenergic analog, induces atypical prostate hyperplasia in rats (Golomb et al, 1998) resembling those of prostate intraepithelial neoplasia (PIN) in men (Bostwick et al, 1993; Epstein, 1995). These lesions were also accompanied by mild fibrosis and a few inflammatory cells (Golomb et al, 1998). Chronic inflammation of longstanding duration has been linked to the development of prostatic carcinoma and proliferative inflammatory atrophy of the human prostate (De Marzo et al, 1999). Recently we defined the evolutionary progression of the PIN lesions, as induced by PE in rats, from 8 hours postinjection until 14 days of treatment (Rosenzweig et al, 2004). It was found that PE induced a biphasic reaction: a subacute inflammatory exudate that took place in the prostate stroma but that was partially resolved later on. The presence of this inflammatory exudate preceded the epithelial changes that evolve into PIN lesions. In addition, resident macrophages and mast cells, known for their capacity to secrete various growth factors and cytokines, were also found in elevated numbers in the rat prostate (Rosenzweig et al, 2004).

Most of the histopathological studies on pharmacological induction of benign prostatic hyperplasia (BPH) or PIN in rats have so far focused on the epithelial proliferative lesions rather than on the stromal ones. To the best of our knowledge, the behavior of various stromal ele-

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ments has not been reported as yet in the experimental induction of neoplastic events in the rat prostate. However, studies on connective tissue constituents (fibrocytes, myofibroblasts, collagen, and elastic fibers) in human prostate stroma were reported in both normal and carcinoma patients, in whom the stromal mass is predominant and much denser (Chung and Davies, 1996; Chagas et al, 2002; Zhang et al, 2003) than in rodents (Scolnik et al, 1994; Nemeth and Lee, 1996).

To better understand the microenvironmental role of prostate stroma in the initiation and promotion of aberrant epithelial growth, we have investigated the pathogenetic evolution of major connective tissue fibrillar and cellular component changes by quantitative histochemical and immunohistochemical (IHC) methods following PE-induced PIN lesions and have compared these within control rats.

Materials and Methods

Experimental Animals

Wistar adolescent rats (45 days old) were maintained under standard laboratory conditions with a 12-hour light-dark cycle and isothermal conditions of 22°C–24°C; rats were permitted free access to chow pellets and tap water. The experiment was approved by the University Committee for the Humane Use of Laboratory Animals.

The animals were randomly divided into 3 groups, comprising 1 experimental (9 rats) and 2 control (4 rats each) groups. The experimental group was treated daily with PE (Sigma Chemical Co, Rehovot, Israel) dissolved in saline (10 mg/mL), subcutaneously injected at a dose of 10 mg/kg body weight (BW)/d for a period of 30 days. The control groups consisted of untreated rats and those that received an equivalent volume of saline for the same period of time. Since no histological differences were encountered between these 2 groups, they were unified into 1 control group.

For the assessment of the cellular proliferation rate, a single dose of 5′-bromo-2′-deoxyuridine (BrdU; 50 mg/kg BW) (Sigma) was injected IP 48 hours prior to the end of the experimental period (Yu et al, 1992).

Histopathology Processing

The rats were euthanized with an overdose of pentobarbital (IP). The ventral prostate was excised and fixed overnight in Stieve solution at 4°C (Lillie, 1965). Afterward, the samples were histologically processed, and paraffin-embedded sections were stained by Harris hematoxylin-eosin for pathological diagnosis. In addition, toluidine blue staining for identification of the mastocyte metachromasia was used (Sheehan and Hrapchak, 1980). For a general examination of the prostatic stroma, the Gomori trichrome stain was used (Lillie, 1965). To obtain evidence of fine elastic fibrils, the Pinkus method was used (Pinkus and Hunter, 1960). The quantitative analysis of the collagen fibers was performed using picro-sirius red (PSR) stain visualized under polarized light (Junqueira et al, 1979), using cross-Nicol prisms (Olympus, Tokyo, Japan). A spectra of colors ranging from red-orange to yellow-green micropatches (Junqueira et al, 1979; Whittaker et al, 1994) was noted. For the quantitative analysis, the frequency of each color spot was established (see ''Statistical Analysis'' section).

IHC Staining

The stromal cells were visualized by IHC methods as follows: deparaffinized and rehydrated tissue sections were blocked for endogenous peroxidase using a mixture of methanol 3% H_2O_2 solution. Antigen retrieval was achieved by heating in a programmable microwave (Amana, Newton, Iowa). The sections were placed in 0.1 M citric acid buffer solution (pH 6.5) and microwaved twice (5 minutes each time) in a pressure cooker (Nordic Ware, Minneapolis, Minn).

For the characterization of the stromal cells, the following antibodies at their respective dilutions were used: for fibrocytes and myofibroblasts, both mouse monoclonal anti-vimentin (1:100; Novocastra, Newcastle, United Kingdom) and goat polyclonal anti–actin isoforms of broad range (1:75; Santa Cruz, Calif); for monocyte-derived macrophages, mouse monoclonal anti-ED2 (1:50; IQ Products, Groningen, The Netherlands); for the identification of S-phased proliferating cells, monoclonal anti-BrdU (1:200; Dako, Glostrup, Denmark). For the immunoreaction, biotinylated secondary antibodies (rabbit anti-goat, goat anti-mouse; Sigma) and the HRP-avidin system (Zymed, Calif) were used. The color reaction was detected by DAB-H_2O_2 substrate (Zymed), according to manufacturer protocols. For each immunoreaction, negative controls of adjacent sections were performed by omitting either the primary antibody or both primary and secondary antibodies.

Statistical Analysis

The comparative quantitative assessment for positive immunoreactive cells was performed for each histological section on 4 different, randomly chosen fields. Thereafter the individual values were regrouped and their mean values were calculated. For cell examination, a 200× final magnification was used. Cell identification was conducted according to their positive immunoreactivity, and morphological patterns and their number per unit surface area (1 mm^2) were assessed.

Similarly, the color spectra from polarized PSR-stained sections were taken by Olympus DP-50 digital camera using a 10× objective. Color separation of the red, orange, and yellow collagen fibers was performed using the Adobe Photoshop 7.0 ME. For the quantitative study, the selected colors were copied to another file to generate a new gray-scale picture (Tiff format), which was examined using NIH image 1.63 software. In order to avoid interference with the broken cells or fibrils and, eventually, the microscopic dust particles, a minimal reliable unit of 15 pixels per particle size was chosen. The sum of pixels of a given color was calculated for each section, and the group means (± standard deviation) was statistically compared between the treated and untreated rats.

The statistical significance of the means was assessed by multiple pairwise Student's *t* test ($P < .05$).

Results

The stromal cell population of the control rat prostates constitutes a few elongated cells and isolated round-shaped leukocytes. The macrophages, characterized by their positive immunoreactivity (IR) to ED2 antibody, were rarely seen (Figure 1A), whereas toluidine blue–stained mastocytes were identified by their metachromasia and were found near the vasculo-nervous network (Figure 1C). The stroma is constituted mainly of 2 type-elongated actin- or vimentin-positive cells that were randomly dispersed in the stroma as well as in the neighborhood of the acini (Figure 1E and G). The smooth muscle of the arterioles stained more intensively to actin. Based on these findings, an attempt to assess the arteriolar density was made (see further). Vimentin IR was also found among endothelial cells (Figure 1G).

The prostates of the PE-treated rats show an increased IR intensity among all the stromal cells, as expressed both qualitatively (Figure 1B, D, F, and H) and quantitatively (Figure 2A). The increased number of fibrocytes immunoreactive to actin were mainly found at the proximity of the acinar borders, where they stained more intensively than those observed in the mid-core of interacinar space (Figure 1F). In addition, the mastocytes and epithelial cells were also found to express actin IR (Figure 1F).

The frequency of the ED2-positive macrophages as well as of the mastocytes was significantly increased among the PE-treated rats compared to the untreated ones (Figure 2A) ($P < .01$). In addition, the mesenchymal actin-positive and vimentin-positive cell frequency was also found to be increased following the PE treatment ($P < .001$). The assessment of arteriolar density was found to be significantly increased ($P < .05$) among the PE-treated prostates compared to the control ones (Figure 2B).

The increase in mesenchymal cell numbers after PE treatment was substantiated by their high proliferative rate (Figure 3A through C). The control prostates showed isolated BrdU-positive cells in both epithelial and stromal compartments (Figure 3A). After the PE treatment, their frequency in absolute numbers was significantly increased ($P < .05$ and .025 respectively) (Figure 3B and C). However, when their relatively increased ratio was considered, the stromal cells presented a higher proliferative rate ($P < .05$) compared to the epithelial cells (Figure 3C, insert).

The Gomori trichrome stain of control ventral prostates revealed a random alignment of short and delicate collagen fibers in the periacinar area, intermingled among the few stromal cells (Figure 4A). In the PE-treated rats, these fibers were more numerous and variable in thickness, while their alignment followed a more orderly pattern (Figure 4B).

In the control prostate, the elastic fibrils were quite imperceptible in the interacinar spaces, but a few delicate fibrils were seen in the interlobular areas (Figure 4C). The affected prostates showed a definite increase in elastica fibril number and in deposition patterns similar to the collagen fibrils, some with longitudinal splits (Figure 4D). It is noteworthy that the internal elastic lamina of arterioles did not change after the PE treatment (Figure 4D).

Picro-sirius red selectively stained the collagen fibrils. Analyzed under the polarized light, they appear as a well-structured network in both interacinar and interlobular spaces, being more conspicuous among the treated than the control animals (Figure 4E and F). The collagenous material is disposed as thin lamellae presenting various diffraction colors ranging from red to orange and yellow patches. The computerized digital analysis of these colored patches (Figure 4G) reveals an increase in yellow and orange patches in the treated groups. This increase is indicative of a de novo synthesis induced by PE in the prostate stroma.

Discussion

The present findings reveal that PE has a dual role in the development of PIN lesions, affecting both epithelial and stromal constituents of the rat ventral prostate. The epithelial changes that we previously reported (Golomb et al, 1998; Rosenzweig et al, 2004) look similar to the PIN lesions described in human prostates (Bostwick et al, 1993; Epstein, 1995). Similarly, an increase in frequencies of IR stromal cells among PE-treated rats was also found and substantiated by their high proliferation rate. The relative ratio of the BrdU-positive cells is an interesting finding, since stromal cells are relatively fewer in the normal rat prostate and yet they duplicate their number following treatment, in a higher ratio, as compared to epithelial cell proliferation.

The increase in stromal cell population can be attributed to the sympathomimetic stimulation of PE. A genetic excessive-evoked sympathetic activation is a major feature in the development of high blood pressure among the spontaneously hypertensive rats (SHR) (Magee and Schofield, 1994; Julius, 1996). When fibroblasts and vascular smooth muscle cells originating from the SHR strain are grown in vitro, they show an increased proliferative rate (Paquet et al, 1989; Guicheney et al, 1991) as well as an increase in the number of their prostatic epithelium cells (Matityahou et al, 2003). Moreover, we have previously reported that SHR develop BPH spontaneously (Golomb et al, 2000). When mesenchymal stromal cells of human BPH origin are treated in vitro with norepinephrine (Smith et al, 1999) or PE (Boesch et al, 1999), they show an increased expression of smooth muscle cytoskeleton proteins such as actin and myosin, as expressed

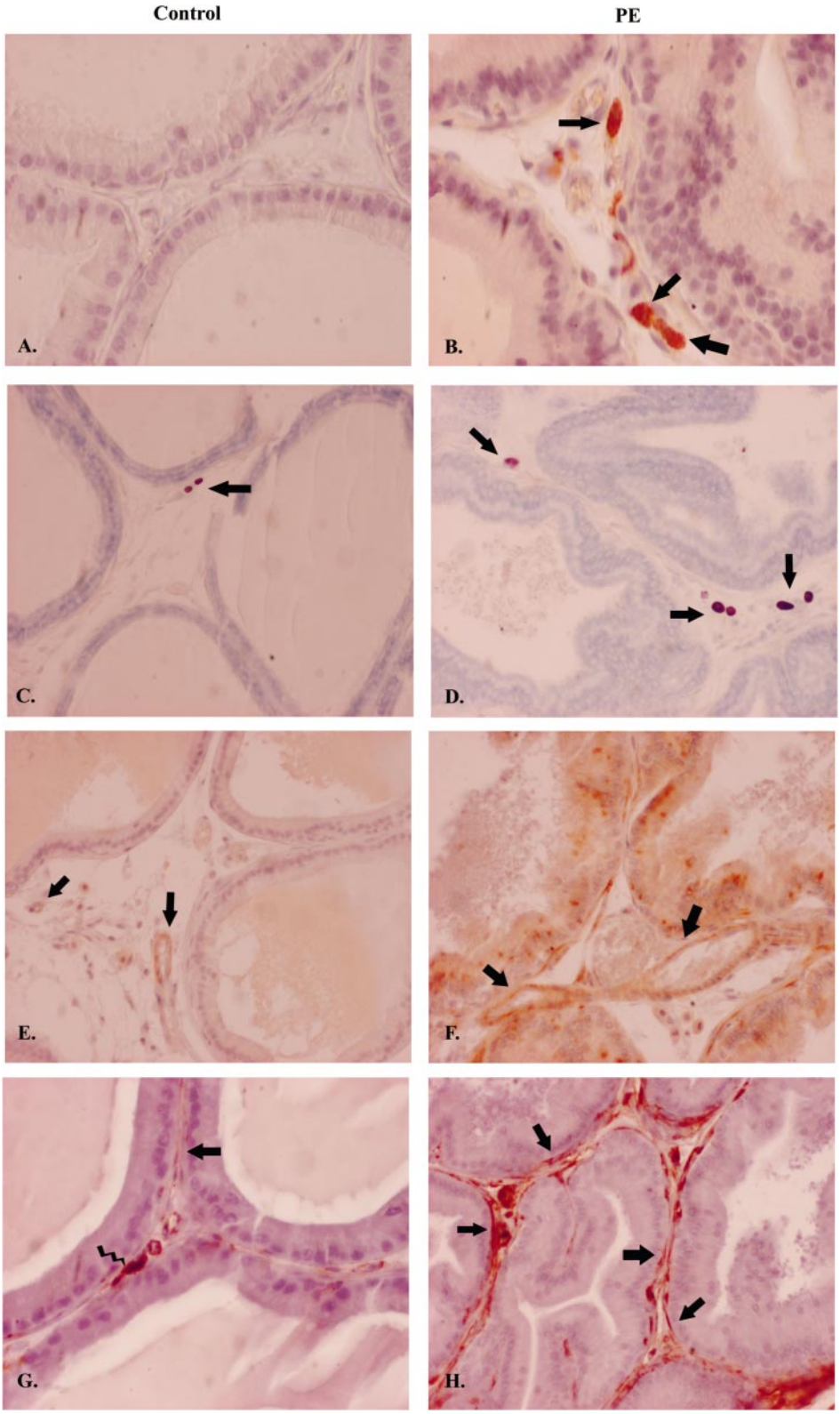

Figure 1. Identification of relatively few stromal cells seen in control rats' prostates **(A, C, E, G)** as compared to their increased number after phenylephrine (PE) administration **(B, D, F, H)**. Residual mononuclear-derived macrophages showed positive immunoreactivity to ED2 **(B**, arrows); mastocytes metachromasia is higher in the treated group **(C, D**, arrows). Actin immunostaining showed a weak reactivity for fibrocytes and epithelial cells, while the vascular smooth muscle cells (bold arrows) reacted more intensively **(E)**. Numerous actin immunoreactive cells were evident after PE treatment; smooth muscles of small and larger arterioles were also positively immunostained (bold arrows) **(F)**. Myofibroblasts, like cells (bold arrows) and their fibroblast precursors, reacted positively to vimentin in both control **(G)** and PE-treated prostates **(H)**. Note the positive reactivity of isolated mast cells **(G**, lightning symbols). Original magnification: A, B, G, H: 400×; C–F: 200×.

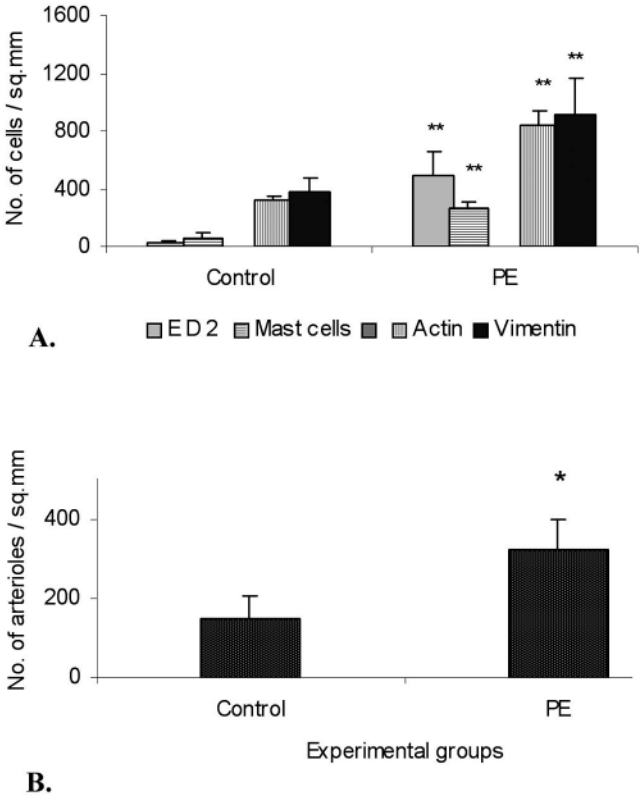

Figure 2. Frequencies of different stromal cells **(A)** and vascular density **(B)** among control and phenylephrine (PE)–treated rats. * $P < .05$; ** $P < .01$.

by their differentiation into smooth muscle phenotype (Magee et al, 2000). This effect was inhibited by doxazosin, an alpha-1 adrenergic antagonist (Boesch et al, 1999; Smith et al, 1999; Magee et al, 2000). The alpha-1 adrenergic blockade down-regulates the myosin heavy-chain mRNA expression in human BPH (Lin et al, 2001). According to Kanagawa et al (2003), norepinephrine increases the DNA synthesis and, subsequently, cell proliferation of the human prostate stromal cells by activating the MAP kinase (MAPK) signaling pathway. However, this stimulatory effect was less effective on the epithelial cells (Kanagawa et al, 2003).

It is noteworthy that steroid sex hormones do not influence the expression of the contractile filaments and therefore neither enhanced nor inhibited the norepinephrine and doxazosin effect (Magee et al, 2000). It seems that the prostatic smooth muscle cells are less responsive to hormonal alteration than the fibroblasts (Zhao et al, 1992).

Alpha smooth muscle actin expression is considered a marker of choice for myofibrillar modulation and is also transiently expressed by myofibroblasts during experimental wound healing (Gabbiani et al, 2003). It was suggested (De Wever and Mareel, 2003) that the myofibroblasts might play a causal role in the transition from the noninvasive toward the invasive tumor phenotype, as has been observed in various tumors including human prostatic carcinoma (PCa) (Rowley 1998–99; Tuxhorn et al, 2002). Nowadays, the fibroblasts are considered to *trans*-differentiate into myofibroblasts by expressing both alpha actin as well as vimentin micro filaments (De Wever and Mareel, 2003). In such a case, myofibroblasts and their precursor fibrocytes may well be involved in the synthesis of stromal fibrillar constituents. The overgrowth of these cells could obviously lead to prostatic hyperplasia directly and/or through local mesenchymal epithelial interaction (Cunha, 1994; Thomson et al, 2002).

It is well accepted that ECM serves as a microenvironmental reservoir for the various growth factors necessary for the growth and remodulation of the tissue, thus offering the anchoring scaffolding for the surrounding ductoacinar complex. This can explain the inability of primary human prostatic epithelial cells to proliferate in vitro in the absence of stromal cells (Hall et al, 2002). On the other hand, the paucity of stromal elements that characterizes different strains of rat prostates might be attributed to their innate refractivity to develop hyperplastic lesions spontaneously with advancing age (Scolnik et al, 1994).

We have shown that continuous PE administration by itself favors the maintenance of the inflammatory exudate over the acute reactive phase in the rat prostate (Rosenzweig et al, 2004). In fact, a persistent chronic inflammatory exudate promotes fibroblast proliferation and angiogenesis, ultimately leading to scar organization and fibrosis. The repair process begins as early as 24 hours postinjury (Kumar et al, 2005).

The increase in the monocyte-derived macrophages and, to a lesser extent, the mast cells may well explain their role in fibrotic scar formation (Frangogiannis et al, 2002), as these cells are known to produce cytokines and growth factors. Fibrotic changes were observed in human BPH and PCa (Chagas et al, 2002; Zhang et al, 2003). The smooth muscle cells that were found subadjacent to the hyperplastic acini and also in well-differentiated PCa are totally absent in the poorly differentiated carcinoma and metastases (Deering et al, 1995). Various conditions have been reported to accompany prostatic fibrosis in men, such as chronic ischemia (Kozlowski et al, 2001), proliferative inflammatory atrophy (De Marzo et al, 1999; Putzi and De Marzo, 2000), as well as in cases of chronic administration of testosterone in baboons (Karr et al, 1984) and estradiol in rats (Zhao et al, 1992). A detailed review of the literature regarding the functional resemblance between wound repair and reactive stroma in many cancers, including prostatic cancer, was offered by Rowley (1998–99).

The increase in the microvascular density, as observed among PE-treated rats, is indicative of the existence of neoangiogenesis, which occurs during the healing stage

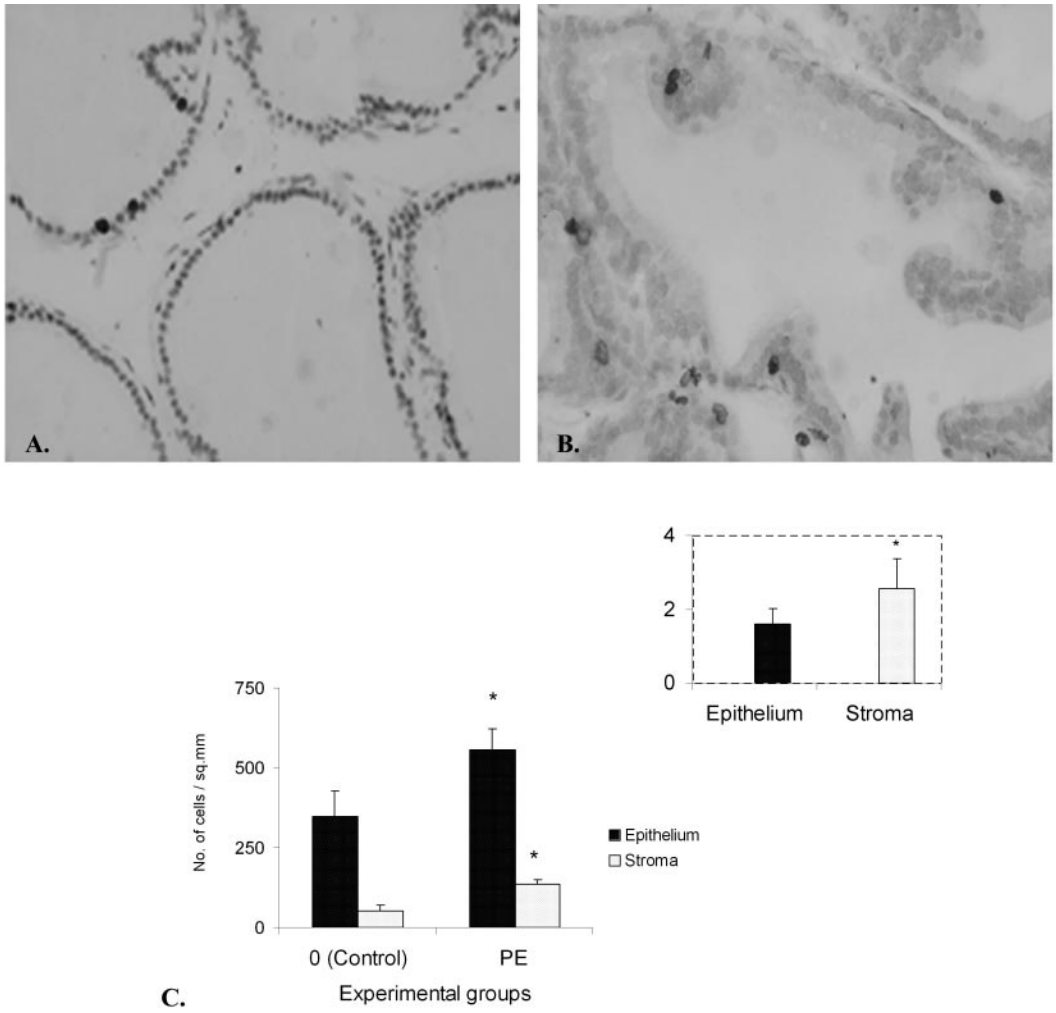

Figure 3. Immunohistochemistry (IHC) expression of 5′-bromo-2′-deoxyuridine (BrdU) incorporation involves both stromal and the epithelial cells in control **(A)** as well as in the phenylephrine (PE)–treated rats **(B)**. The epithelial and stromal cell frequencies are both increased by their absolute numbers after PE treatment **(C)**. When the relative ratio between treated vs control is considered (T:C ratio), the increase in the stromal cell population is predominant (insert). Original magnification: A, B: 200×. * $P < .05$.

observed following the early phase of PE effect (Rosenzweig et al, 2004). Tumors are known to alter their own microenvironment by creating new blood vessels to hasten their growth (Guiot et al, 2003). According to Bostwick and Iczkowski (1998), the microvascular density is a good criterion for the assessment of the degree of angiogenesis and is therefore considered an independent prognostic factor for patients with PCa. Blood vessel density in human PCa was found to be twice as high as in normal prostate (Deering et al, 1995). Angiogenesis could be accelerated both in BPH and PCa via epidermal growth factor induction of vascular endothelial growth factor gene expression. In an IHC study it was found that vascular endothelial growth factor was absent in the normal human prostate, and only the epithelia from BPH and, to a lesser extent, in PCa cell lines exhibited strong immunoreactivity (Ravindranath et al, 2001). A recent review of tumor angiogenesis is offered by Verheul et al (2004).

It is known that collagen is the most ubiquitous protein in animals and the key molecule of the ECM organization. The trichrome stain is a classical routine technique to identify the presence of connective tissue as a whole, but it cannot afford the identification of fine fibrillar collagen. Moreover, it is known that the normal rat prostatic stroma contains a negligible amount of connective tissue elements (Lin et al, 2001). Therefore, we approached a more adequate technique, such as polarized-light picrosirius red staining, in order to obtain a better resolution for the collagen fibrils. The color spectra of the collagen fibers depend on their size (Junqueira et al, 1979; Modis, 1991) or their packing alignment and orientation in the ECM (Nimni, 1980; Pickering and Boughner, 1991). The presence of yellow fibers is considered to reflect decreased packing density or size, therefore corresponding to thin and young fibers, whereas the orange-red ones that are thicker do represent polymerized, old fibers (Dayan

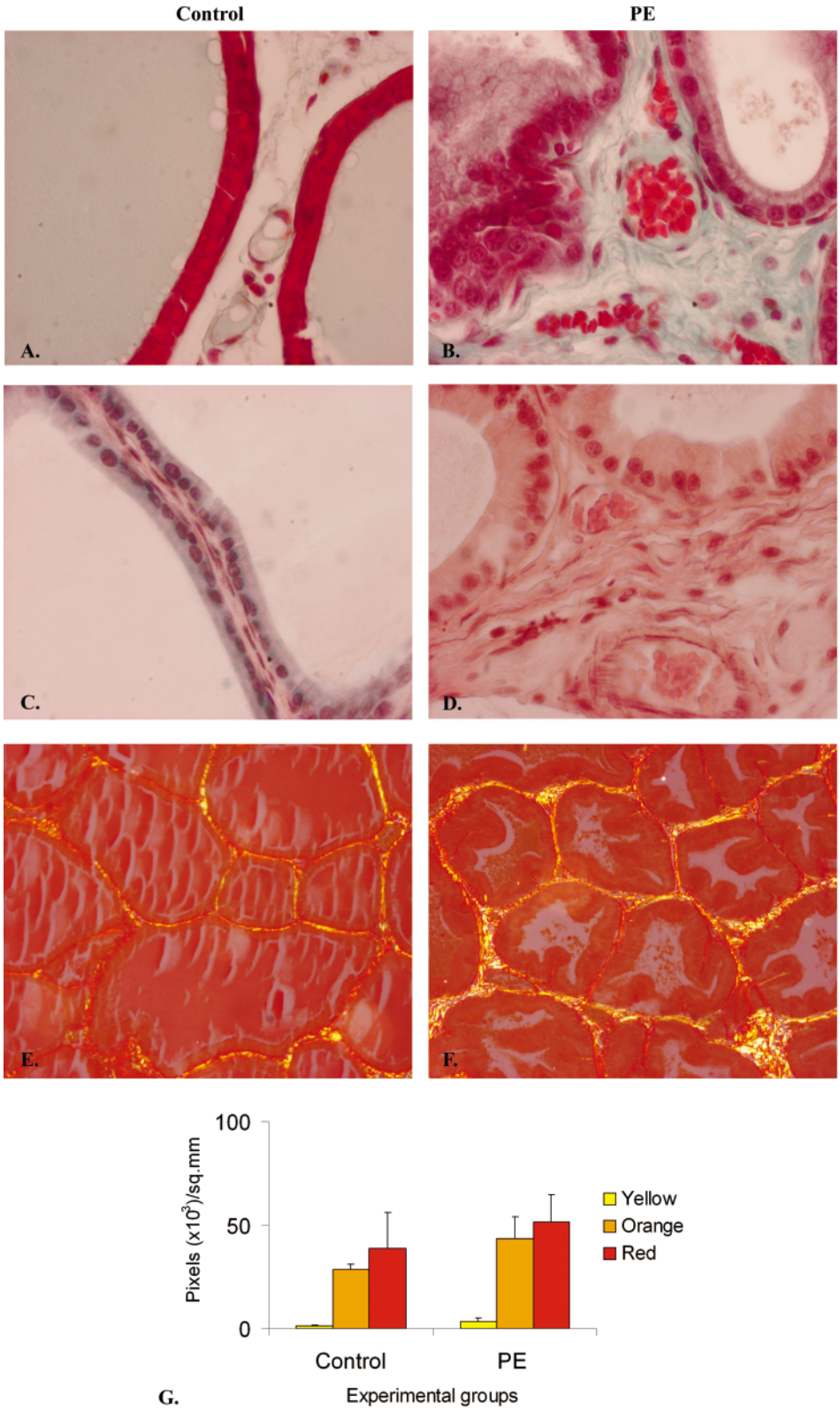

Figure 4. A scanty fibrillar stroma was identified in the control rats **(A, C, E)**, which become conspicuous in a more ordered alignment in the phenylephrine (PE)–treated group **(B, D, F)**. Connective tissue stroma was evident upon use of the Gomori trichrome stain **(A, B)**; fine elastic fibrils were visualized by the Pinkus method **(C, D)**. Polarized light dichroism of collagen fibrils was stained by picro-sirius red **(E, F)**. Original magnification: A–D: 400×; E, F: 100×. The quantitative analysis of the color dichroism distribution **(G)** presented an increase in the yellow and orange range of the PE-treated prostate, compared to control group.

et al, 1989). These data seems to reflect the evolution of collagenetic process as encountered in our model. The significant increased amounts of the yellow and orange fibers found among PE-treated rats correspond to the synthesis of new, young, relatively unorganized collagen fibers. At the beginning the fibrils are short (1–3 μm), but later on, as a result of a fusion process, larger fibrils are generated, creating a shaft network (Kadler et al, 2000). The continuous deposition of new collagen fibers creates thickened fibers, thus leading to denser ECM material. Such increases in the collagen content corroborate with the increase in the fibrocytes and in the myofibroblasts number, since these cells are known to be involved in collagen synthesis and secretion (Bosman and Stamenkovic, 2003). In human BPH, this collagen network is much increased and appears denser (Chagas et al, 2002), but it is relatively reduced in PCa, in which some disintegration of the fibers was reported (Morrison et al, 2000). The latter was correlated to the Gleason score and is regarded as a predictor of patient survival (Burns-Cox et al, 2001).

Little information is as yet available with regard to the amount and location of the elastic fibers in the prostate gland, since the fibers are known to intermingle among the collagen fibers (Scott and Vesely, 1995). This may explain their similar location patterns, which are similar to the collagen fibers. Although the presence of fine elastic fibrils appears in scar tissues (Roten et al, 1996) as well as in normal human prostate and PCa (Zhang et al, 2003), the time course of their appearance has not been elucidated. However, a study of human skin scar formation revealed that focal appearances of thin elastic filaments do appear relatively late in the third month after the initiation of the lesion (Roten et al, 1996).

In conclusion, chronic PE administration was found to induce definite stromal changes, such as inflammatory leukocytic exudate, as well as a significant increase in both cellular and fibrillar elements, including neoangiogenesis. According to Mareel and Leroy (2003), the combination of all these lesions is considered as a desmoplastic process frequently encountered in various neoplastic conditions. The findings presented here raise the question as to whether other xenobiotics capable of inducing prostatic hyperplasia might also promote similar stromal lesions in rats. Understanding the factors capable of stimulating or monitoring stromal growth may open new therapeutic strategies in the prevention of the fibrotic process, with a view to attenuating the pathogenesis and evolution of prostatic disorders.

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References

Boesch ST, Corvin S, Zhang J, Rogatsch H, Bartsch G, Klocker H. Modulation of the differentiation status of cultured prostatic smooth muscle cells by an alpha1-adrenergic receptor antagonist. *Prostate.* 1999; 39:226–233.

Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. *J Pathol.* 2003;200:423–428.

Bostwick DG, Iczkowski KA. Microvessel density in prostate cancer: prognostic and therapeutic utility. *Semin Urol Oncol.* 1998;16:118–123.

Bostwick DG, Srigley J, Grignon D, Maksem J, Humphrey P, van der Kwast TH, Bose D, Harrison J, Young RH. Atypical adenomatous hyperplasia of the prostate: morphologic criteria for its distinction from well-differentiated carcinoma. *Hum Pathol.* 1993;24:819–832.

Burns-Cox N, Avery NC, Gingell JC, Bailey AJ. Changes in collagen metabolism in prostate cancer: a host response that may alter progression. *J Urol.* 2001;166:1698–1701.

Chagas MA, Babinski MA, Costa WS, Sampaio FJ. Stromal and acinar components of the transition zone in normal and hyperplastic human prostate. *BJU Int.* 2002;89:699–702.

Chung LW. The role of stromal-epithelial interaction in normal and malignant growth. *Cancer Surv.* 1995;23:33–42.

Chung LW, Davies R. Prostate epithelial differentiation is dictated by its surrounding stroma. *Mol Biol Rep.* 1996;23:13–19.

Cunha GR. Role of mesenchymal-epithelial interactions in normal and abnormal development of mammary gland and prostate. *Cancer.* 1994;74:1030–1044.

Dayan D, Hiss Y, Hirshberg A, Bubis JJ, Wolman M. Are the polarization colors of picrosirius red–stained collagen determined only by the diameter of the fibers? *Histochemistry.* 1989;93:27–29.

Deering RE, Bigler SA, Brown M, Brawer MK. Microvascularity in benign prostatic hyperplasia. *Prostate.* 1995;26:111–115.

De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol.* 1999;155:1985–1992.

De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. *J Pathol.* 2003;200:429–447.

Epstein JI. Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. *Hum Pathol.* 1995;26:223–229.

Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res.* 2002;53:31–47.

Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol.* 2003;200:500–503.

Golomb E, Kruglikova A, Dvir D, Parnes N, Abramovici A. Induction of atypical prostatic hyperplasia in rats by sympathomimetic stimulation. *Prostate.* 1998;34:214–221.

Golomb E, Rosenzweig N, Eilam R, Abramovici A. Spontaneous hyperplasia of the ventral lobe of the prostate in aging genetically hypertensive rats. *J Androl.* 2000;21:58–64.

Guicheney P, Wauquier I, Paquet JL, Meyer P. Enhanced response to growth factors and to angiotensin II of spontaneously hypertensive rat skin fibroblasts in culture. *J Hypertens.* 1991;9:23–27.

Guiot C, Degiorgis PG, Delsanto PP, Gabriele P, Deisboeck TS. Does tumor growth follow a ''universal law''? *J Theor Biol.* 2003;225:147–151.

Hall JA, Maitland NJ, Stower M, Lang SH. Primary prostate stromal cells modulate the morphology and migration of primary prostate epithelial cells in type 1 collagen gels. *Cancer Res.* 2002;62:58–62.

Julius S. The evidence for a pathophysiologic significance of the sympathetic overactivity in hypertension. *Clin Exp Hypertens.* 1996;18: 305–321.

Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polar-

ization microscopy, a specific method for collagen detection in tissue sections. *Histochem J.* 1979;11:447–455.

Kadler KE, Holmes DF, Graham H, Starborg T. Tip-mediated fusion involving unipolar collagen fibrils accounts for rapid fibril elongation, the occurrence of fibrillar branched networks in skin and the paucity of collagen fibril ends in vertebrates. *Matrix Biol.* 2000;19:359–365.

Kanagawa K, Sugimura K, Kuratsukuri K, Ikemoto S, Kishimoto T, Nakatani T. Norepinephrine activates P44 and P42 MAPK in human prostate stromal and smooth muscle cells but not in epithelial cells. *Prostate.* 2003;56:313–318.

Karr JP, Kim U, Resko JA, Schneider S, Chai LS, Murphy GP, Sandberg AA. Induction of benign prostatic hypertrophy in baboons. *Urology.* 1984;23:276–289.

Kozlowski R, Kershen RT, Siroky MB, Krane RJ, Azadzoi KM. Chronic ischemia alters prostate structure and reactivity in rabbits. *J Urol.* 2001;165:1019–1026.

Kumar V, Abbas AK, Fausto N, eds. *Robbins and Cotran: Pathologic Basis of Disease*. 7th ed. Philadelphia, Pa: Elsevier Science Publishers Inc; 2005: 87–118.

Lepor H, Tang R, Meretyk S, Shapiro E. Binding and functional properties of alpha 1 adrenoceptor in different regions of the human prostate. *J Urol.* 1993;150:253–256.

Lillie RD. *Histopathologic Techniques and Practical Histochemistry*. 3rd ed. New York, NY: McGraw-Hill Inc; 1965:547.

Lin VK, Benaim EA, McConnell JD. Alpha-blockade downregulates myosin heavy chain gene expression in human benign prostatic hyperplasia. *Urology.* 2001;57:170–175.

Magee JC, Schofield GG. Alteration of synaptic transmission in sympathetic ganglia of spontaneously hypertensive rats. *Am J Physiol.* 1994; 267:1397–1407.

Magee JC, Schofield P, Rhodes NP, Ke Y, Foster CS. Modulating effect of estrogen and testosterone on prostatic stromal cell phenotype differentiation induced by noradrenaline and doxazosin. *Prostate.* 2000; 44:111–117.

Mareel M, Leroy A. Clinical, cellular, and molecular aspects of cancer invasion. *Physiol Rev.* 2003;83:337–376.

Matityahou A, Rosenzweig N, Golomb E. Rapid proliferation of prostatic epithelial cells in spontaneously hypertensive rats: a model of spontaneous hypertension and prostate hyperplasia.*J Androl.* 2003;24:263–269.

McVary KT, McKenna KE, Lee C. Prostate innervation. *Prostate.* 1998; 8:2–13.

Modis L. *Organization of ECM: A Polarization Microscopic Approach.* Boca Raton, Fla: CRC Press; 1991:2–7, 58–60.

Morrison C, Thornhill J, Gaffney E. The connective tissue framework in the normal prostate, BPH and prostate cancer: analysis by scanning electron microscopy after cellular digestion. *Urol Res.* 2000;28:304–307.

Nemeth J, Lee C. Prostatic ductal system in rats: regional variation in stromal organization. *Prostate.* 1996;28:124–128.

Nimni ME. The molecular organization of collagen and its role in determining the biophysical properties of the connective tissues. *Biorheology.* 1980;17:51–82.

Paquet JL, Baudouin-Legros M, Marche P, Meyer P. Enhanced proliferating activity of cultured smooth muscle cells from SHR. *Am J Hypertens.* 1989;2:108–110.

Pickering JG, Boughner DR. Quantitative assessment of the age of fibrotic lesions using polarized light microscopy and digital image analysis. *Am J Pathol.* 1991;138:1225–1231.

Pinkus H, Hunter R. Simplified acid orcein and Giemsa technique for routine staining of skin sections. *Arch Dermatol.* 1960;82:699–700.

Putzi MJ, De Marzo AM. Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. *Urology.* 2000;56:828–832.

Ravindranath N, Wion D, Brachet P, Djakiew D. Epidermal growth factor modulates the expression of vascular endothelial growth factor in the human prostate. *J Androl.* 2001;22:432–443.

Rosenzweig N, Horodniceanu J, Abramovici A. Phenylephrine-induced neurogenic prostatitis facilitates the promotion of PIN-like lesions in rats. *Prostate.* 2004;59:107–113.

Roten SV, Bhat S, Bhawan J. Elastic fibers in scar tissue. *J Cutan Pathol.* 1996;23:37–42.

Rowley DR. What might a stromal response mean to prostate cancer progression? *Cancer Metastasis Rev.* 1998–99;17:411–419.

Scolnik MD, Servadio C, Abramovici A. Comparative study of experimentally induced benign and atypical hyperplasia in the ventral prostate of different rat strains. *J Androl.* 1994;15:287–297.

Scott M, Vesely I. Aortic valve cusp microstructure: the role of elastin. *Ann Thorac Surg.* 1995;60:S391–S394.

Sheehan D, Hrapchak B. *Theory and Practice of Histotechnology*. 2nd ed. Columbus, Ohio: Battelle Press; 1980:282.

Smith P, Rhodes NP, Ke Y, Foster CS. Influence of the alpha1-adrenergic antagonist, doxazosin, on noradrenaline-induced modulation of cytoskeletal proteins in cultured hyperplastic prostatic stromal cells. *Prostate.* 1999;38:216–227.

Thomson AA, Timms BG, Barton L, Cunha GR, Grace OC. The role of smooth muscle in regulating prostatic induction. *Development.* 2002; 129:1905–1912.

Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res.* 2002;8:2912–2923.

Verheul HM, Voest EE, Schlingemann RO. Are tumours angiogenesis-dependent? *J Pathol.* 2004;202:5–13.

Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. *Basic Res Cardiol.* 1994;89:397–410.

Wong YC, Wang YZ. Growth factors and epithelial-stromal interactions in prostate cancer development. *Int Rev Cytol.* 2000;199:65–116.

Yu CC, Woods AL, Levison DA. The assessment of cellular proliferation by immunohistochemistry: a review of currently available methods and their applications. *Histochem J.* 1992;24:121–131.

Zhang Y, Nojima S, Nakayama H, Jin Y, Enza H. Characteristics of normal stromal components and their correlation with cancer occurrence in human prostate. *Oncol Rep.* 2003;10:207–211.

Zhao GQ, Holterhus PM, Dammshauser I, Hoffbauer G, Aumuller G. Estrogen-induced morphological and immunohistochemical changes in stroma and epithelium of rat ventral prostate. *Prostate.* 1992;21: 183–199.