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CELLULAR AND CYTOSKELETAL HETEROGENEITY ALONG THE RAT VENTRAL PROSTATIC DUCT

BY

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submitted in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry

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" Des soirées entières sur un mot ... et quelque fois une simple conjonction. A la rigueur, c'est assez facile de choisir entre *mais* et *et*. C'est déjà plus difficile d'opter entre *et* et *puis*. La difficulté grandit avec *puis* et *ensuite*. Mais, assurément, ce qu'il y a de plus difficile, c'est de savoir s'il faut mettre *et* ou s'il ne le faut pas."

Albert Camus (La Peste)

ABSTRACT

The prostate gland is under androgenic control. However, the different cell types present in the gland do not respond in the same manner to androgens: Epithelial cells are far more affected than stromal cells after castration. In addition, the epithelial cells forming the ductal-acinar network in the prostate display a different behaviour depending on their location relative to the prostatic urethra. Cells at distal tips possess a high degree of protein and DNA synthetic activity and die soon after androgen ablation while the cells in the proximal region of the ducts, closer to the urethra, are unaffected by the withdrawal of androgens.

In this study, the ductal heterogeneity in the rat ventral prostate was further investigated in terms of structural elements that make up the different cell types of the prostate as well as cell populations found along prostatic ducts. In addition, effects of androgens were analyzed in specific locations of the ductal-acinar network. The cytokeratin content of the prostate was characterized biochemically and by immunofluorescence and was used as a marker to monitor the behaviour of each cell type after androgen depletion. It was found that the cytokeratin content of basal and luminal epithelial cells are characteristic for each cell type and that these structural proteins constitute good markers for these cell types. Using antibodies specific for basal and luminal epithelial cells, the ratio basal to luminal epithelial cells was shown to vary along the prostatic duct as well as after castration, suggesting a preferential loss of luminal epithelial cells. Based on the observations of the cellular dynamics in the prostate of intact versus castrated rats, a model of regression is proposed.

RESUME

La prostate est sous le contrôle des androgènes. Toutefois, les différents types de cellules qui composent cette glande ne répondent pas de la même manière à la présence des androgènes: Les cellules épithéliales sont beaucoup plus affectées par la castration que les cellules stromales. De plus, les cellules épithéliales, formant le réseau de canaux de la prostate, se comportent différemment selon leur position le long du canal prostatique. Les cellules à l'extrémité des canaux (relativement à l'urètre prostatique) synthétisent activement ADN et protéines puis meurent rapidement en absence d'androgènes, contrairement aux cellules épithéliales le long des canaux prostatiques dans la région plus rapprochée de l'urètre.

Dans ce projet, l'hétérogénéité cellulaire le long des canaux prostatiques du rat a été étudiée en ce qui a trait aux éléments structuraux composant les différent types de cellules épithéliales de la prostate de même qu'au niveau des populations de cellules retrouvées le long de ces canaux. De plus, les effets causés par les androgènes ont été analysés dans différentes régions du réseau de canaux prostatiques. Le contenu de la prostate en cytokératines a été analysé biochimiquement et par immunofluorescence. Les cytokératines ont été également employées comme marqueur dans le but de suivre le comportement des cellules épithéliales de chaque type suite à l'ablation des androgènes. Les résultats démontrent que le contenu en cytokératines est spécifique et différent pour les cellules épithéliales basales et luminales et ces protéines structurales constituent donc de bons marqueurs cellulaires. Une étude du rapport des cellules épithéliales basales versus luminales à l'aide d'anticorps spécifiques pour les cellules epithéliales basales et luminales a démontré une variation de ce rapport le long des canaux prostatiques. Ces résultats suggèrent que les cellules épithéliales luminales sont affectées de façon préférentielle par l'absence des androgènes. Un modèle de régression de la prostate suite à la castration

est proposé, basé sur les observations de la dynamique cellulaire de cette glande.

Ce travail est dédié à mes parents et à François
en guise de reconnaissance de leurs encouragements
et de leur précieux support moral.

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INTRODUCTION

1. Historical Background :

Since Berthold's (1849) demonstration of an internal secretory action of the testis, the field of hormone research has developed rapidly. Target(s) of testis secretion, causes and mechanism of action were and are still fully investigated. In the early 1900's, the focus of the research was directed toward the identification of the active ingredient of hormone secretion and its function. Early studies examined the effects of castration and injection of testicular homogenates on a number of psychological parameters, including the inclination to mate and the tendency to fight. These characteristics were found to be as much dependent on housing conditions and the presence of female as on the presence of testicular hormones (Steinach, 1920; Moore and Gallagher, 1930). The effects of testicular hormones on other parameters, such as body weight (Moore, 1920), hair coat (Steinach, 1920; Moore, 1920), bone length (Poncet, 1903; Bouin and Ancel, 1906) and spermatozoon motility (Moore, 1928) demonstrated that of these parameters, only the spermatozoon motility was influenced by the testicular hormones. However, the most dramatic finding demonstrated that removal of the testes caused the involution of several accessory sexual glands including the seminal vesicles, coagulating glands and the prostate (Moore and Gallagher, 1930). This observation focused research on the analysis of the relationship between testicular hormones and accessory sexual gland structure and function. This research was initiated by monitoring the function of seminal vesicles and prostate in guinea pigs by measuring the amount of ejaculate produced weekly by castrated animals and by castrated animals injected with testicular extract. Ejaculate production was lost in long term castrated animals but this could be prevented by a daily injection of lipoid bull testis extract (Moore and Gallagher, 1930). At the same time, Moore, Price and Gallagher (1930) looked at the effects of castration on the cytology of the rat seminal vesicles and prostate glands. They found that dramatic cellular changes occurred, especially within the prostate gland. Following castration, epithelial cells appeared to occupy only one half to one third of their normal size while relative

number and volume of stromal cells increased. However, upon daily injection of the testicular extract, newly castrated animals maintained normal cytological appearance while long term castrates returned to normal cytology within 20 days of injection. These first morphological observations revealed the direct importance of the testicular hormones on accessory glands and have subsequently been used as model systems to study the effects of androgens.

Several components with hormonal activity, such as androsterone (McGee, 1927; Butenandt, 1931) and testosterone (David, Dingemanse, Freud and Laqueur, 1935) were successfully isolated from testis extracts and crystallized. Soon after their characterization, several synthetic procedures for the preparation of androgens were developed, facilitating further investigation on the mechanism of androgen action. The research of this era culminated in the demonstration by Huggins and Hodges (1941) that human prostatic carcinoma was activated by androgen injections while its dissemination was inhibited by castration.

2. Androgen Dependence of Enzymatic Activities in the Prostate:

Many enzymatic activities have been assayed in normal and androgen depleted animals in order to narrow down the possible targets for androgens. Glycolytic and respiratory chain enzymes were found to be modulated by androgen supply. Fumarase, aconitase, malic dehydrogenase (William-Ashman, 1954), cytochrome oxidase, succinic dehydrogenase (Davis, Meyer and McShaw, 1949), transaminase (Awapara, 1952) and aldolase (Bulter and Schade, 1958) all have a decreased activity in the prostate of castrated animals.

However, a number of prostate specific enzymes whose activities are directly related to prostate function and therefore to hormonal supply have also been identified. Acid phosphatase, which is found in large amount in the human prostate gland (Kutsher and Wolbergs, 1935) showed a higher activity in prostatic carcinoma (Gutman, Sproul and Gutman, 1936).

Although the lysosomal form of the enzyme is present in most other tissues including spleen (Manning, Babson, Butler and Priester, 1966), kidney (Iype and Heidelberger, 1968; Goldstone and Koenig, 1973), liver (Barka, 1961; Manning *et al.*, 1966) and testis (Vanha-Perttula, 1970), the prostate is believed to be the only gland that contains a secretory form of the enzyme (Woodward, 1959) and marked rise of the prostatic isoenzyme in serum is invariably associated with disseminated prostatic carcinoma (Huggins and Hodges, 1941). The secretory acid phosphatase was found to be highly dependent on androgens for its synthesis (Helminen, Ericsson, Rytoluoto and Vanha-Perttula, 1975) and therefore this makes it a very good androgen dependent marker of the prostate (Tenniswood, Bird and Clark, 1976).

Prostate steroid-binding protein (PSBP), first identified by Heyns and De Moor (1977) as a low affinity steroid-binding protein secreted by the rat prostate into the lumen, has also been shown to be under androgenic control (Heyns, Van Damme and De Moor, 1978). More recently, it has been demonstrated that PSBP is the most abundant protein synthesized in the rat ventral prostate and that the androgenic control of its synthesis is at the level of transcription and post-transcriptional hnRNA and mRNA stability (Parker and Scrace, 1979; Page and Parker, 1982; Zhang and Parker, 1985; Bossyns, Delaey, Rombauts and Heyns, 1986). This has made the protein a particularly useful marker of androgen dependent gene expression in the rat prostate.

3. Structural Compartments of the Prostate :

3.1 Morphological Aspects

Following the early histological observations on the prostate (Moore *et al.*, 1930) numerous reports have provided more detailed information on the structural organization of the prostate. The rodent gland is composed of distinct compartments, referred to as prostatic lobes. Price, Mann and Lutwak-Mann (1955), Gunn and Gould (1957) and Schrodt (1961) have shown

that histologically, the lateral, dorsal and ventral lobes of the rat prostate are different. This has been confirmed by Sugimura, Cunha and Donjacour (1986a), who demonstrated that each lobe originates from a specific portion of the urogenital sinus at specific times during embryogenesis. The fine structure of the epithelial cells of each lobe, particularly of the rough endoplasmic reticulum and the Golgi vacuoles content, also distinguishes each prostatic compartment (Brandes and Groth, 1961; Schrodt, 1961).

3.2 Cell Types in the Rat Ventral Prostate

Three distinct cell populations have been identified in the rat ventral prostate gland which has been described as acini formed by luminal secretory and basal epithelial cells surrounded by a connective tissue made of stromal cells.

The vast majority of the morphological studies of the prostate have focused on luminal epithelial cells due to their obvious and important function as secretory cells. In the normal prostate, these cells are characterized by their tall columnar shape and basally located nuclei (Moore *et al.*, 1930; Brandes, 1966; Helminen and Ericsson, 1971). Numerous desmosomes are observed between adjacent cells, at the apical side (Brandes, 1966). In the ventral lobe, luminal secretory cells have characteristic Golgi apparati and numerous secretory granules at the apical side of cells. The rough endoplasmic reticulum (RER) is highly developed and its complexity has been often commented on.

Due to their location and their sparse distribution, the basal cells of the prostate were not noticed or described until quite recently. These triangular or flat elongated cells lie between the stroma and the secretory epithelium (Franks and Barton, 1960; Rowlatt and Franks, 1964; Brandes, Kerchheim and Scott, 1964). They sometimes intercalate between luminal cells or are spread over the basal side of these cells. Although their function is not known, Rowlatt and Franks (1964) suggested that they

act as myoepithelial cells involved in the secretion of prostatic products. Ichihara, Kallio and Pelliniemi (1978) suggested that they act as transporters of material between secretory epithelial cells and the extracellular space. Brandes *et al.* (1964) had suggested that they are the stem cells of the gland, based on their morphological observations, although more recently Evans and Chandler (1987a, b) and English, Santen and Isaacs (1987) have demonstrated that this is unlikely to be the case. Other investigators have associated basal cells with a number of pathological conditions and have suggested that they may be the target for neoplastic agents (Brawer, Peehl, Stamey and Botswick, 1985).

Stromal cells have received little attention relative to epithelial cells, probably due to the assumption that they serve solely as a stable scaffold to maintain the structural integrity of the prostatic acini.

3.3 Hormonal Dependence of the Rodent Prostate Gland

The response of the dorso-lateral and ventral lobes of the mouse prostate to androgens have been investigated by Sugimura, Cunha and Donjacour (1986b). After castration, all lobes show similar degree of regression. However, while re-administration of androgen caused the growth of the ventral lobe to its former size and morphology, the dorsal and lateral lobes developed into excessive epithelial infoldings and ductal distension.

Prostate regression is in part due to the cell shrinkage early described by Moore *et al.* (1930). As later confirmed by others (Helminen and Ericsson, 1971, 1972), epithelial cells are atrophied while stromal cells do not appear to be affected. The organelles within the epithelial cells are decreased in number and size, especially those involved in protein synthesis and secretion, such as RER and Golgi complex. The tall columnar shape of epithelial cells in the normal rat ventral prostate becomes flat and cuboidal following castration. However, the cell shrinkage is not sufficient to account for the dramatic decrease in the

prostate size. It was shown that over 80% of ventral prostatic cells are lost following castration (Coffey, Shimazaki and William-Ashmann, 1968; DeKlerk and Coffey, 1978). This cell loss cannot occur solely through normal rate of prostatic cell turnover, which is only about 8% in three days in the rat ventral prostate (Bruchovsky, Lesser, van Doorn and Craven, 1975). Stanisic, Sadowski, Lee and Grayhack (1978) have been able to demonstrate that cell loss requires active RNA and protein synthesis. Kerr and Searle (1973) and Wyllie (1987) described this process of cell death as apoptosis, or programmed cell death. This active process follows a specific sequence of morphological and biochemical changes which require an increase in a number of enzymatic activities including transglutaminases (Fesus, Thomazy and Falus, 1987), endonuclease (Kyprianou, English and Isaacs, 1988) and protease (Engel, Lee and Grayhack, 1980; Lee, 1981). The synthesis of some of these proteins appears to be induced *de novo*, as is the synthesis of some mRNA species (Léger, Montpetit, Tenniswood, 1987), even though most of the transcription and protein synthesis are shut off.

Androgen replacement will however reverse the cell shrinkage and inhibit apoptosis (Brandes, 1966; Helminen and Ericsson, 1971; Kerr and Searle, 1973), and the size of the prostate will be restored to normal. In the rat, further growth will not proceed, even with injection of massive doses of additional androgens, to either intact or castrated animals (Berry and Isaacs, 1984). Since androgens are clearly necessary to maintain the size of the prostate, androgens were proposed to act at two levels on the rat ventral prostate: they act agonistically, on cell proliferation, but also antagonistically, on cell death (Isaacs, 1984; Tenniswood, 1986; Isaacs, 1987). During net prostatic growth, the rate of cell proliferation is greater than the rate of cell death. The opposite is true during prostatic involution, and at homeostasis, the rates of cell proliferation and cell death are equal. The classical mechanism of androgen action (Mainwaring, 1977) has been used to explain the effects of these steroids in prostatic function. However, it has been suggested that other factors must be involved in order to obtain such a broad array

of effects within the same organ (Tenniswood, 1986).

4. Stromal-Epithelial Cell Interactions in the Rodent Ventral Prostate:

While morphological studies were principally focused on the epithelial cells of the prostate, as described in the previous section, enzymatic activities and metabolite concentrations have largely been measured in total prostate homogenates (Awapara, 1952; Iype *et al.*, 1968; Shimazaki, Matsushita, Furuya, Yamaka and Shida, 1969). In an early study, Shimazaki *et al.* (1969) measured the activity of the enzyme Δ^4-5α-reductase in prostate homogenates. This enzyme is the key enzyme in the reduction of testosterone to its active metabolite 5α-dihydrotestosterone (DHT). The 5α-reductase activity was measured in the prostate at different times following castration, and it was shown that the enzyme activity is androgen dependent. However, these studies ignored the variations that may exist between stromal and epithelial cells since prostatic homogenates were used. More rigorous studies by Cowan, Cowan, Grant and Elder (1977) and Bruchovsky and Dunstan-Adams (1985), using isolated epithelial and stromal cell fractions, clearly demonstrated that the majority of enzyme activity is localized in the nuclear membrane of the stromal cell fraction. These findings contributed to the realization that stromal cells of the prostate play a significant role in the control of prostate function, and lead to a closer examination of the biochemistry of stromal cells and on the possible interactions between the two cell types.

The role of stroma or mesenchyme in embryonic development of the mouse prostate has been investigated in detail by Cunha and his colleagues (Cunha, Donjacour, Cooke, Mee, Bigsby, Higgins and Sugimura, 1987). During embryogenesis, the ventral lobe of the mouse prostate arises as epithelial outgrowths (prostatic buds) from the ventral side of the urogenital sinus (UGS). This embryonic structure consists of an epithelium (UGE) surrounded by an abundant mesenchyme (UGM) into which the epithelium will grow when stimulated. Epithelial outgrowths from different region of the UGS will form the urethra, bulbourethral gland, coagulating glands or the prostate

lobes (Cunha, Chung, Shannon and Reese, 1980). This development is under the control of fetal androgens (Jost, 1953) and their absence will cause the UGS to differentiate into female organs.

Cunha and his colleagues have developed a very elegant procedure, referred to as "tissue recombination", to explore the role of mesenchyme in transmitting androgenic influences to the epithelium during embryogenesis. In one of their early experiments, the UGE was mechanically separated from the UGM and was recombined with skin mesenchyme (SM) or UGM. Under androgenic stimulation, the UGE/UGM hybrid underwent prostatic organogenesis while UGE combined with SM was keratinized, a process specific to the epithelial cells of epidermis (Cunha, 1972). Likewise, UGM combined with urinary bladder epithelium (BLE) of embryonic mice induced the epithelium to form prostate-like acini lined by simple columnar cells when the hybrid organ was grafted in an adult male host (Cunha and Lung, 1978). The UGM also has the capacity to direct re-differentiation of adult bladder or vaginal epithelium into prostatic epithelium (Cunha, 1975; Cunha and Lung, 1978). In addition, the importance of the mesenchyme was further highlighted using UGM and UGE of mice with testicular feminization (Tfm). In Tfm/Y male mice, although androgens are present in normal amounts, the internal secondary sex organs and external genitalia are female, due to a mutation in the androgen receptor gene (Gehring, Tomkins and Onho, 1971; Onho, 1977). When UGM from Tfm monolayers were recombined with male wild-type UGE, the UGE developed as a vagina, even though the epithelial cells contain a normal, functioning androgen receptor. In contrast, the wild-type UGM/Tfm UGE recombinant leads to prostatic morphological differentiation (Cunha and Lung, 1978). Similar experiments in the rat urogenital sinus led Lasnitzki and Mizuno (1979) to identical conclusions. These results have lead to the rather startling conclusion that the morphogenetic function of the epithelial cells of the prostate is dictated by stromal cells.

The nature of the stromal-epithelial relationship in the prostate has not yet been defined. Epithelial-stromal interactions in the adult

are more difficult to study due to the complexity of interactions that occur *in vivo*. A hypothesis has recently been proposed which suggests that the control of prostate homeostasis involves two growth factors: one stromally derived (SDGF), one epithelially derived (EDGF). An inhibiting factor of epithelial origin (EDIF), which interacts to insure that a stimulation-feedback loop exists between stromal and epithelial cells, is also involved (Tenniswood, 1986). This hypothesis further suggests that during development, androgens stimulate SDGF synthesis which in turn directs epithelial cell proliferation. EDGF is synthesized in the epithelial cells to promote the coordinated proliferation of stromal cells. The epithelial cells are assumed to synthesize androgen receptors just prior to puberty. Under androgenic control, EDIF will be produced to inhibit SDGF synthesis. Homeostasis will be established in the prostate and the rates of cell proliferation and cell death will be equal.

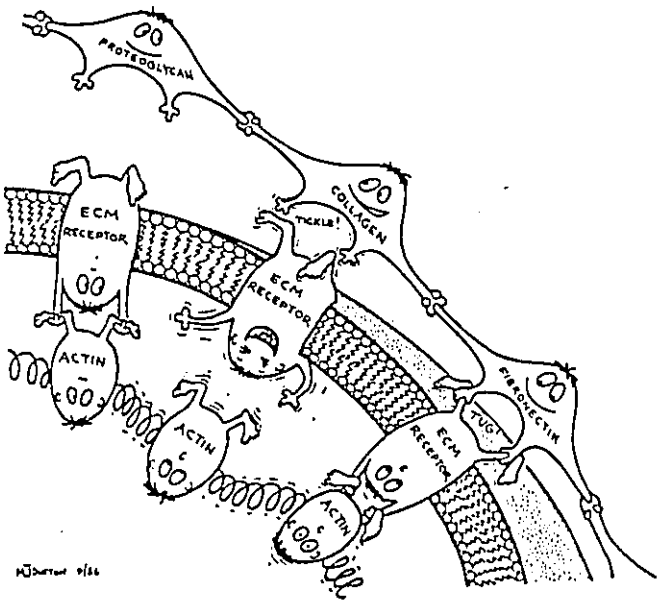
5. Stromal-Epithelial Physical Links:

The nature of the stromal and epithelial factors remain to be identified. One of the most likely mechanisms involves the interaction between stromal and epithelial cells via the extracellular matrix (ECM) and the cell cytoskeleton.

5.1 Extracellular Matrix

The ECM is made of glyco- and sulfo-protein components secreted by both stromal and epithelial cells. The principal components of the ECM are collagen, proteoglycans, heparan sulfate (Hay, 1981). Two other components of the ECM are probably important in signal transduction. Fibronectin, which is principally synthesized by the stromal cells, acts as a linker between the stromal cells and the ECM. The fibronectin molecule possesses several binding domains, each of which binds specifically either to receptors on the cell membrane or to components of the ECM (Yamada, 1981). Similarly, another important component of the ECM, laminin, possesses numerous binding domains which allows a physical

interaction between the cell membrane and the ECM. However, laminin is not synthesized by stromal cells (Foidart, Bere, Yaar, Rennard, Gullino, Martin and Katz, 1980) and, therefore, the prostatic laminin is synthesized uniquely by epithelial cells. The binding of fibronectin and laminin to cell membranes has been extensively studied during the past decade.



Trends in Biochem. Sci., 11, p. 484 (1986)

Extracellular fibronectin and intracellular actin have been shown to be linked physically by at least one transmembrane protein, integrin (Tamkun, DeSimone, Fonda, Patel, Buck, Horwitz and Hynes, 1986) and by the cytoskeletal proteins vinculin (Burridge and Feramisco, 1980; Singer, 1982) and talin (Burridge and Cornell, 1983; Horwitz, Duggan, Buck, Beckerle and Burridge, 1986). Laminin has been shown to interact with integrin (Tamkun *et al.*, 1986) and also with another fibronectin binding protein (Horwitz, Duggan, Greggs, Decker and Buck, 1985), as well as with several specific laminin-binding proteins isolated from the plasma membrane of tumor cells (Malinoff and Wicha, 1983), macrophages (Huard, Malinoff and Wicha, 1986) and neutrophils (Yoon, Boxer, Mayo, Yang and Wicha, 1987).

An analysis of the primary and secondary structure of integrin has identified a potential site for tyrosine phosphorylation in its cytoplasmic domain, and it has been suggested that phosphorylation of integrin affects its interactions with the cell cytoskeleton (Tamkun *et al.*, 1986).

It has been demonstrated that not all stromal cells synthesize the

same ECM molecules *in vitro* and that they may modify the array of ECM proteins produced in response to changes in their environment (Conrad, Dessau and von der Mark, 1980; Hay, 1980). The numerous proteins of the ECM allows a great amount of variability in its composition and, therefore, it has been often suggested that this provides a mean to transmit information from one cell type to the other. It was shown that, in the rat ventral prostate, collagen synthesis from stromal cells is stimulated by androgens (Müntzing, 1981). In addition, it was demonstrated that within two weeks following castration of guinea pigs, the collagen content of the ventral prostate is reduced by 36% (Mariotti and Mawhinney, 1981).

5.2 Cytoskeleton and Karyoskeleton

The interconnection of the ECM components with the plasma membrane (through fibronectin and laminin) and with the cell cytoskeleton (by integrin, vinculin and talin) ensures a physical link between stromal and epithelial cells in the prostate.

The cell cytoskeleton includes three major fibrous protein systems, microtubules, microfilaments and intermediate filaments which interact with each other by means of "associated proteins". Microtubules and microfilaments are produced by homopolymerization of their respective subunits, tubulin and actin. In contrast, intermediate filaments are composed of several subunits, which vary according to the cell type (see section 5.3).

Early descriptions of the cytoskeleton depicted it as a static complex of proteins whose function was to maintain cellular integrity. However, little by little, these low solubility filaments were found to be involved in the dynamic functioning of the cell as well. The protein synthetic machinery (mRNA, initiation factors, aminoacyl-tRNA synthetase complexes and the CAP binding protein) was found associated with the cytoskeletal framework during mRNA translation (Lenk, Ransom, Kaufmann and

Penman, 1977; Ornelles, Fey and Penman, 1986). Further investigation led to the observation that cytoskeletal fibres, which span the entire cytoplasm, are in fact physically linked to the plasma membrane at one extremity and to the karyoskeleton (or nuclear matrix) at the other (Goldman, Goldman, Green, Jones, Lieska and Yang, 1985; Georgatos and Blobel, 1987). The nuclear matrix is known to direct the functional organization of DNA into domains. It is associated with specific DNA sequences and therefore provides sites for the specific control of nucleic acid transcription (Nelson, Pienta, Barrack and Coffey, 1986).

Therefore, these findings suggested that the ECM is linked to the karyoskeleton through the cytoskeleton. This led Gospodarowicz, Greenburg and Birdwell (1978) to propose that the ECM determines the shape of the cell and regulates its proliferative potential. Others (Penman, Fulton, Capco, Ben Ze'ev, Wittelsberger and Tse, 1981; Isaacs, Barrack, Isaacs and Coffey, 1981) have suggested that the interactions between the ECM and the cell membrane are transmitted to the nuclear matrix via the cytoskeleton by a process referred to as "tensegrity" (Ingber and Jamieson, 1985). Folkman and Moscona (1978) have correlated cell shape with DNA synthesis and cell growth. More recently, casein gene expression *in vitro* has been found to be dependent on the collagenous substrata on which mouse mammary epithelial cells were grown (Lee, Parry, Bissell, 1984; Lee, Lee, Kaetzel, Parry and Bissell, 1985). Blum and Wicha (1988) have demonstrated that the expression of laminin induced milk protein genes in rat mammary cultures depends on the integrity of the cytoskeleton and suggests that laminin exerts its effects on gene expression through the cytoskeleton. The importance of overall cell morphology in regulating tissue-specific gene expression has been clearly demonstrated, since liver specific gene expression and cell morphology have been closely related to the nature of the culture substratum (cell-matrix interactions) as well as to the density of growing cells (cell-cell interactions) (Ben Ze'ev, Robinson, Bucher and Farmer, 1988).

These findings provide a general scheme of how stromal-epithelial

interactions may occur in the prostate, since, through the structural networks, the nuclei of both cell types are linked. Although no clear answers are yet available on how androgens act to mediate their effects, androgen binding sites have been identified on the nuclear matrix in the rat ventral prostate (Barrack and Coffey, 1982; Lefebvre, Howell and Golsteyn, 1985). It may appear that the attachment of the androgen to its receptor causes structural changes in the cytoskeletal components of the cell and therefore transmit a signal to the neighbouring cells.

5.3 Intermediate Filaments

Cytoskeletal filaments are well conserved among species. However, while microtubule and microfilament subunits are highly conserved among different cell types and tissues, intermediate filament subunits vary according to the cell type. To date, five classes of intermediate filaments (IF) have been described and are currently characterized at the biochemical and functional levels (Lazarides, 1980, 1982; Osborn and Weber, 1986). Neuronal cells synthesize up to three neurofilaments, astrocytes contain only one glial fibrillary acidic protein, mesenchymal cells express vimentin and muscle cells contain desmin while a complex family of at least 20 cytokeratins (CKs) are present in epithelial cells (Moll, Franke, Schiller, Geiger and Krepler, 1982). It has been recently proposed that there is a sixth class of IF. Although the lamins compose the filaments of the inner nuclear membrane and are common to all cell types, their structure is very similar to that of the cited IFs and it has been proposed that the lamins A, B and C also belong to that family (Hoger, Krohne and Franke, 1988).

In addition to their high level of complexity, IF proteins are among the most insoluble constituent of the cells. Extraction of tissue or cultured cells with high salt solutions and nonionic detergents removes membranes and most other proteins and cellular components including microfilaments and microtubules. What remains is a "skeleton" consisting largely of IFs.

These and other cytoskeletal filaments provide a link between the plasma membrane and the nucleus. They have been shown to be attached to the karyoskeleton, through nuclear pores, associated with the lamins (Lazarides, 1980; Jones, Goldman, Steinhert, Yuspa and Goldman, 1981; Georgatos and Blobel, 1987; Carmo-Fonseca, Cidadao, David-Ferreira, 1987; Katsuma, Swierenga, Marceau and French, 1987). In epithelial cells, the IFs span the entire cytoplasm and terminate at the plasma membrane, where they converge towards the desmosomal plaques, which are involved in the tight association between adjacent cells. The two major proteins of the desmosomes, which interact with the intermediate filaments, are desmoplakin I and desmoplakin II (Steinert and Roop, 1988).

As mentioned above, the human cytokeratin family is composed of at least 20 polypeptides (Moll *et al.*, 1982). The identification of the numerous cytokeratins has required the analysis of the insoluble polypeptides of a large number of tissues since each tissue or cell type expresses only a small subset of the known cytokeratins. The cytoskeletal content of epithelial cells may vary depending on cell type, period of embryonic development, cellular growth environment, disease state or histological differentiation (Moll *et al.*, 1982; Woodcock-Mitchell, Eichner, Nelson and Sun, 1982; Nelson and Sun, 1983; Green, Fuchs and Watt, 1982). The molecular weights of the human cytokeratins (CKs) range from 40 to 68 kDa and the pI from 5 to 8.5 (Moll *et al.*, 1982). According to their charge, their immunological reactivity to two monoclonal antibodies, AE1 and AE3, and to tryptic peptide maps, the CKs have been subdivided into acidic (A) and basic (B) groups (Woodcock-Mitchell *et al.*, 1982; Tseng, Jarvinen, Nelson, Huang, Woodcock-Mitchell and Sun, 1982; Schiller, Franke and Geiger, 1982). CKs of the A group have a pI between 4.9 and 5.7 and all share an AE1 determinant, while CKs of the basic group have a pI ranging between 6 and 8.5 and all react with the antibody AE3 (Woodcock-Mitchell *et al.*, 1982; Eichner, Bonitz and Sun, 1984; Sun, Eichner, Schermer, Cooper, Nelson and Weiss, 1984). Acidic and basic cytokeratins are structurally related since CK filament formation involves

the polymerization of a member of the A family with one of the B family (Sun *et al.*, 1984; Kulesh and Oshima, 1988). Except for the smallest of the cytokeratin polypeptide (40 kDa in human, CK 19), all acidic cytokeratins have a basic homologue which is on average of 8 kDa larger (Sun *et al.*, 1984). Analysis of the primary and secondary structure of CKs has revealed that this size difference is due to two well conserved additional protein domains in the basic polypeptides (Steinert, Steven and Roop, 1985). The level of each cytokeratin within a pair appears to be coupled, that is the amount of each member of one CK pair may be coordinately regulated (Fuchs, Hanukoglu, Marchuck, Grace and Kim, 1985; Domejoud, Jorcano, Breuer and Alonso, 1988; Kulesh and Oshima, 1988).

5.4 Cytoskeletal Content as Marker of Epithelial Cell Differentiation

As mentioned previously, the cytoskeletal content is highly dependent on the cell type and its state of differentiation. Sun *et al.* (1984) have provided an extensive review of the expression of cytokeratin pairs in relation to their tissue specificity. The smallest polypeptide of each subfamily, the basic 52 kDa (CK 8) and the acidic 45 kDa (CK 18) form a pair whose expression is characteristic of simple epithelium (Moll *et al.*, 1982; Sun *et al.*, 1984) and is the first cytokeratin pair found in the embryo (Jackson, Grund, Schmid, Bürki, Franke and Illmensee, 1980; Jackson, Grund, Winter, Franke and Illmensee, 1981; Duprey, Morello, Vasseur, Babinet, Condamine, Brulet and Jacob, 1985). Larger cytokeratin pairs such as basic 58 kDa (CK 5) and acidic 50 kDa (CK 14) are not synthesized in simple epithelium but rather are components of stratified squamous epithelium, such as epidermal basal cells (Sun *et al.*, 1984; Woodcock-Mitchell *et al.*, 1982; O'Guin, Galvin, Schermer and Sun, 1987). Based on such observations, each cytokeratin pair has been proposed to be a marker for a particular cell type or state of differentiation. With respect to the examples cited above, CK 8/18 pair is thought to be a marker of simple epithelium while CK 5/14 pair is a marker for all stratified epithelia (Moll *et al.*, 1982; Tseng *et al.*, 1982; Nelson *et al.*, 1984).

The complexity of the cytokeratin family is striking. Several groups of investigators have used the epidermis as a model to study the differential expression of the CKs. The epidermis consists of several layers of epithelial cells which become more specialized as they move further towards a suprabasal location. The cytokeratin content of these cells changes with their physical movement (Fuchs and Green, 1980; Banks-Schlegel, 1982). It has been proposed, based on the epidermis development, that an evclutionary pathway of cytokeratins accounts for the increasing size of the CK pairs with the cell type in which they are expressed (Sun *et al.*, 1984). In the early embryo, the epidermis consists of a simple epithelium containing the smallest CK pair (8/18). During the development of epidermis stratified epithelia appears, probably due to the piling up of simple epithelium. Larger cytokeratin pairs are synthesized, due to the induction of transcription of new CK genes (Fuchs, Tyner, Giudice, Marchuck, RayChaudhury and Rosenberg, 1987). This has led to the proposition that smaller CKs are characteristic of "primitive" or less differentiated epithelial cells while larger CK pairs populate more "complex", or differentiated cells. This hypothesis has received much attention and increasing evidence supports it (Dale, Holbrook, Kimball, Hoff and Sun, 1985; Lane, Bartek, Purkis and Leigh, 1985; Van Muijen, Warnaar and Ponec, 1987). Therefore, cytokeratins are suggested to be markers of the state of differentiation.

5.5 Cytokeratins of the Human and Rat Prostates

Cytokeratin content of the human prostate has been characterized biochemically and immunologically by few investigators. Luminal cells, lining prostatic ducts, were defined as simple epithelial cells, since they were found to contain CKs 8 and 18 (Nagle, Ahmann, McDaniel, Paquin, Clark and Celniker, 1985; Achtstätter, Moll, Moore and Franke, 1985; Wernert, Seitz and Achtstätter, 1987). Trace amounts of CKs 7 and 19 were detected in these cells and in basal cells (Wernert *et al.*, 1987). CK 5, on the other hand, was reported to be present in the prostate in very low

amounts (Achtstätter *et al.*, 1985). It was however not assigned to a particular cell type. At the time this study was initiated, no information was available on the CK content of the rat prostate. However, an analysis of the CK content of the rat ventral prostate was recently published (Verhagen, Aalders, Ramaekers, Debruyne and Schalken, 1988). Rat equivalent to human CK 18 was found in luminal cells, while CK 5 was also found present in the rat ventral prostate.

6. Heterogeneity in the Rodent Prostate:

Although castration causes a rapid decrease in prostate size (Coffey *et al.*, 1968) long term castration clearly indicates that a subpopulation of androgen independent prostatic cells remains. It has been shown that stromal cells are far less affected by androgen ablation than epithelial cells but histological analysis of the prostate from long term castrated rats has shown that small cuboidal epithelial cells are also present (Moore *et al.*, 1930). This indicates that some epithelial cells do not require androgens for their survival while others do. Therefore, the cellular heterogeneity in the rodent prostate appears more complex than just stromal/epithelial subpopulations.

The first observation suggesting that a certain heterogeneity exists in the androgen responsiveness of the cells in the rat ventral prostate was made by Burkhart (1942). She noticed that after castration and androgen re-administration to castrated rats:

> "Changes in the gland do not proceed at equal rates throughout the whole gland: both hypertrophy and mitosis start earlier along the periphery of the gland".

She suggested that:

> "Heterogeneity of response to androgens within the gland indicates an intimate relationship with the morphology of the gland as a whole".

However, her observations went essentially unnoticed until recently when the analysis of the behaviour of epithelial cells in different locations along the prostatic ducts revealed that the extremities (i.e. distal tips) are far more affected by androgen ablation than are cells at the region closer to the prostatic urethra (Sugimura *et al.*, 1986b).

When DNA synthetic activity was measured along the mouse prostatic ducts, it was found to be higher in the distal tips than in the proximal region during early postnatal development (10-15 days) and to be totally confined to distal tips later on (30 days). In adult animals, DNA synthesis is limited but it is still detectable in cells at the distal tips of ducts (Sugimura, Cunha, Donjacour, Bigsby and Brody, 1986c).

Androgen dependence of cell survival and DNA synthesis vary considerably on a regional basis within the prostate. Another aspect of the heterogeneity was identified by the observation that luminal epithelial cell shape vary along the ducts. Cells lining the ducts in the distal region are tall columnar while the proximal region is made of a small cuboidal epithelium (Cunha *et al.*, 1987).

7. Research Objectives:

While biochemical studies using prostatic homogenates and histological studies using prostate random sections have provided a great deal of information regarding the biochemistry, morphology and androgen dependence of the prostate, a better understanding of the gland now requires a consideration of the architectural heterogeneity of the prostate. The general purpose of this study was to further characterize the diversity found in the rat ventral prostate as well as to test the hypothesis that stromal-epithelial interactions may be mediated through the cytoskeleton.

I have attempted to:

1) Identify the intermediate filaments of the normal rat ventral

prostate.

2) Determine IF markers of the different epithelial cell types in normal rat ventral prostate.
3) Identify the changes that occur in intermediate filament components after castration of the rat.
4) Identify the cellular behaviour of the different cell types of the rat ventral prostate following castration.
5) Determine the distribution of IF components along the prostatic ducts in the normal rat.
6) Determine the behaviour of each cell type along the rat prostatic duct following castration and re-administration of androgens.

METHODS

1. Animals

Adult male Sprague-Dawley rats (250-300 g) were obtained from Charles River Inc. (Montréal, Québec). The animals were housed in groups of three or four in the Animal Care Facility of the Health Sciences Building at the University of Ottawa. The light cycle was 14 h on/10 h off. The rats were fed with Purina rat chow and water *ad libitum*. The rats were allowed to acclimate for at least four days before any study was started. The rats were castrated via the scrotal route under fluothane anesthesia. Where indicated, the rats were injected with 5α-dihydrotestosterone propionate (10 mg/ml in sesame oil) at a dose of 2 mg/day. They were sacrificed at indicated times by cervical dislocation.

2. Cytokeratin Extraction

The extraction of cytokeratins from the rat ventral prostate procedure was based on the method of Aschtstätter, Hatzfeld, Quinlan, Parmelee and Franke (1986). The ventral prostate was excised, the fat capsule removed and the prostates were immediately plunged in precooled isopentane. The tissues in isopentane were stored in liquid nitrogen until all the samples were available for the extraction. The protein extractions were performed in parallel for each group of ventral prostates. A starting material weight of 0.5g for each group of ventral prostate was used. The samples were minced and homogenized in 10 volumes of cold "homogenization buffer" (96.0 mM NaCl, 8.0 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1.5 mM KCl, 10 mM Na_2EDTA, 1.0 mM β-mercaptoethanol, 0.4 mM PMSF, pH 6.8) in a Dounce homogenizer by 15 strokes with a loose fitting pestle. The homogenates were filtered through four layers of gauze, and 3 volumes of cold "very high salt buffer" (0.2 M NaCl, 2.0 M KCl, 10.0 mM Tris, 1.0 mM β-mercaptoethanol, 0.4 mM PMSF, pH 7.4) were added. This solution was stirred for 30 min. at 4°C. The suspension was homogenized by 10 additional strokes in the same homogenizer and centrifuged at 9250 rpm for 20 min. at 4°C in a Beckman JA-20 rotor. The pellet was homogenized with 10 strokes in the same homogenizer in the same volume of cold "high salt buffer" (0.14 M NaCl, 1.5 M KCl, 10.0 mM Tris, 5.0 mM Na_2EDTA, 0.5 % (w/v) Triton X-100, 1.0 mM β-mercaptoethanol, 0.4 mM PMSF, pH 7.6) relative to

the volume of very high salt buffer used in the previous step. The suspension was stirred for 30 min. at 4°C and centrifuged as above. The pellet was suspended in high salt buffer, homogenized, stirred and centrifuged a second time. The final pellet was washed in PBS (140 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4) and solubilized in a modified sample buffer (2.0% (w/v) SDS, 20% (v/v) glycerol, 5 mM Tris-HCl(pH 7.4), 2.0 mM Na_2EDTA, 4.0 mM PMSF). The solubilization was aided by homogenization and sonication for 10 sec. at low intensity with the Biosonik III sonicator (Bronwill Scientific, Rochester, NY). The sample was then centrifuged in an Eppendorf microfuge for 10 min. at 12,000 rpm at room temperature. The supernatant, which contained the solubilized cytokeratin fraction, was characterized.

The protein concentration of each preparation was determined by the Folin phenol method (Paterson, 1983), a modification of the original method of Lowry (Lowry, Rosebrough, Farr and Randall, 1951).

3. Electrophoresis

The Bio-Rad Mini-Protean II electrophoresis system was used to perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the cytokeratin enriched protein samples. Eight μg of each protein sample in modified sample buffer was mixed with 2 μl of each 1 M Tris-HCl (pH 7.4) and β-mercaptoethanol and the volume of each sample was brought up to 15 μl with the modified sample buffer and loaded onto an 8% polyacrylamide gel. The samples were electrophoresed at a constant current of 20 mA in the stacking gel and 40 mA in the separating gel. Molecular weight markers, rabbit muscle actin and vimentin standards were solubilized in sample buffer (20% (v/v) glycerol, 2.0% (w/v) SDS, 0.1 M Tris-HCl pH 7.4, 2.0 mM Na_2EDTA, 1.43 M β-mercaptoethanol, 4.0 mM PMSF, bromophenol blue crystals) and were co-electrophoresed with the cytokeratin samples. The proteins were visualized by staining for one hour with agitation in Coomassie Blue solution (25% (v/v) isopropanol, 10% (v/v) glacial acetic acid, 0.5% (w/v) Coomassie blue dye). To perform Western analysis, the proteins were transferred from the polyacrylamide

gel to 0.45μ nitrocellulose filters without prior staining. The transfer was achieved in the mini Trans-Blot electrophoretic transfer cell at a current of 0.3 A for 1 h, in a transfer buffer containing 25 mM Tris, 192 mM glycine and 20% (w/v) methanol.

4. Western Analysis

Nitrocellulose filters were rinsed in water. The efficiency of transfer was checked by staining for 5 min. with agitation in Ponceau red solution (0.2% (w/v) Ponceau Red S, 3.0% (w/v) trichloroacetic acid) and destaining in distilled water to visualized the protein bands. Filters were then completely destained in PBS for 10 min. and were either stored at -20°C in a sealed plastic bag until use or immediately incubated for one hour in 0.5% (w/v) skim milk powder in PBS (Blotto) at room temperature. The filters were subsequently incubated overnight at 4°C with one of the antibodies listed in Table I at its working dilution in 0.5% Blotto. After three washes of 10 min. at room temperature in 0.1% (v/v) Tween 20 in PBS the filters were incubated for 3 h at room temperature with the appropriate goat anti-mouse antibody (directed against IgG or IgM antibodies) conjugated to horseradish-peroxidase (diluted 1:2000 in PBS). Three washes of 20 min. in 0.1% Tween 20 in PBS were followed by a rinse in Tris buffer saline (0.9% (w/v) NaCl, 10.0 mM Tris, pH 7.4). All the above incubations were performed with agitation.

To check for non-specific binding of goat anti-mouse antibodies to the nitrocellulose membrane, filters were sequentially incubated with 0.5% Blotto and with goat anti-mouse IgG or IgM conjugated with horseradish-peroxidase and washed as above.

Antibody binding was detected in 0.05% (w/v) 4-chloronaphtol, 16.6% (v/v) methanol in Tris buffer saline, pH 7.4 to which H_2O_2 was added to a final concentration of 0.01% (v/v). To halt the peroxidase reaction, the filter was immersed in water for 10 min. and dried on Whatman paper. Filters were stored in the dark to reduce fading of the signal.

Antibody	Against[a]	Type	Working Dilution[b]	
			Immuno-fluorescence	Western
PKK1	CKs 8, 18, 19	Mouse IgG	1:10	1:2000
PKK2	CKs 7, 16 17, 19	Mouse IgG	1:10	1:2000
PKK3	CK 18	Mouse IgG	1:10	———
EAB 903	CKs 1, 5 10, 11	Mouse IgG	1:4000	1:1000
CK 8.60	CKs 10, 11, (1)	Mouse IgG	1:20	1:500
312C8-1	CK 14	Mouse IgM	1:1000	1:100
C4	Actin	Mouse IgG	1:400	1:2000
DP-2.17	Desmo-plakin I	Mouse IgG	1:10	1:100

5. Tissue Samples for Random Sections

The ventral prostates of individual rats were excised and teased free of the fat capsule. The prostates were covered with Tissue Tek and quickly plunged in isopentane precooled in liquid nitrogen. The tissues were stored in liquid nitrogen until sectioning into 5 μm thick sections with a Damon microtome at -20°C. The sections were placed on a coverslip and air dried for 10 min. at room temperature. The sections were rinsed in PBS, fixed for 10 min. in cold acetone, rinsed again in PBS and stained for analysis by immunofluorescence.

6. Tissue Samples for Longitudinal Sections

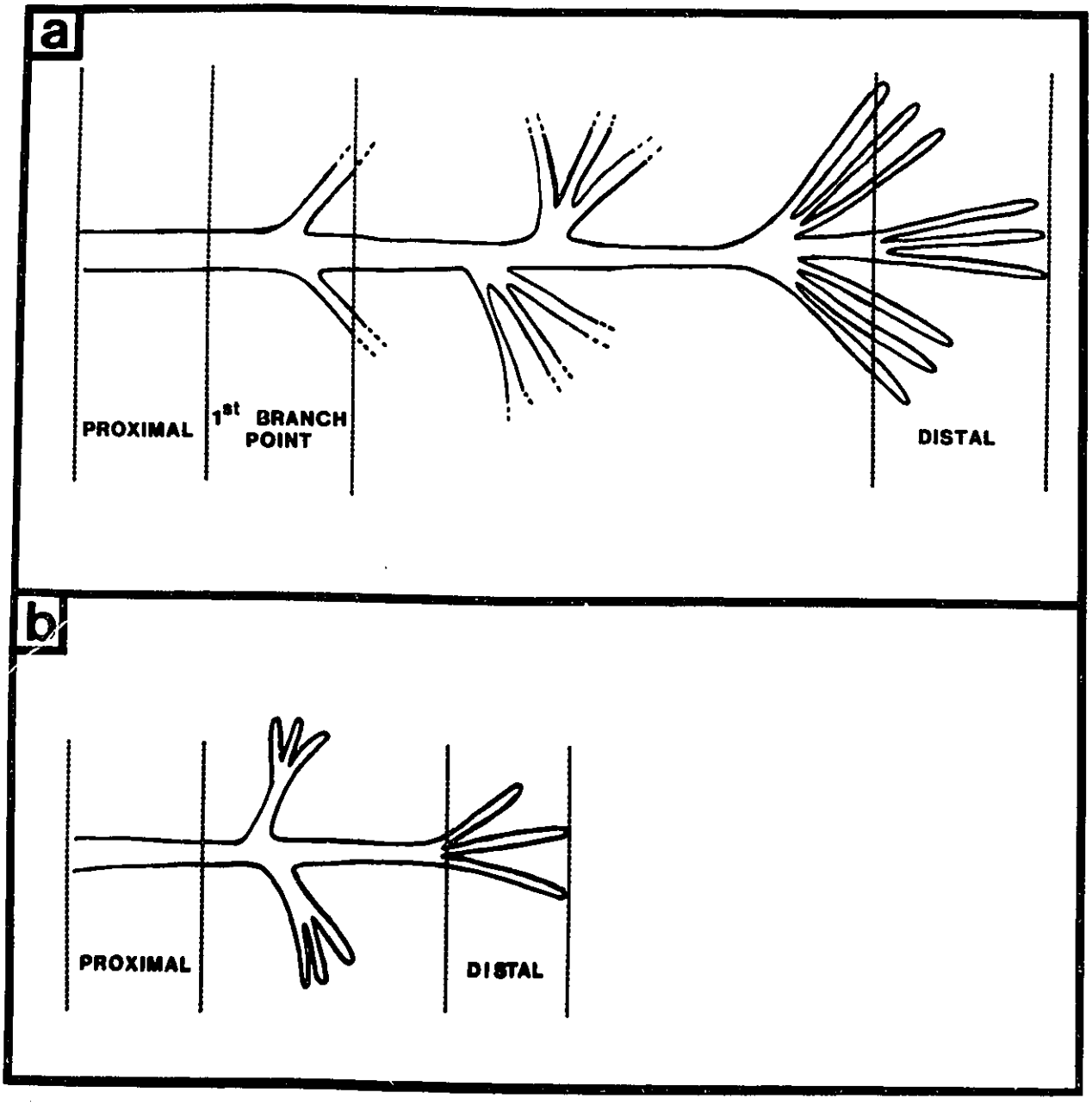
For these experiments, the ventral prostate was excised with the bladder for orientation purposes. The fat capsule of the prostate and the distal portion of the bladder were removed. The prostate was micro-dissected into individual ducts as previously described (Sugimura *et al.*, 1986a). The tissue was rinsed in calcium-magnesium free (CMF) Hank's solution (140 mM NaCl, 5.4 mM KCl, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 1 g/L glucose) and immersed in a solution of 1% collagenase Type IV (in CMF Hank's) for 10 min. at room temperature to allow the digestion of the connective tissue. During this time, the prostatic ducts were delicately teased apart with fine electron microscopy forceps and a surgical knife under a Zeiss stereomicroscope. The prostate was transferred to CMF Hank's and further micro-dissected. The remaining portion of the bladder was removed. Portions of proximal (closer to the urethra) and distal (closer to the tips) ducts were excised and each was placed on a small piece of stainless steel covered with Tissue Tek. Figure 1 shows a schematic representation of micro-dissected ducts from normal and castrated animals. The ducts of each piece were teased apart, laid straight on the metal piece and frozen at -20°C. Immediately after freezing, 5 μm tissue sections were prepared as for the random sections and air dried for 30 min. at room temperature. The sections were rinsed in PBS, fixed for 10 min. in cold acetone and rinsed again in PBS.

Figure 1: Schematic representation of micro-dissected ducts from rat ventral prostates.

Panel a: Prostatic ducts from normal rats

Panel b: Prostatic ducts from castrated rats

The labelled areas indicate the relationship between proximal and distal regions as mentioned in the text.


a
PROXIMAL
1st BRANCH POINT
DISTAL
b
PROXIMAL
DISTAL

7. Immunofluorescence staining

The sections were incubated in a humidity chamber at room temperature or at 37°C for 1 to 1.5 h, according to the recommendation of the supplier, with one of the antibodies listed in Table I, diluted in PBS to the appropriate working dilution. The sections were washed three times for four min. each in PBS at room temperature and subsequently incubated for 1.5 h at 37°C in a humidity chamber with the appropriate goat anti-mouse secondary antibody conjugated to fluorescein isothiocyanate (FITC) or rhodamine isothiocyanate (RITC) (both diluted 1:100 in PBS). The sections were washed three times for four min. in PBS and mounted on a microscope slide in 50% glycerol in PBS containing 1% p-phenylenediamine to reduce fading of the fluorescence. For double immunostaining, the sections were sequentially incubated with PKK1 or EAB 903, a goat anti-mouse IgG antibody conjugated to FITC, 312C8-1 and finally with a goat anti-mouse IgM antibody conjugated to RITC. The incubation and washing times were as above.

Controls for non-specific binding of secondary antibodies were prepared by incubating prostate sections under the same conditions as above with one of the goat anti-mouse IgG or IgM secondary antibodies and mounted. For double immunostaining, the non-specific binding was checked by incubating sections with PKK1 or EAB 903 followed by goat anti-mouse IgG-FITC conjugated antibody and then incubated with goat anti-mouse IgM-RITC conjugated and mounted.

The antibody staining was visualized using a Zeiss Universal microscope equipped for epifluorescence with a Xenon light and appropriate filters for FITC and RITC. All pictures were taken on Ilford XP1 400 black and white films or Kodak Ektachrome 400 colour films. Black and white pictures were printed on Kodak paper and color pictures on Cibachrome paper.

RESULTS

1. Biochemical Characterization of the Rat Ventral Prostate Cytoskeletal Proteins:

To determine the composition of the structural proteins of the prostate and the changes that may occur upon androgen ablation and re-administration, the insoluble components of the prostate were extracted. A sequential separation of soluble from insoluble proteins with increasing salt concentrations in the extraction buffers allowed the recovery of a protein extract enriched in cytokeratins but which also contained desmosomal proteins and the actin subunits of the microfilaments. The cytoskeletal protein composition of ventral prostates from intact, castrated and androgen re-administrated rats was analyzed by SDS-polyacrylamide gel electrophoresis.

The cytoskeletal elements of the ventral prostate from intact rats and from rats castrated five and eight days previously were separated on SDS-PAGE. The cytoskeletal components of the ventral prostate from a second group of rats, which were castrated, left for 14 days and then injected for three and eight days with 2 mg/day testosterone propionate, were analyzed on the same gels. The cytokeratin enriched protein extract from the intact rat ventral prostate (Fig. 2, Panel A, lane 2) is composed of at least 13 major elements, among which a minimum of eight proteins belong to the cytokeratin family (molecular weight ranging between 40 and 68 kDa). Actin subunits are located at 43 kDa, as confirmed by the comigration of a commercially purified actin sample (Fig. 2, Panel A, lane 1) and by the Western analysis with an anti-actin monoclonal antibody (Fig. 3, lane 1 and 2). By Western analysis using PKK1 antibody, which was raised against human CKs 8, 18 and 19, protein bands with a molecular weight of 51.5 and 49.5 kDa are identified in the normal rat prostate protein extract (Fig. 4, lane 2). According to their molecular weight and their immunoreactivity, these proteins are the rat equivalent to human CKs 8 and 18 respectively (rat CKs which correspond to human proteins will be referred to as "rat CK" followed by the number of the human CK, as catalogued by Moll *et al.*, 1982).

Figure 2: Coomassie Blue staining of cytoskeletal proteins from rat ventral prostate gland separated by SDS-PAGE.

Lane 1: Purified rabbit actin
Lane 2: Cytokeratin enriched protein extract from intact rat ventral prostate
Lane 3: Cytokeratin enriched protein extract from the ventral prostate of rats castrated 5 days previously
Lane 4: Cytokeratin enriched protein extract from the ventral prostate of rats castrated 8 days previously
Lane 5: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 3 days with testosterone propionate
Lane 6: Cytokeratin enriched protein extract from ventral prostates of rats castrated 14 days previously and injected for 8 days with testosterone propionate
Lane 7: Purified bovine vimentin

Panel A: Short run (1.5 h)
Panel B: Long run (4 h)

Molecular weight marker positions are indicated on the left. The position of the rat equivalent to CKs 8 and 18 are indicated by *dots*, that of desmoplakin I and II by a *star*, that of desmoglein by an *arrow*, that of actin by an *horizontal bar* and vimentin by an *arrow head*.

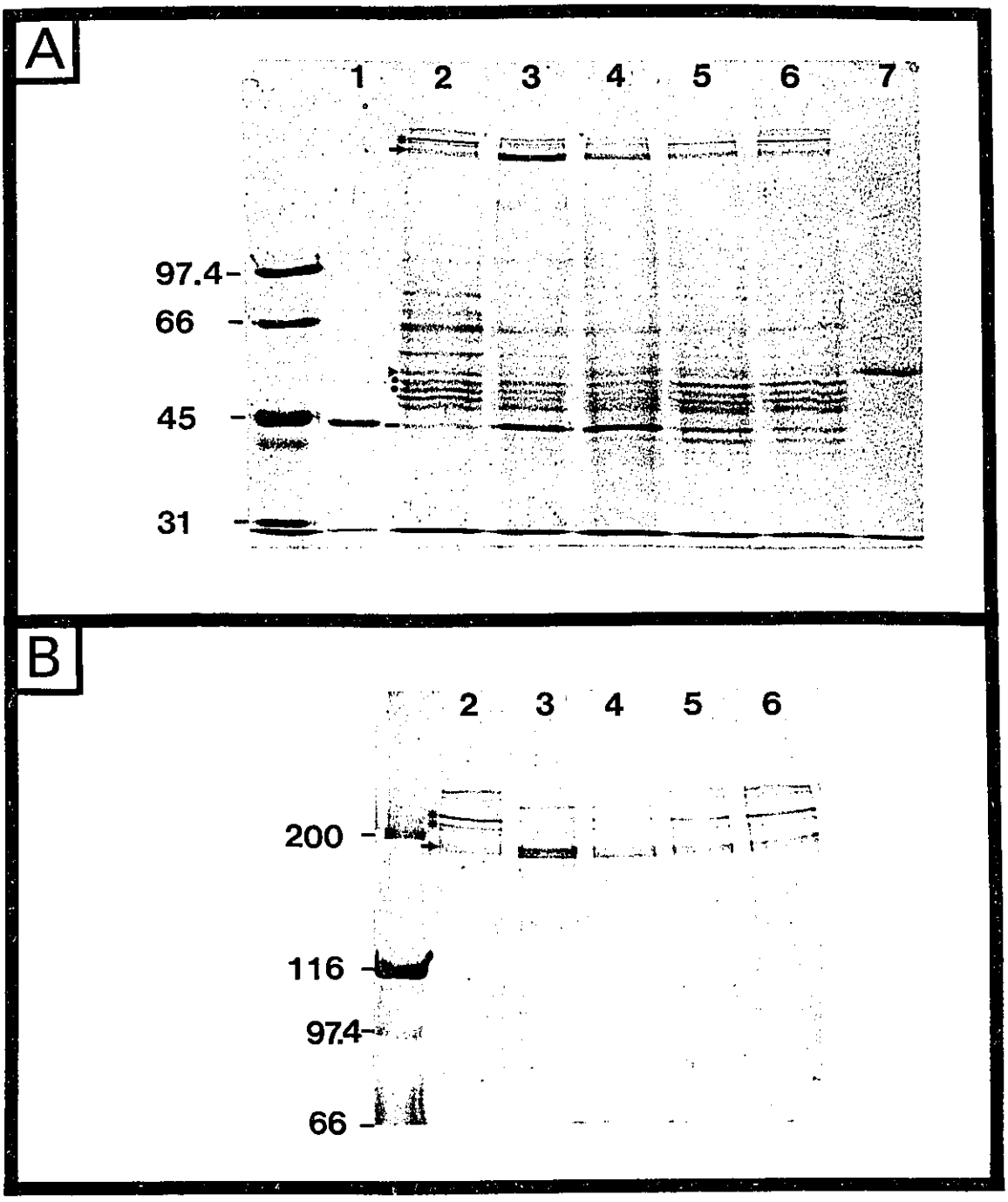
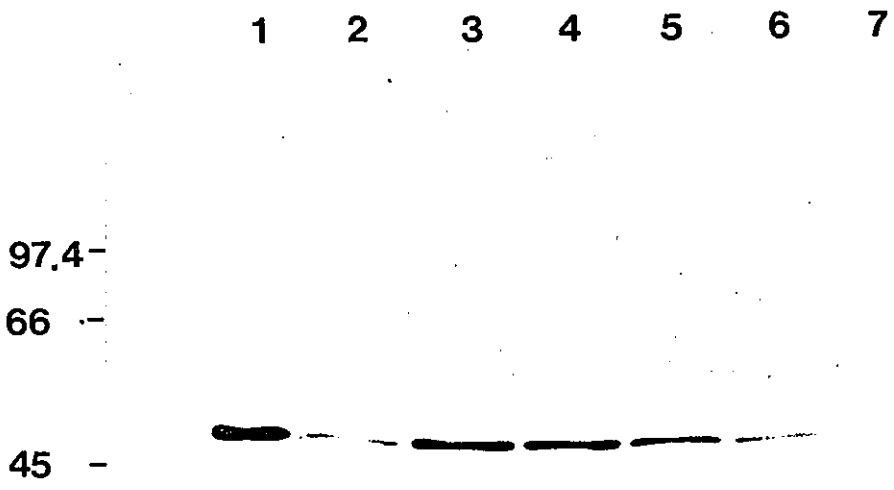

A
1
2
3
4
5
6
7
97.4
66
45
31
B
2
3
4
5
6
200
116
97.4
66

Figure 3: Western analysis of actin content of cytokeratin enriched protein extracts of the ventral prostate from intact, castrated and androgen re-administrated rats.

Lane 1: Purified rabbit actin
Lane 2: Cytokeratin enriched protein extract from intact rat ventral prostate
Lane 3: Cytokeratin enriched protein extract from ventral prostate of rats castrated 5 days previously
Lane 4: Cytokeratin enriched protein extract from ventral prostate of rats castrated 8 days previously
Lane 5: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 3 days with testosterone propionate
Lane 6: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 8 days with testosterone propionate
Lane 7: Purified bovine vimentin

Molecular weight marker positions are indicated on the left.


1
2
3
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5
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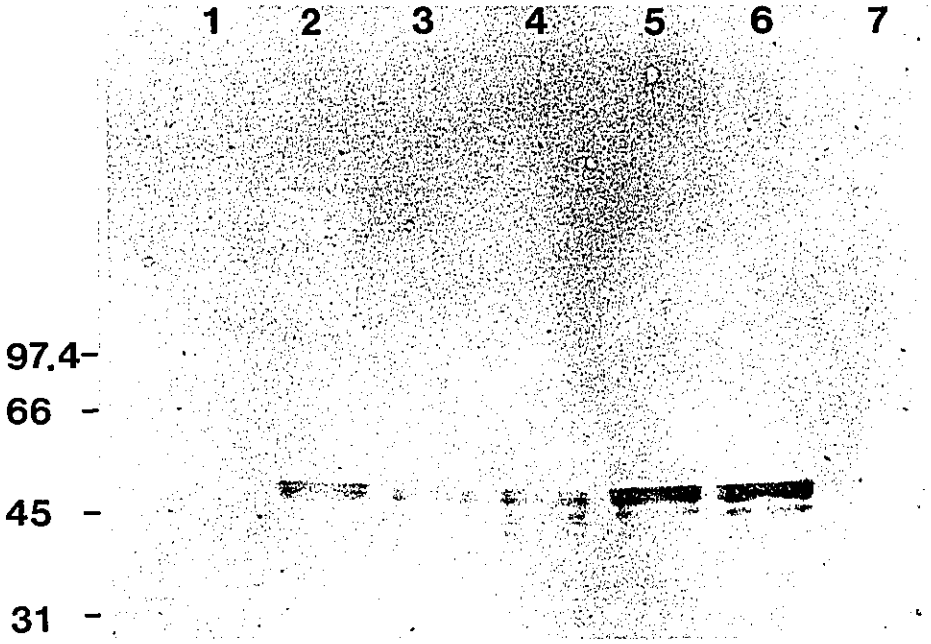
7
97.4
66
45

Figure 4: Western analysis of rat CKs 8, 18 and 19 with PKK1 antibody in cytokeratin enriched protein extracts of the ventral prostate from intact, castrated and androgen re-administrated rats.

Lane 1: Purified rabbit actin
Lane 2: Cytokeratin enriched protein extract from intact rat ventral prostate
Lane 3: Cytokeratin enriched protein extract from ventral prostate of rats castrated 5 days previously
Lane 4: Cytokeratin enriched protein extract from ventral prostate of rats castrated 8 days previously
Lane 5: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 3 days with testosterone propionate
Lane 6: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 8 days with testosterone propionate
Lane 7: Purified bovine vimentin

Molecular weight marker positions are indicated on the left.


1
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97.4
66
45
31

Similar attempts to identify the basal cell specific cytokeratins of intact rat ventral prostate extract by Western analysis with the antibodies were unsuccessful since neither of these antibodies reacted with cytokeratins on nitrocellulose (Fig. 5, lane 2).

High molecular weight proteins, which are components of the desmosomal plaques, were also identified in the rat ventral prostate. On the short migration gel (Fig. 2, Panel A) these proteins are stacked close to the origin. However, by a comparison of the band pattern with that published by Owaribe, Kartenbeck, Rungger-Brändle and Franke (1988), they could be identified as desmoplakin I and II and desmoglein (Fig. 2, Panel A). Their molecular weights calculated from a longer migration of a similar set of protein extracts with appropriate markers, confirm the identity of these bands (Fig. 2, Panel B).

While no antibody against rat vimentin was available, the co-migration of a purified bovine vimentin sample suggests that the 54.5 kDa protein band of the cytoskeletal extract is the IF characteristic of stromal cells, vimentin (Fig. 2, Panel A).

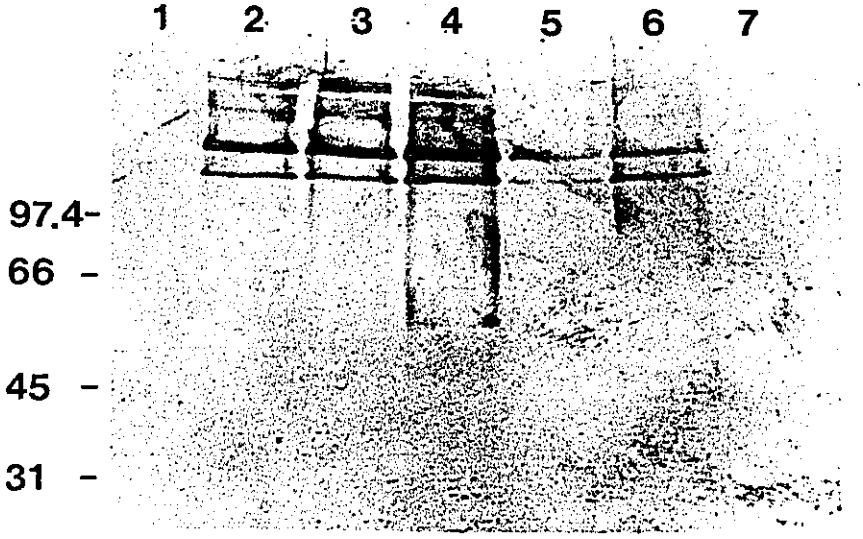
Castration is known to cause a dramatic decrease in size of the prostate, due to active cell death (Kerr and Searle, 1973; Sandford, Searle and Kerr, 1984). We were interested in looking at the changes occurring in the cytoskeletal components of the prostate and to determine if these changes were general to all cytoskeletal components or affecting only a small subset of them.

A comparison of the elements of the cytoskeletal extracts before and after castration of the rat reveals that changes occur rapidly among the structural proteins. Rat CKs 8 and 18 are less abundant (lane 3 and 4, Fig. 2 and Fig. 4). Five days following castration, this CK pair is barely detected although the two components are still represented in equal quantities. Eight days following castration, the amount of CKs 8/18 appeared to increase slightly over the level seen at five days after

Figure 5: Western analysis of rat basal cell cytokeratins with 312C8-1 antibody in cytokeratin enriched protein extracts of the ventral prostate from intact, castrated and androgen re-administrated rats.

Lane 1: Purified rabbit actin
Lane 2: Cytokeratin enriched protein extract from intact rat ventral prostate
Lane 3: Cytokeratin enriched protein extract from ventral prostate of rats castrated 5 days previously
Lane 4: Cytokeratin enriched protein extract from ventral prostate of rats castrated 8 days previously
Lane 5: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 3 days with testosterone propionate
Lane 6: Cytokeratin enriched protein extract from ventral prostate of rats castrated 14 days previously and injected for 8 days with testosterone propionate
Lane 7: Purified bovine vimentin

Molecular weight marker positions are indicated on the left.


1
2
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97.4-
66 -
45 -
31 -

castration but is still lower than in intact rat prostate. In addition, two other protein bands are detected, at very low levels, by the PKK1 antibody, with molecular weights of approximately 47 and 42 kDa. While the 42 kDa protein may be a degradation product due to its smaller size, the 47 kDa protein may be the rat CK 19, which PKK1 is able to detect. Moreover, like CKs 8/18, CK 19 is characteristic of simple epithelium and its presence in the cytoskeletal extract would not be surprising.

The immunoblot obtained by incubation with the basal cell specific antibody 312C8-1 revealed several protein bands of high molecular weight. This was the case for all lanes containing cytoskeletal extracts. However, it reacted with an additional protein band in the eight-day-castrated rat protein extract, of a molecular weight of 59.5 kDa, which may correspond to the rat equivalent of CK 14 (Fig. 5).

The desmosomal protein pattern shows significant variations after castration. The desmoglein content appears to increase in the prostate of rats castrated five days previously relative to the intact control, and then to decrease again by day eight. Desmoplakin I, and to a lesser extent desmoplakin II, dramatically decreases after castration (Fig. 2, Panel B, lane 2 and 3). On the other hand, the actin subunits show a marked increase five days following castration. This level is maintained in rats castrated eight days previously (Fig. 3, lane 3 and 4).

For all Western analysis, an appropriate control of non-specific binding of antibodies were performed and did not reveal any protein bands (Fig. 6).

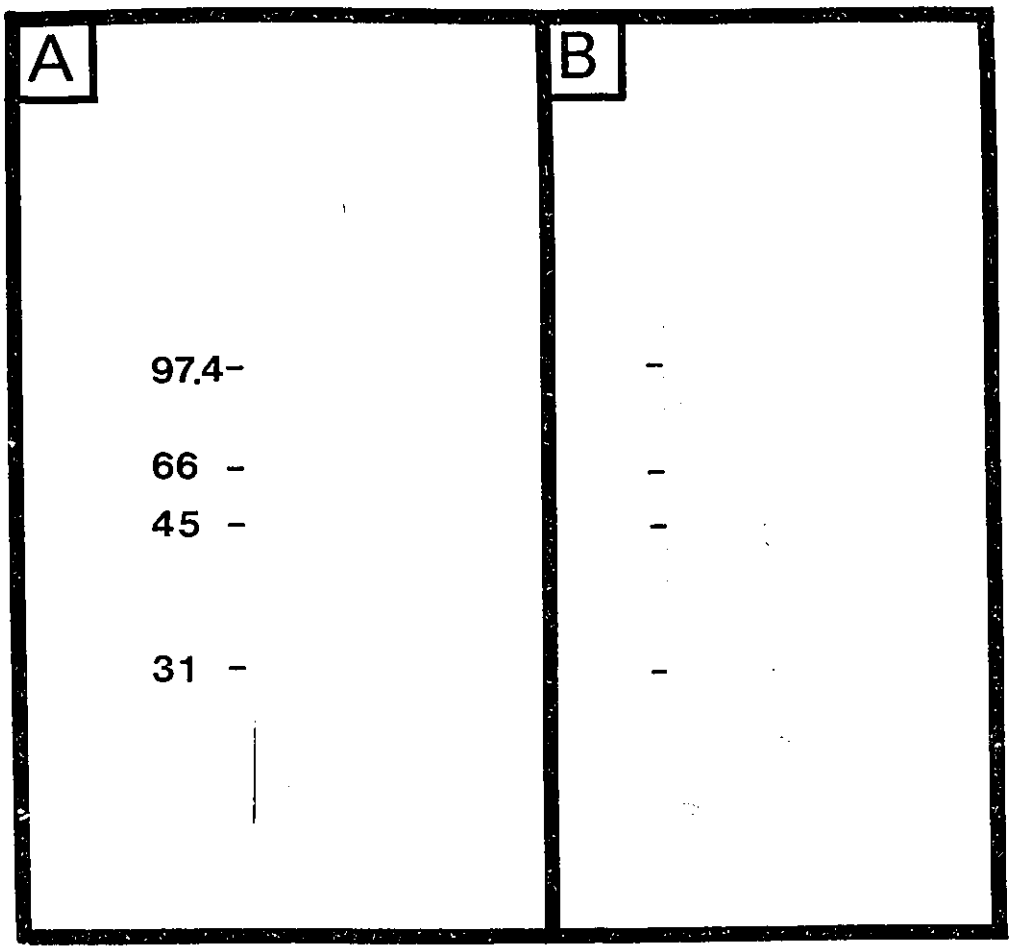
To determine whether androgen administration also restores the cytoskeletal components of the rat ventral prostate, rats castrated for 14 days were injected with 2 mg testosterone propionate daily and the cytoskeletal components were extracted from the ventral prostate after three and eight days of treatment. The 51.5 and 49.5 kDa cytoskeletal proteins (rat CKs 8/18) increase as soon as three days after androgen re-

Figure 6: Control of non-specific binding of secondary antibodies to nitrocellulose membrane.

Panel A: Non-specific binding of goat anti-mouse IgG antibody to a cytokeratin enriched protein extract transferred to a nitrocellulose filter

Panel B: Non-specific binding of goat anti-mouse IgM antibody to a cytokeratin enriched protein extract transferred to a nitrocellulose filter

Molecular weight marker positions are indicated on the left.


A
B
97.4-
66 -
45 -
31 -

administration. Both components increase in parallel (Fig. 4, lane 5 and 6). On the other hand, the 42 kDa component detected eight days after castration by PKK1 is not detectable as soon as three days after androgen administration. The 47 kDa protein (presumed to be CK 19) is increased significantly at both three and eight days following androgen replacement (Fig. 4, lane 5 and 6). This 47 kDa cytoskeletal protein band increases until at least eight days of androgen administration, in contrast to the CK pair 8/18 and to the actin levels which return gradually to nearly normal levels (Fig. 2, Panel A, lane 5 and 6; Fig. 3, lane 5 and 6). Similarly, desmosomal proteins are restored to normal levels (Fig. 2, Panel B, lane 5 and 6).

While this biochemical characterization did not identify all of the cytoskeletal components extracted from the rat ventral prostate, it demonstrates without a doubt that modifications in the cytoskeletal components of the prostate do occur after castration as the gland involutes. Since these changes do not follow the same pattern (some components decrease while others increases after castration), it indicates that cytoskeletal structures have different susceptibilities to androgens. It further suggests, but does not prove, that the heterogeneity of the rat ventral prostate extends to the structural organization of the cytoskeleton.

It was therefore of interest to determine if some of the cytoskeletal components were specific to individual cell types in the gland and to identify those CKs which could be used as markers to follow the cell behaviour and dynamics after androgen ablation and re-administration.

2. Intermediate Filaments Content of Epithelial Cells in the Rat Ventral Prostate :

Immunofluorescent staining of random sections of prostates from normal rats and rats castrated eight days previously shows that luminal

epithelial cells have a cytokeratin content characteristic of that of simple epithelium. The antibodies PKK1 and PKK3 are able to recognize the rat CKs 8, 18 and 19 and CK 18, respectively. The filaments stained by these antibodies span the entire cytoplasmic region of the tall columnar epithelial cells of the ventral prostate of control animals (Fig. 7, Panel a and c; Fig. 8, Panel a and c) as well as the small cuboidal luminal epithelial cells of the castrated rat (Fig. 7, Panel b and d; Fig. 8, Panel b and d). The basal cell specific antibody, EAB 903, which recognizes CKs 1, 5, 10 and 11, does not stain any filaments of the tall columnar cells in the intact rat ventral prostate, confirming their identity as simple epithelial cells (Fig. 9, Panel c). On the other hand, basal cells are specifically stained by EAB 903 in the normal ventral prostate. These cells are elongated or triangular, lie over, or intercalate between, luminal epithelium at the basal region (Fig. 9, Panel c). The staining pattern within these cells in normal and castrated rats is similar, although the abundance of basal cells is much greater in the castrated animals (Fig. 9, Panel d). In contrast, the antibody CK 8.60, which recognizes CKs 10 and 11 (and slightly CK 1), is unreactive to the cells of the ventral prostate (Fig. 10, Panels c and d) as well as to cytokeratin enriched protein extracts on nitrocellulose (not shown).

Appropriate controls of non-specific binding of the fluorescent secondary antibodies were made in parallel and do not show any staining of the prostate sections (Fig. 11).

Luminal and basal epithelium can therefore be differentiated on the basis of their cytokeratin content. While PKK1 and PKK3 are "markers" of luminal epithelial cells, EAB 903 is specific to basal cells in the rat ventral prostate.

The monoclonal antibody PKK2, which is specific for CKs 7, 16, 17 and 19, stains filaments sparsely distributed at the basal side of columnar epithelial cells in a similar fashion to the basal cell specific antibody EAB 903 in the intact rat ventral prostate (Fig. 12, Panels e and

Figure 7: Immunofluorescent staining with PKK1 of random transverse sections of the ventral prostate from intact and castrated rats.

Panel a: Section of ventral prostate from intact rat
b: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 80 μm

Panel c: Section of ventral prostate from intact rat
d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.

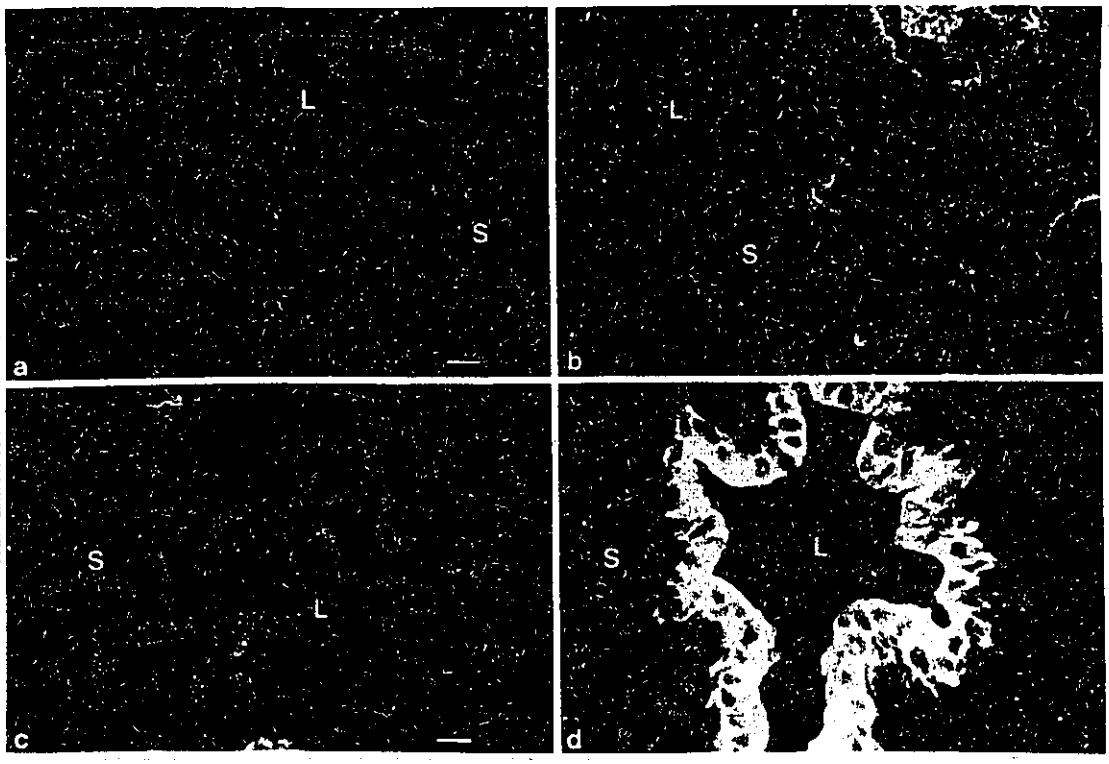

L
S
a
L
S
b
S
L
c
L
S
L
d

Figure 8: Immunofluorescent staining with PKK3 of random transverse sections of the ventral prostate from intact and castrated rats.

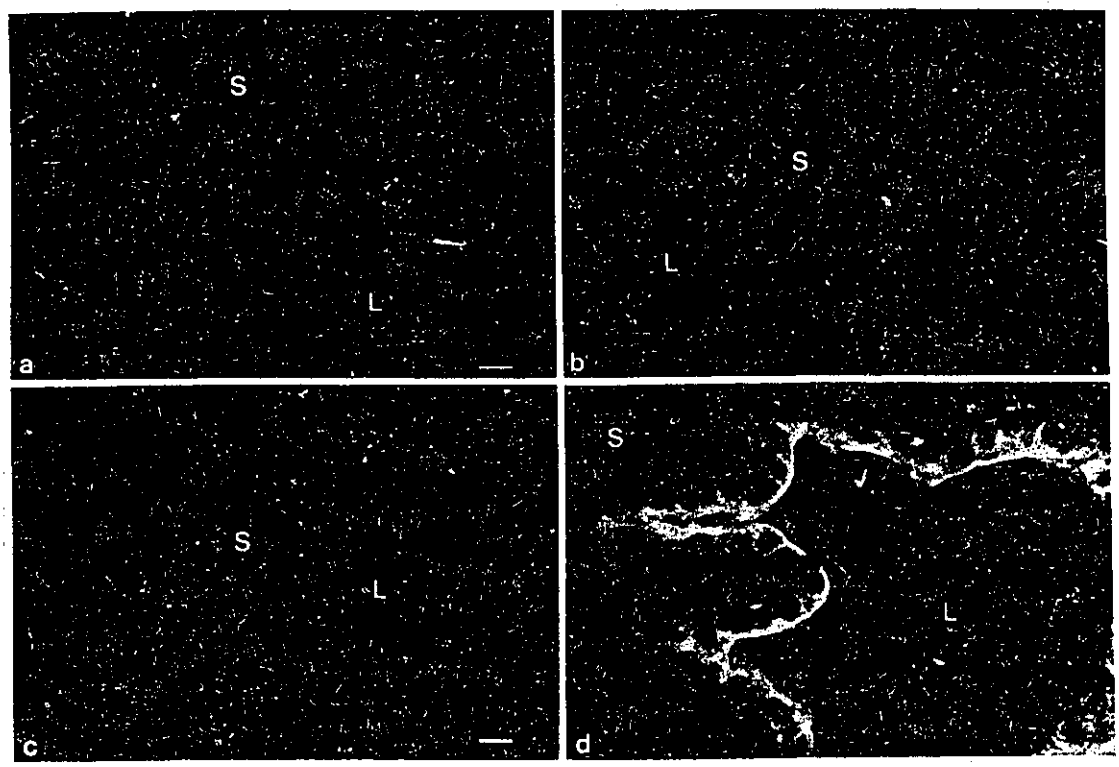
Panel a: Section of ventral prostate from intact rat
b: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 80 μm

Panel c: Section of ventral prostate from intact rat
d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.


S
L
a
S
L
b
S
L
c
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d

Figure 9: Immunofluorescent staining with EAB 903 of random transverse sections of the ventral prostate from intact and castrated rats.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat
Panels b and d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.

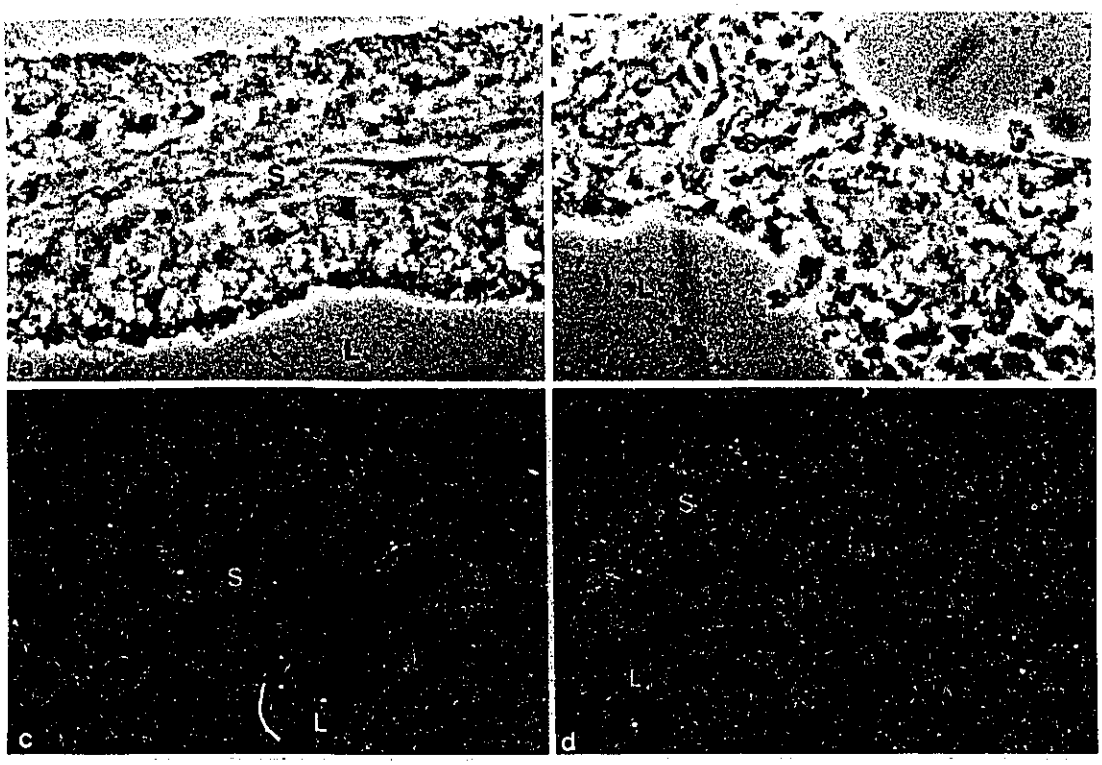
S
L
S
L
c
d

Figure 10: Immunofluorescent staining with CK 8.60 of random transverse sections of the ventral prostate from intact and castrated rats.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat
Panels b and d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.

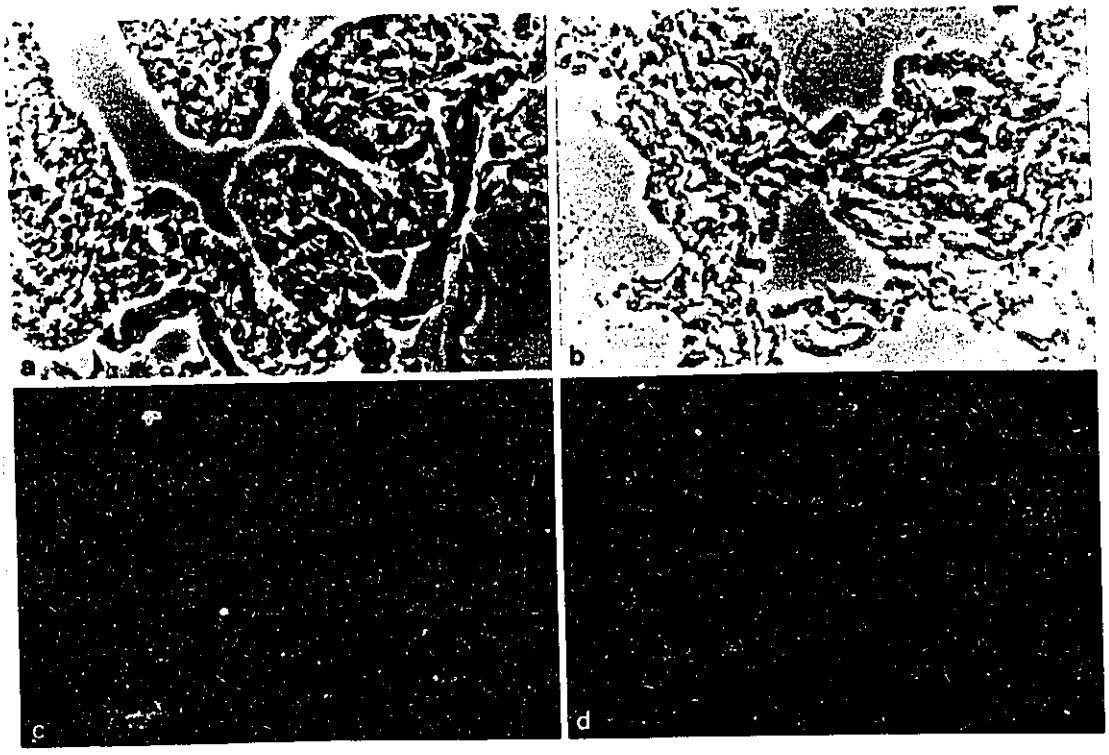
a
b
c
d

Figure 11: Non-specific binding of secondary antibodies to random sections of rat ventral prostate.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat
Panels b and d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.

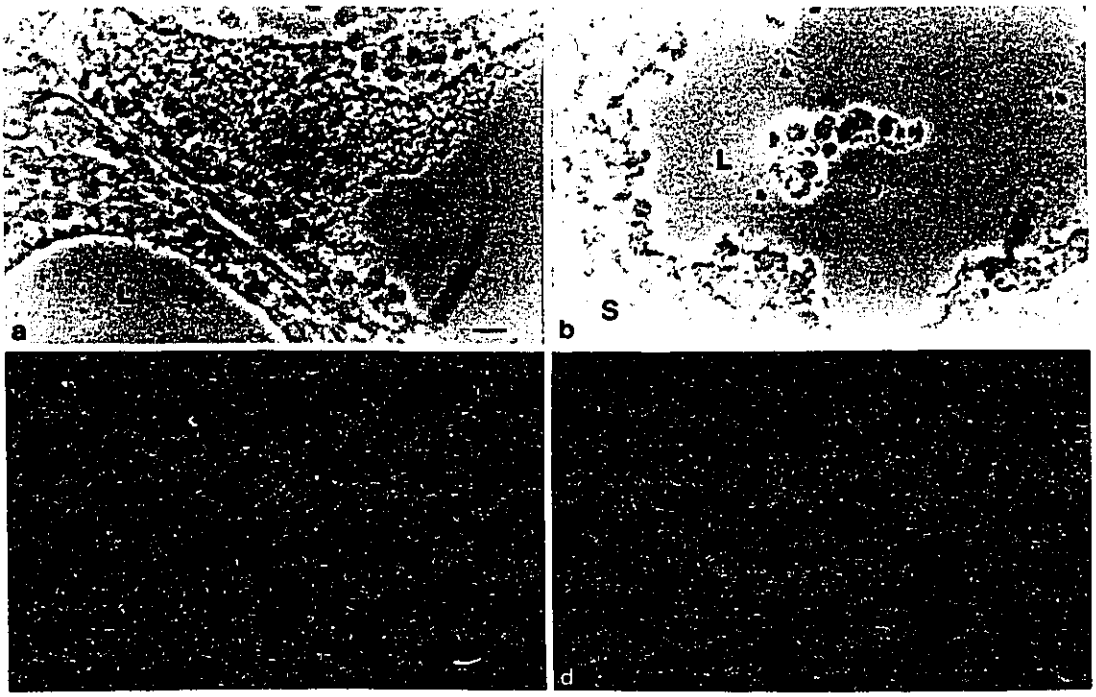
a
b
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Figure 12: Immunofluorescent staining with PKK2 of random transverse sections of the ventral prostate from intact and castrated rats.

Panels a, b, e and f: Phase contrast photomicrographs
Panels c, d, g and h: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat. The luminal border of the tall columnar epithelial cells is indicated by *arrows*

Panels b and d: Section of ventral prostate from rat castrated 8 days previously

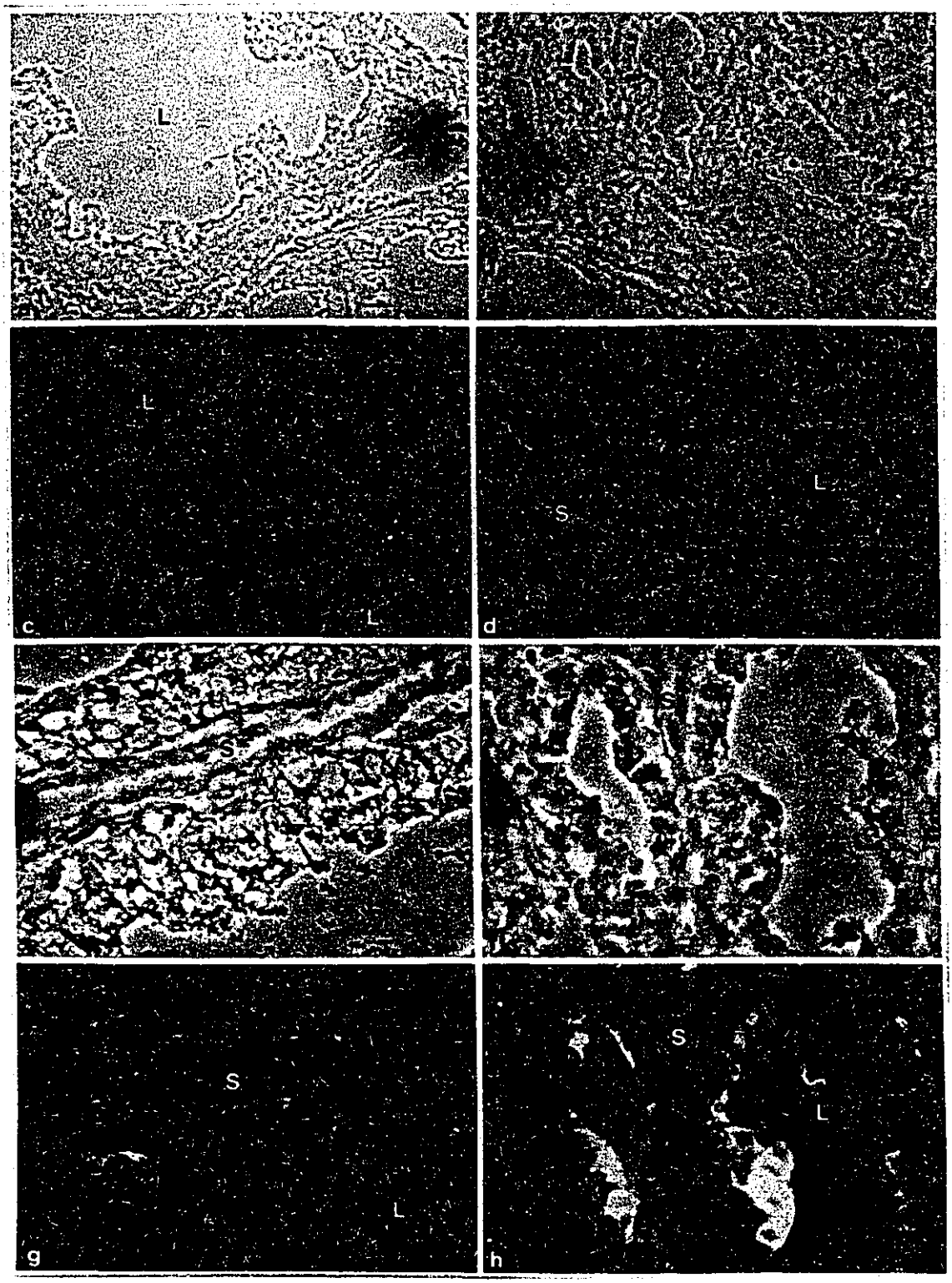
Scale bar: 80 μm

Panels e and g: Section of ventral prostate from intact rat.

Panels f and h: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.


L
L
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S
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g
S
L
S
L
h

g). This suggests that PKK2 may react with the basal cells of the intact rat ventral prostate. Androgen ablation greatly modifies this staining pattern. Eight days after castration, the cuboidal luminal cells of the rat ventral prostate contain the proteins recognized by PKK2, which are located in the cytoplasmic region (Fig. 12, Panels d and h). It is not clear, from these photomicrographs, whether basal cells are also immunoreactive. A lower magnification view of the section of the ventral prostate from intact animals clearly demonstrates that the sparse distribution of PKK2 staining around the prostatic ducts is not the only staining pattern found, although it is the most common (Fig. 12, Panel c). In this section, a large duct is lined by small cuboidal epithelial cells that are also stained by PKK2 (Fig. 12, Panel a and c). The neighbouring ducts harbour the general pattern of staining of tall columnar cells. This raises the possibility that the heterogeneity of the prostate may extend to individual ducts.

3. Actin :

Since Western analysis suggested that the level of actin increased after castration, the location of actin filaments was investigated both in normal and rats castrated eight days previously. As Fig. 13 (Panel c) shows, the actin filaments present in the prostate are localized around the basal margin of the cells lining the ducts. They appear to be primarily localized within the stromal cells, although the luminal epithelial cells also contain some actin filaments at their apical borders (seen best in Fig. 13, Panel d). The intensity of actin staining increases after castration, correlating well with the observation of an increased relative number of stromal cells at the same period and with the Western blotting result presented previously.

4. Desmosomal Proteins :

Desmoplakin I is one of the components of desmosomal plaques which

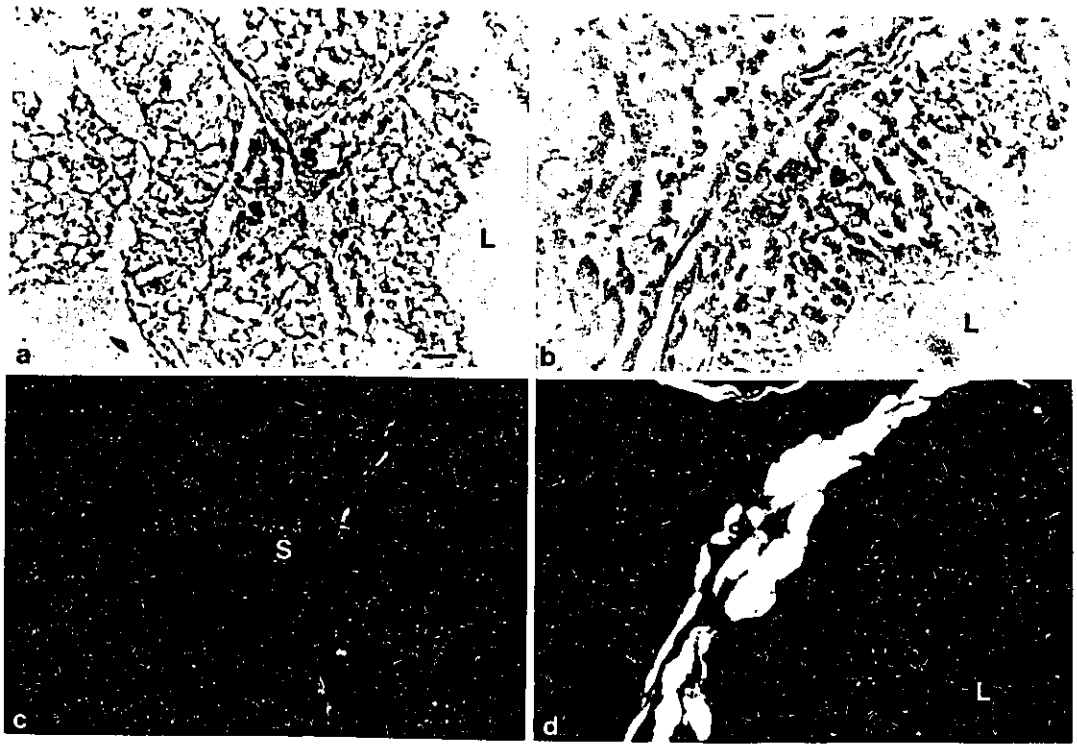
Figure 13: Immunofluorescent staining with anti-actin antibody of random transverse sections of the ventral prostate from intact and castrated rats.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat
Panels b and d: Section of ventral prostate from rat castrated 8 days previously

Scale bar: 30 μm

L: Lumen; S: Stroma.


L
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L
d

are associated with IFs. By gel electrophoresis, it was demonstrated that the level of desmoplakin I decreases dramatically after castration (Fig. 2). The distribution and abundance of desmoplakin I was monitored in sections from ventral prostate of intact and castrated rats. Unfortunately, even though the antibody was used according to the supplier's recommendation, the intensity of the staining is low. In intact animals, desmoplakin I is mainly localized on the apical side of luminal cells, at the junctions between cells (Fig. 14, Panel c). It is also observed around nuclei depending on the sectioning plane. Since desmoplakin I is an obligatory component of desmosomes, this indicates that numerous desmosomes are present in the prostate from intact animals. Following castration, the relative number of desmosomes decreases remarkably (Fig. 14., Panel d). In addition, the intensity of desmoplakin I staining is reduced, suggesting that the desmosomes which remain have a decreased desmoplakin I content following castration.

In summary, these experiments clearly indicate that:

1) luminal cells within the rat ventral prostate can have tall columnar or small cuboidal morphologies and different immunoreactivity toward PKK2.
2) heterogeneity also exists between the basal and luminal epithelial cells with regard to their CK content.
3) the ratio of basal to luminal epithelial cells alters after castration as observed with EAB 903 staining pattern.

Therefore, using immunofluorescence staining on random frozen sections of rat ventral prostate, we have shown that the cellular content of the prostate is modulated by androgens. The staining patterns obtained with PKK1, PKK2, PKK3 and EAB 903 show that each of the different cell types have a specific content of cytokeratins which can be used to monitor the behaviour of each cell type following castration. The basal to luminal cell ratio was shown to increase and the luminal cells altered their morphology after castration.

Figure 14: Immunofluorescent staining with anti-desmoplakin I antibody of random transverse section of the ventral prostate from intact and castrated rats.

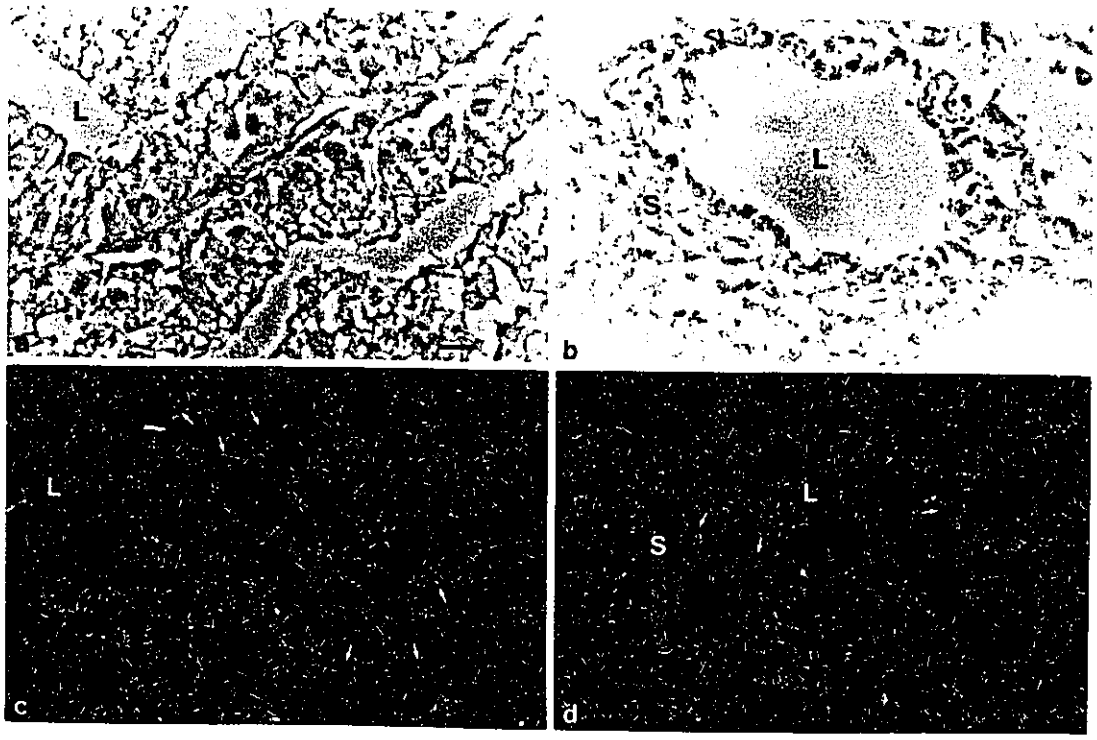
Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of ventral prostate from intact rat
Panels b and d: Section of ventral prostate from rat castrated 8 days previously

Some desmosomes are indicated by *arrows* in Panels c and d.

Scale bar: 30 μm

L: Lumen; S: Stroma.


L
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d

The staining pattern observed with PKK2 also demonstrated that luminal cells in the prostate of an intact rat are of at least two types: a majority of tall columnar epithelial cells that do not contain proteins recognized by PKK2 and a small subpopulation of cells which are grouped around a same duct that contain protein filaments stained by PKK2. This clearly indicates that cellular heterogeneity exists in the prostate, and may reflect ductal heterogeneity.

5. Structural Analysis of Intact Rat Ventral Prostate Longitudinal Sections :

On the basis of the results obtained in random prostate sections, we were intrigued to determine how the heterogeneity is achieved in the rat ventral prostate. The next stage of the study was to explore the possibility that the heterogeneity may extend to entire ducts. Based on the observation in the mouse prostate that the morphology of luminal cells along the same duct varies with the distance of the cells from the origin of the duct (Cunha *et al.*, 1987), longitudinal sections of normal rat ventral prostatic ducts were analyzed in terms of cellular content and cytokeratin content for each cell type.

5.1 PKK2 Staining Along the Prostatic Duct

Since PKK2 antibody revealed a clear difference in CK content of epithelial cells in the rat ventral prostate, it was used to initiate the search for heterogeneity along prostatic ducts. Three different zones of the prostatic ducts were stained with PKK2 (Fig. 15). The proximal zone (Fig. 15, Panels a and b), closer to the urethra, contains luminal cells which are immunoreactive with PKK2 and, therefore, contains one or more of the rat CKs 7, 16, 17 or 19. These cells are cuboidal in shape and show the same pattern of staining as that of the cuboidal shaped luminal epithelium in the random sections of rats castrated eight days previously (Fig. 12). On the other hand, very little staining is observed in more distal regions of the ducts, where the luminal epithelial cells are tall

Figure 15: Immunofluorescent staining with PKK2 of longitudinal sections of micro-dissected ducts from intact rat ventral prostate in the first branch point area.

Panels a, c and e: Phase contrast photomicrographs
Panels b, d and f: Immunofluorescent micrographs

Panels a and b: Section of proximal portion of a prostatic duct

Scale bar: 30 μm

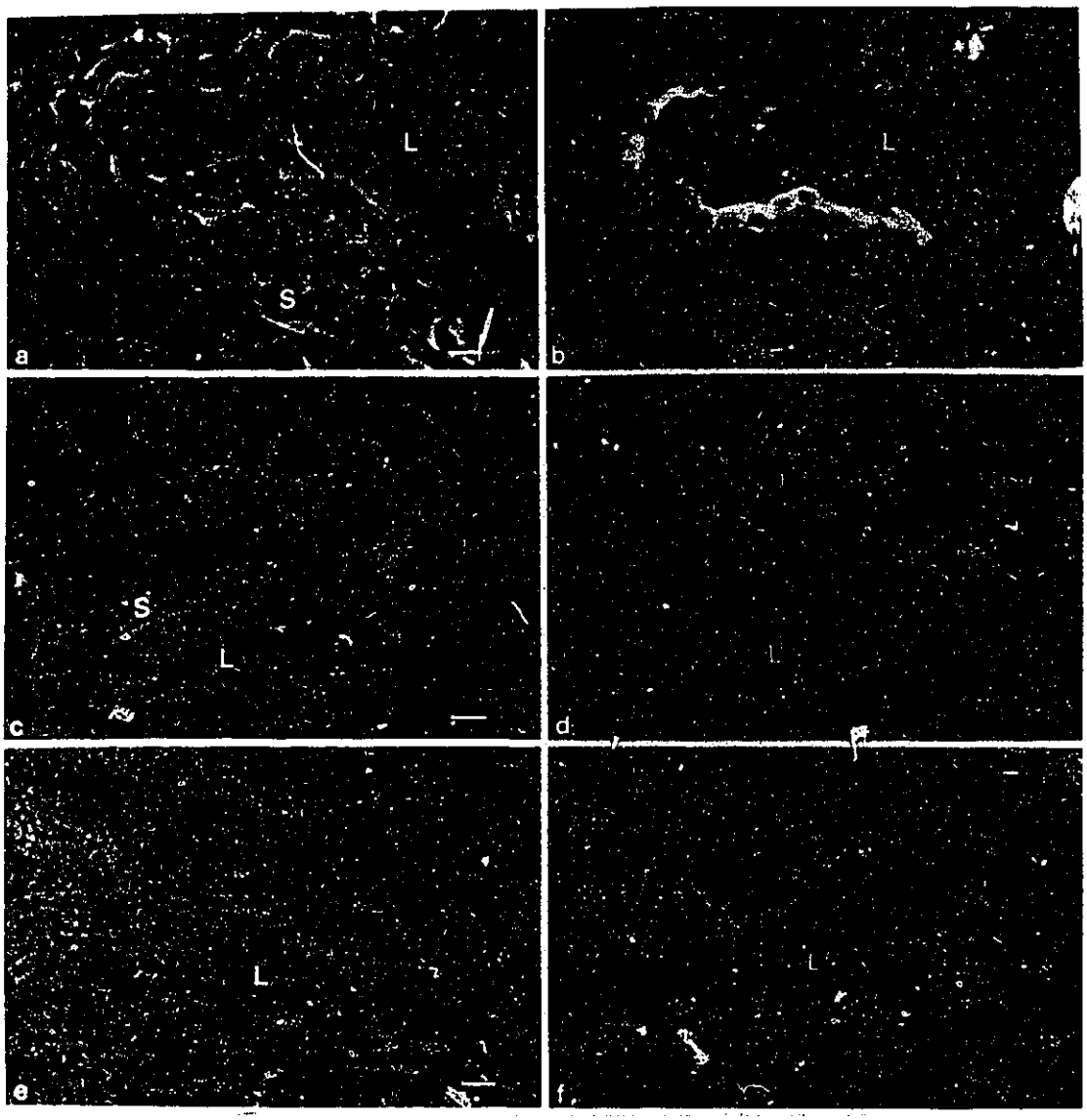
Panels c and d: Section of the first branch point zone of the same prostatic duct as in Panels a and b

Scale bar: 80 μm

Panels e and f: Section of the zone following the branching zone of the same prostatic duct as in Panels a and b

Scale bar: 80 μm

L: Lumen; S: Stroma.


L
S
a
L
b
S
L
c
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d
L
e
L
f

and columnar (Fig. 15, Panels e and f). The staining is located mostly in the basal region, possibly within basal cells. This is similar to what is observed in random sections from control rat ventral prostate, where most luminal epithelial cells possess this shape (Fig. 12, Panel g). Fig. 15, Panels c and d, show the phase contrast and immunofluorescent photographs of the micro-dissected ducts around the first branch point of a prostatic duct. The small cuboidal shaped epithelial cells lining the lumen before the branch point are replaced by tall columnar epithelial cells after the branch point (Panel c). This is coupled with a uniform staining of the luminal cuboidal cells prior to the branch point and by unstained tall columnar epithelial cells characteristic of the distal region immediately after the branch point (Panel d). In the latter region, only basal cells are stained by PKK2.

These observations lead to the hypothesis that cell shape and cytokeratin content are intimately coupled and that both parameters are a function of the ductal location of the cells within the prostate. They also clearly indicate that the cellular heterogeneity of the rat ventral prostate is not a random process in which whole ducts adopt one morphology or another. Rather, the cellular heterogeneity follows a specific pattern of distribution along each duct.

Therefore, it appears that the shape and cellular cytokeratin content of the luminal cells are a function of their location along the prostatic duct.

5.2 Luminal and Basal Cells Along the Prostatic Duct

At this stage of the study, a second antibody specific to basal cells became available, 312C8-1. The antibody was expected to aid in the identification of basal cells CKs on Western analysis (see above) since EAB 903 did not immunoreact with any of the CKs on nitrocellulose membranes. While the results of Western analysis are inconclusive, 312C8-1 appears to react well with rat basal cells in immunofluorescence

studies. To establish that the two basal cell specific antibodies (EAB 903 and 312C8-1) recognize the same cells in the rat ventral prostate, double immunofluorescence staining using FITC (green) and RITC (red) labelled secondary antibodies was performed. Fig. 16 (Panel 1) demonstrates that this is in fact the case, since both antibodies label the same cells, producing an orange colour, rather than discrete green and red stained cells. They also appear to stain identical filaments in the basal cells in both the proximal and distal regions of the ducts. Throughout the remainder of the thesis, these two antibodies were used interchangeably and are referred to as "basal cell specific antibody" without further discrimination.

Since basal cells are located at the basal limit of the luminal cells, it is not clear whether PKK1 is specific to luminal cells or if basal cells also contain the CKs characteristic of the simple epithelium. Double immunofluorescence staining of a longitudinal section from a rat ventral prostate with PKK1 and the basal cell specific antibody is shown in Fig. 16, Panel 2. The analysis of the staining is complicated by the fact that each antibody stains different filaments, which may be contained in the same cell. It is therefore difficult to absolutely associate the stained filament with one cell type, when the staining is located at the interface of two cells. While 312C8-1 does not stain luminal epithelium, it appears that some basal cells may contain the cytokeratins of simple epithelium, as suggested by the fluorescein fluorescence present in some areas of the basal cells. The double immunostaining also demonstrates that basal cells form a continual layer interposed between luminal epithelium and stroma in the proximal region (Fig. 16, Panel 2a) whereas the basal cells containing the 312C8-1 positive cytokeratins are much more dispersed in the distal region (Fig. 16, Panel 2b). In each case, appropriate controls for double immunostaining were prepared. As presented in Fig. 16 (Panel 3), no non-specific staining of the second secondary antibody is obtained. The order in which the primary antibodies are applied on the sections does not affect the pattern of staining observed or the intensity of the non-specific staining (not shown).

Figure 16: Double immunofluorescent staining of longitudinal sections of micro-dissected ducts from intact rat ventral prostate.

Panel 1: Sections were labeled with fluorescein-conjugated goat anti-mouse IgG antibody which recognizes EAB 903, which produces a green fluorescence and with rhodamine-conjugated goat anti-mouse IgM antibody, which recognizes 312C8-1 and produces a red fluorescence.

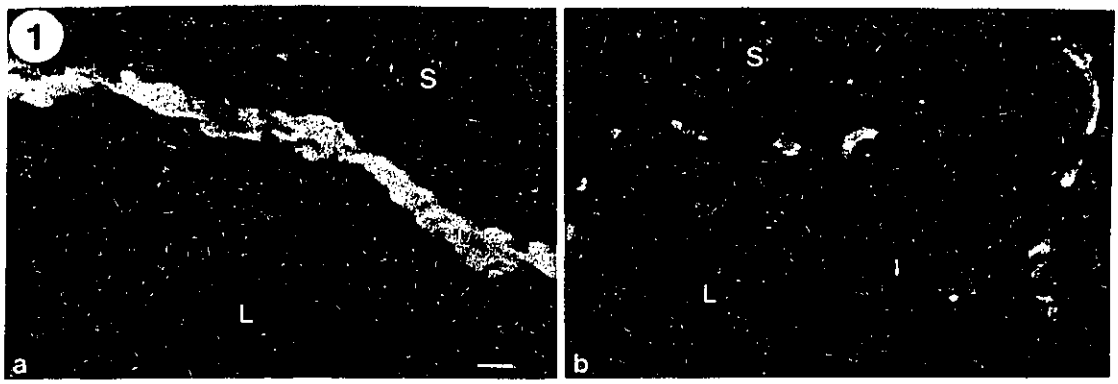
Panel 2: Sections were labeled with fluorescein-conjugated goat anti-mouse IgG antibody, which recognizes PKK1 antibody and produces a green fluorescence, and with rhodamine-conjugated goat anti-mouse IgM antibody, which recognizes 312C8-1 and produces a red fluorescence.

Panel 3: Non-specific immunofluorescent staining of a longitudinal section of micro-dissected ducts from intact rat ventral prostate. The section was labelled with fluorescein conjugated goat anti-mouse IgG antibody, which recognizes PKK1 antibody and produces a green fluorescence, and with rhodamine-conjugated goat anti-mouse IgM antibody without prior incubation with 312C8-1 antibody.

Panel a: Proximal region of the duct
Panel b: Distal region of the duct

Scale bar: 30 μm

L: Lumen; S: Stroma.


1
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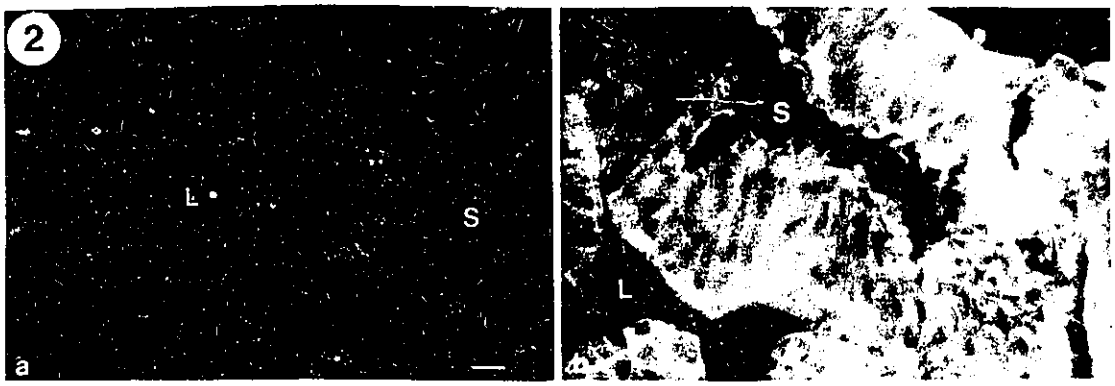
2
L
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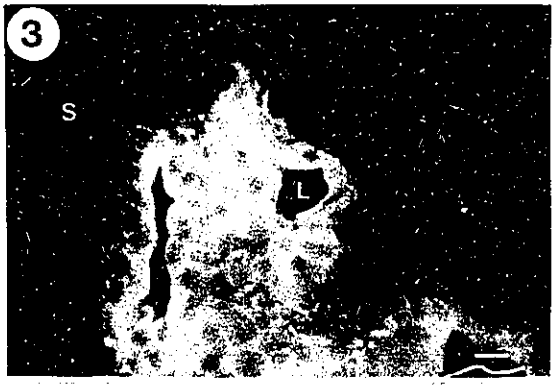
L


3
S
L

In the normal animal, PKK1 staining reveals that luminal cuboidal cells of the proximal region and luminal columnar cells of the distal region both contain CKs 8 and 18, characteristic of simple epithelium. No variations are detected regarding the filament organization or the staining intensity (Fig. 17, Panels c and d). Similarly, basal cells stained by EAB 903 have a similar location and filament distribution (Fig. 18, Panels c and d). However, the abundance of basal cells changes dramatically along the duct. Basal cells form a continuous layer over the luminal cells in the proximal region while they are sparsely distributed in the distal region of the ducts, confirming the results of double immunofluorescent labelling (Fig. 16, Panel 2). It is interesting that the cellular distribution of the distal region closely resembles random section of the normal rat ventral prostate, while the cellular distribution of the proximal region has significant similarities to the random sections from ventral prostate of rats castrated eight days previously (Fig.7).

5.3 Desmosomes Along the Prostatic Duct

The distribution of the desmosomes along the ducts was also investigated using immunofluorescence. Unfortunately, even though the antibody was used according to the supplier's recommendation (and as for random sections), the intensity of the staining is low. To help the visualization of the staining pattern, some indications have been placed on Fig. 19. The location of the desmosomes in both regions of the ducts is similar, i.e. on the apical side of luminal cells, at the junction of neighbouring cells. Although the analysis of the immunofluorescence results is very qualitative, a marked decrease in the intensity of the desmoplakin I staining is observed from the distal region (Fig. 19, Panel c) to the proximal region (Fig. 19, Panel d) and suggests that desmosomes linking tall columnar epithelial cells have a higher content in desmoplakin I than desmosomes linking small cuboidal cells.

Again, a correlation can be made with what was observed in random

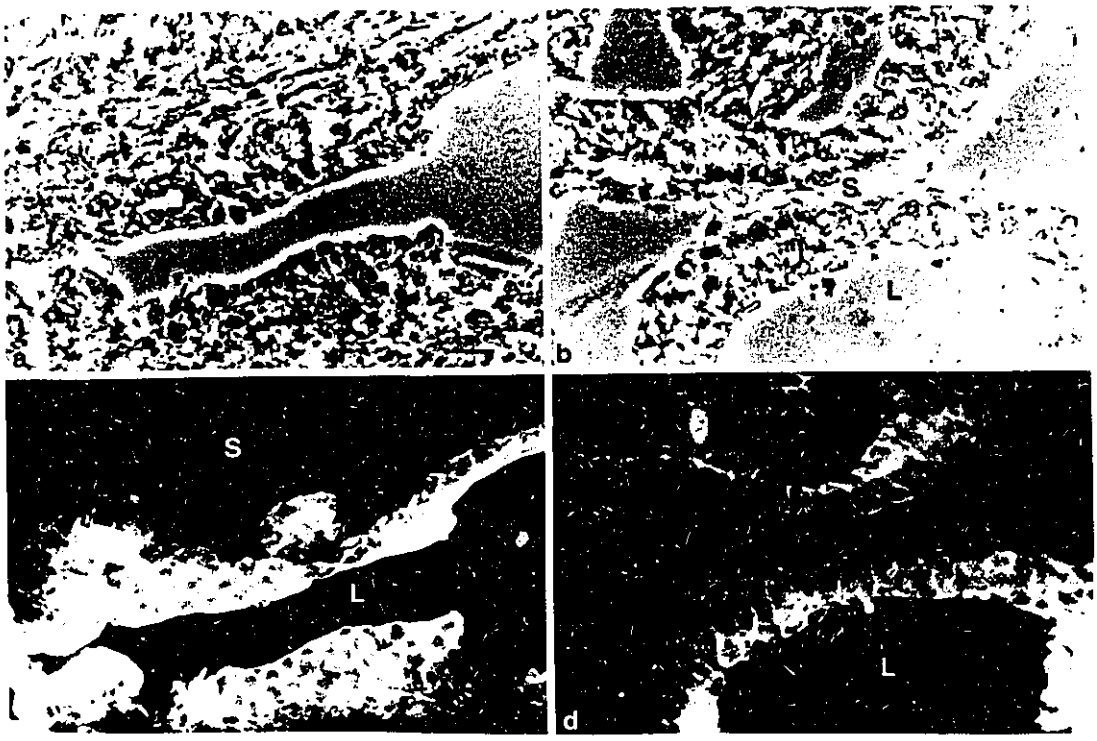
Figure 17: Immunofluorescent staining with PKK1 of longitudinal sections of micro-dissected ducts from intact rat ventral prostate.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of the proximal region of a prostatic duct
Panels b and d: Section of the distal region of a prostatic duct

Scale bar: 30 μm

L: Lumen; S: Stroma.


a
b
S
L
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L
d
L

Figure 18: Immunofluorescent staining with a basal cell specific antibody of longitudinal sections of micro-dissected ducts from intact rat ventral prostate.

Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of the proximal region of a prostatic duct
Panels b and d: Section of the distal region of a prostatic duct

Scale bar: 30 μm

L: Lumen; S: Stroma.

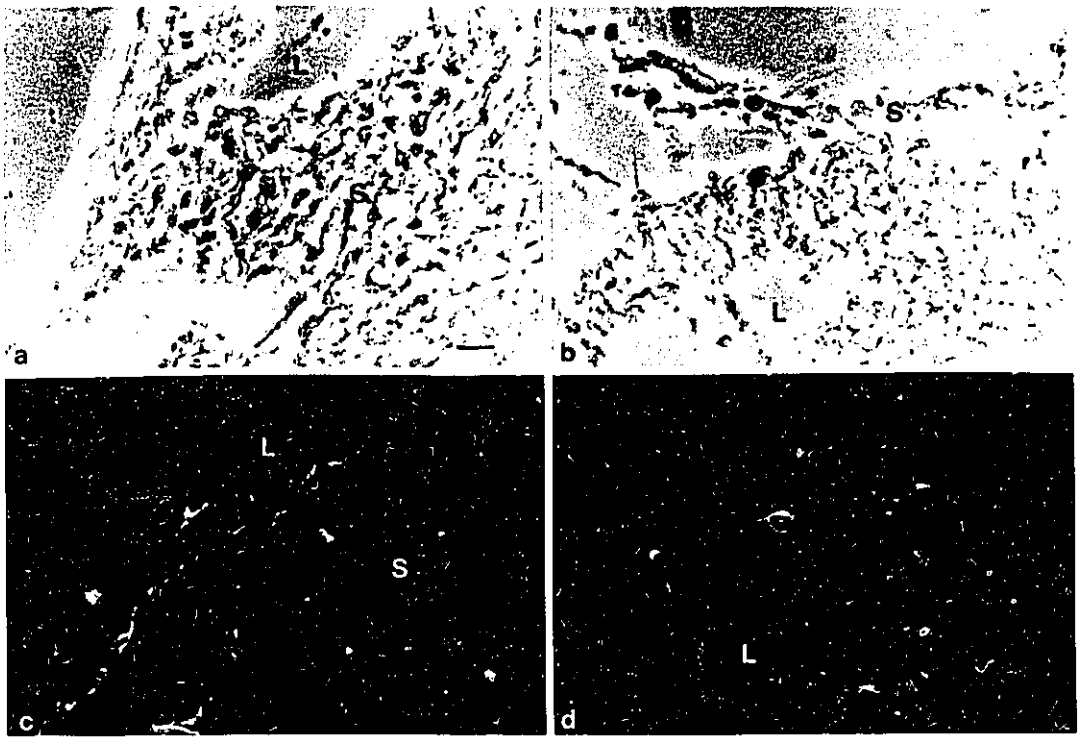

L
S
a
S
L
b
L
S
c
L
d

Figure 19: Immunofluorescent staining with anti-desmoplakin I antibody of longitudinal sections of micro-dissected ducts from intact rat ventral prostate.

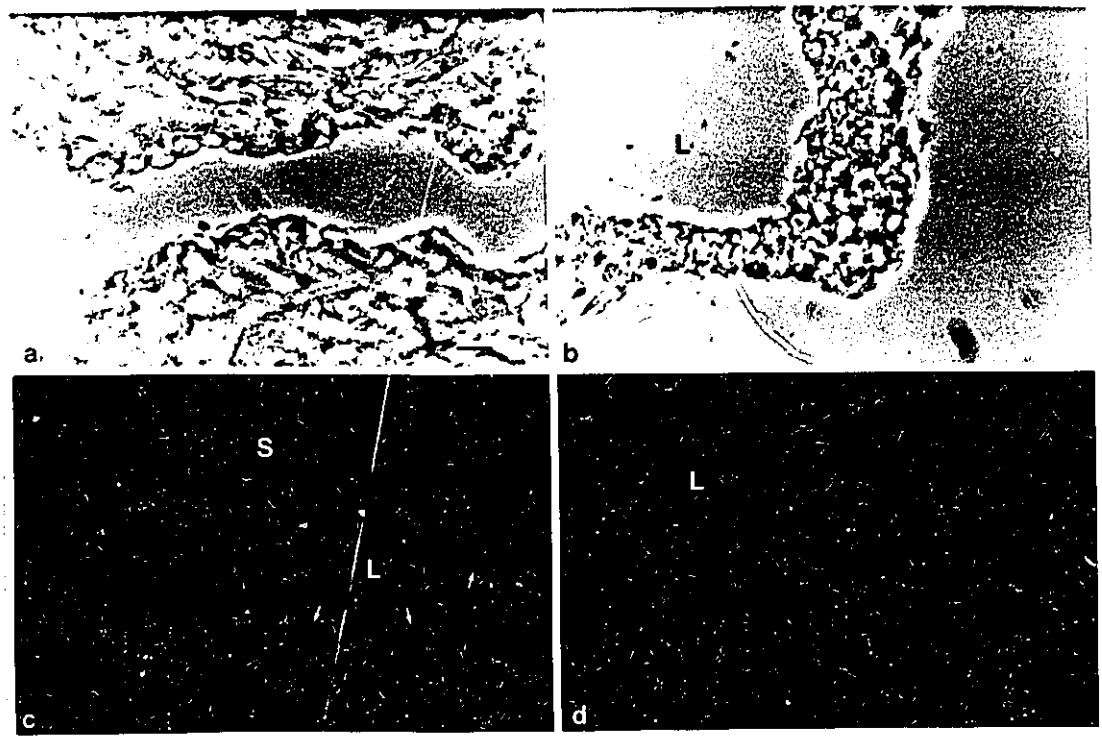
Panels a and b: Phase contrast photomicrographs
Panels c and d: Immunofluorescent photomicrographs

Panels a and c: Section of the proximal region of a prostatic duct
Panels b and d: Section of the distal region of a prostatic duct

Some desmosomes are indicated by *arrows* in Panels c.

Scale bar: 30 μm

L: Lumen; S: Stroma.


S
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sections. The desmoplakin I staining pattern of random sections from normal rat ventral prostate closely resembles the distal region staining of the longitudinal section. Similarly, the proximal region staining with anti-desmoplakin I antibody corresponds to the level of staining in random sections of ventral prostate from a rat castrated eight days previously (Fig. 14).

6. Effects of Androgen Ablation on the Cell Distribution in the Rat Ventral Prostate :

The above results clearly demonstrate that heterogeneity exists in the cellular content of the prostate. The prostatic ducts are lined by luminal cells that vary in shape, from small cuboidal in the proximal region to tall columnar in the distal region and the ratio of basal to luminal epithelial cells is dependent on the ductal location, being higher in the proximal region than in the distal region. Furthermore, the universal component of desmosomal plaques, desmoplakin I, is also present in different amounts in the two regions. In addition, the staining pattern of the distal region resembles that of random sections of the ventral prostate of intact rats while the staining in the proximal region resembles that of random sections of the prostate from rats castrated eight days previously.

To determine if the ductal heterogeneity of the prostate extends to the androgen responsiveness of the gland, longitudinal sections of prostate from castrated rats were stained with PKK1 (specific for simple epithelium cytokeratins) and EAB 903/312C8-1 (specific for basal cells) and with the anti-desmoplakin I antibody. This staining will detect heterogeneity at two different levels: Region-specific androgen dependence and/or cell specific androgen dependence.

To insure that the regression of the prostate was occurring normally in the castrated animals, each ventral prostate sample was weighed (OW) and compared to the body weight (BW) of the donor animal. The curve of the

OW/BW is presented in Fig. 20. A decrease of 50% of prostatic weight occurred within the first two days following castration. Within eight days, the prostate weight decreased to 1/10 of its normal value. No further significant decrease was detected up to 14 days following the castration.

PKK1 staining of longitudinal micro-dissected sections of the rat ventral prostate shows that the distribution of the rat CKs 8, 18 and 19 within the luminal epithelial cells is similar through the ducts during androgen ablation (Fig. 21). However, the cell shape is modified, since the remaining luminal cells in the distal tips are reduced in size after castration apparently due to a reduction in the cytoplasm. By day four after castration a decrease in the size is apparent (Panel d) and by day eight, the size of the luminal cells at the distal tips is similar to luminal cells of the proximal region (Panel h). The changes in size that occur at this region is in marked contrast to the constant cuboidal shape of the luminal epithelial cells in the proximal region (Panels a, c, e and g). The luminal epithelial cells in the distal region of the ducts appear to be far more sensitive to androgen ablation than the luminal cells of the proximal region.

Staining of the longitudinal sections of micro-dissected ducts from control rats with a basal cell specific antibody reveals that the basal cells in the prostate are localized primarily in the proximal region (Fig. 18). The number of basal cells in the proximal region does not appear to be modified by castration in that a single layer of cells appears to lie between the luminal epithelial cell and the stroma (Fig. 22A, Panels b, d, f and h). After castration, there is an increasing number of basal cells in the distal region (Fig. 22B, Panels b, d, f and h) and by day eight this region resembles the proximal region since the ratio of basal to luminal epithelial cells is almost equal to the ratio found in the proximal region of ducts.

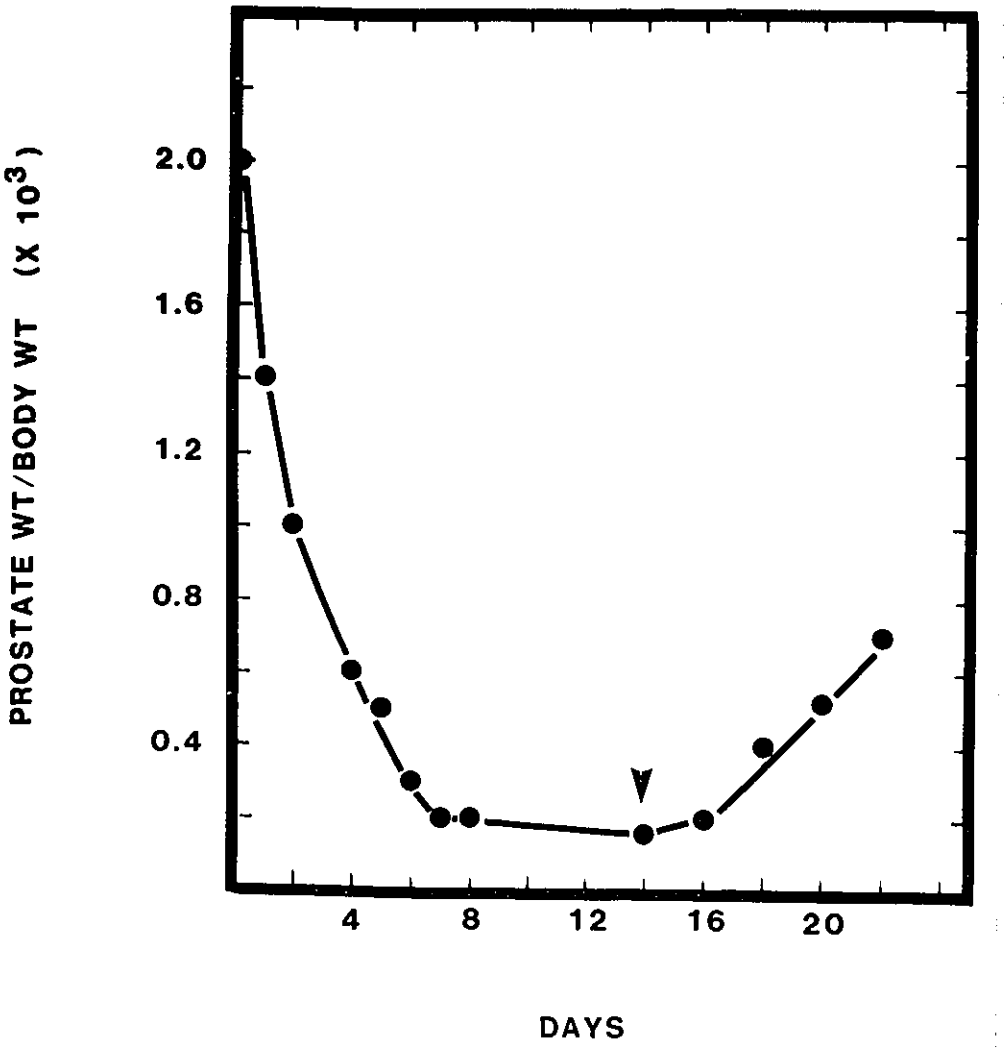
It appears from the staining pattern of PKK1 and basal cell specific

Figure 20: Changes in prostatic weight/body weight for the rat ventral prostate after castration and androgen re-administration.

Rats were sacrificed at the specified days after the surgery or after daily injections starting 14 days following castration. The ventral prostatic lobes were removed and weighed and compared to the body weight (BW) of the donor animal.

The first day of androgen re-administration is indicated by an *arrow head*.

The OW/BW ratio was calculated as described by Jackson, Tenniswood, Bird and Clark (1977).


PROSTATE WT/BODY WT (X 10³)
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20
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Figure 21: Immunofluorescent staining with PKK1 of longitudinal sections of micro-dissected ducts from ventral prostates of rats at various times after castration.

Panels a, c, e and g: Sections from proximal region of the prostatic ducts.
Panels b, d, f and h: Sections from distal region of the prostatic ducts.

Panels a and b: 2 days after castration
Panels c and d: 4 days after castration
Panels e and f: 6 days after castration
Panels g and h: 8 days after castration

Scale bar: 30 μm

L: Lumen; S: Stroma

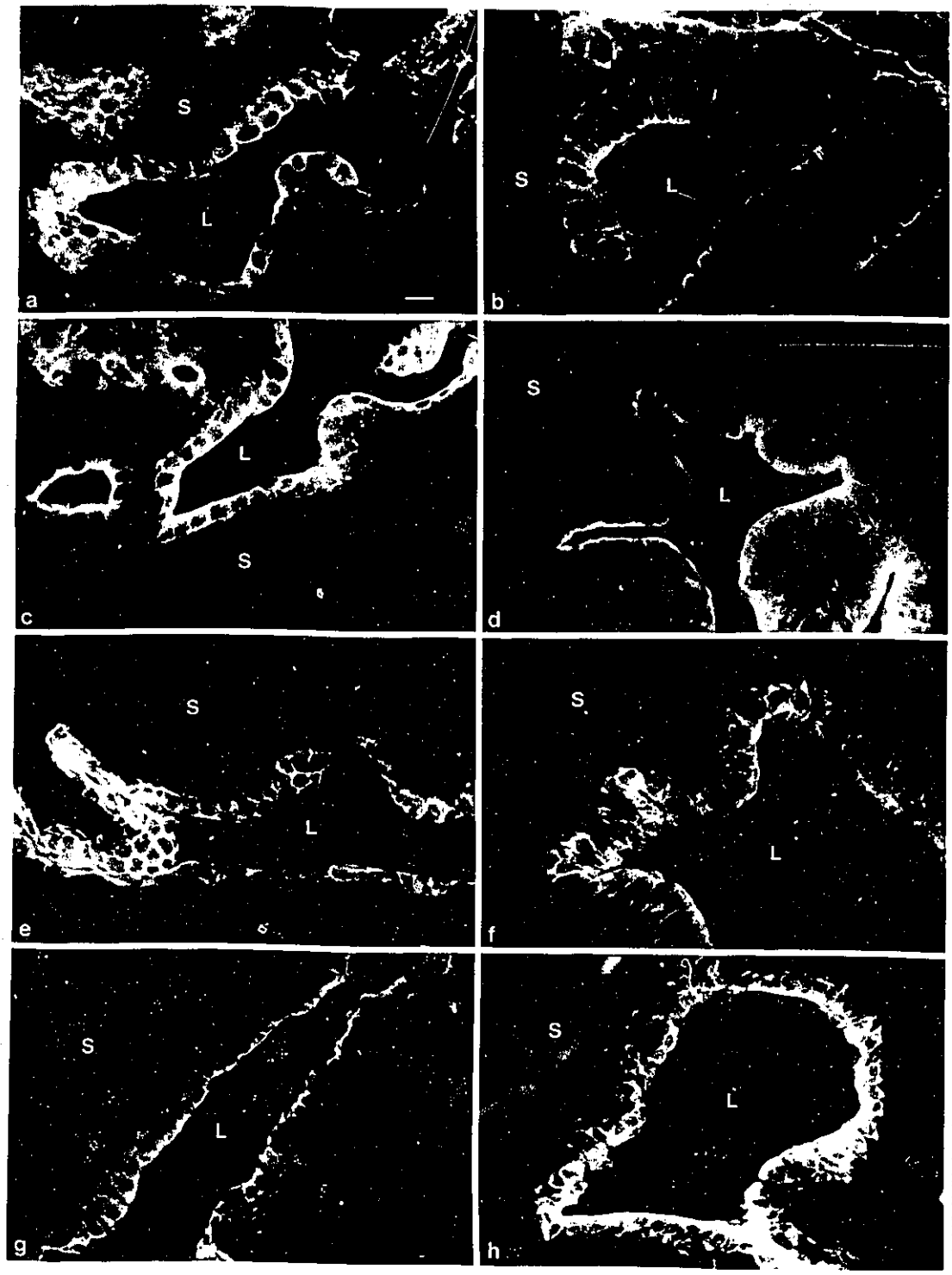
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Figure 22: Immunofluorescent staining with a basal cell specific antibody of longitudinal sections of micro-dissected ventral prostates of rats at various times after castration. A. Proximal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after castration
Panels c and d: 4 days after castration
Panels e and f: 6 days after castration
Panels g and h: 8 days after castration

Scale bar: 30 μm

L: Lumen; S: Stroma

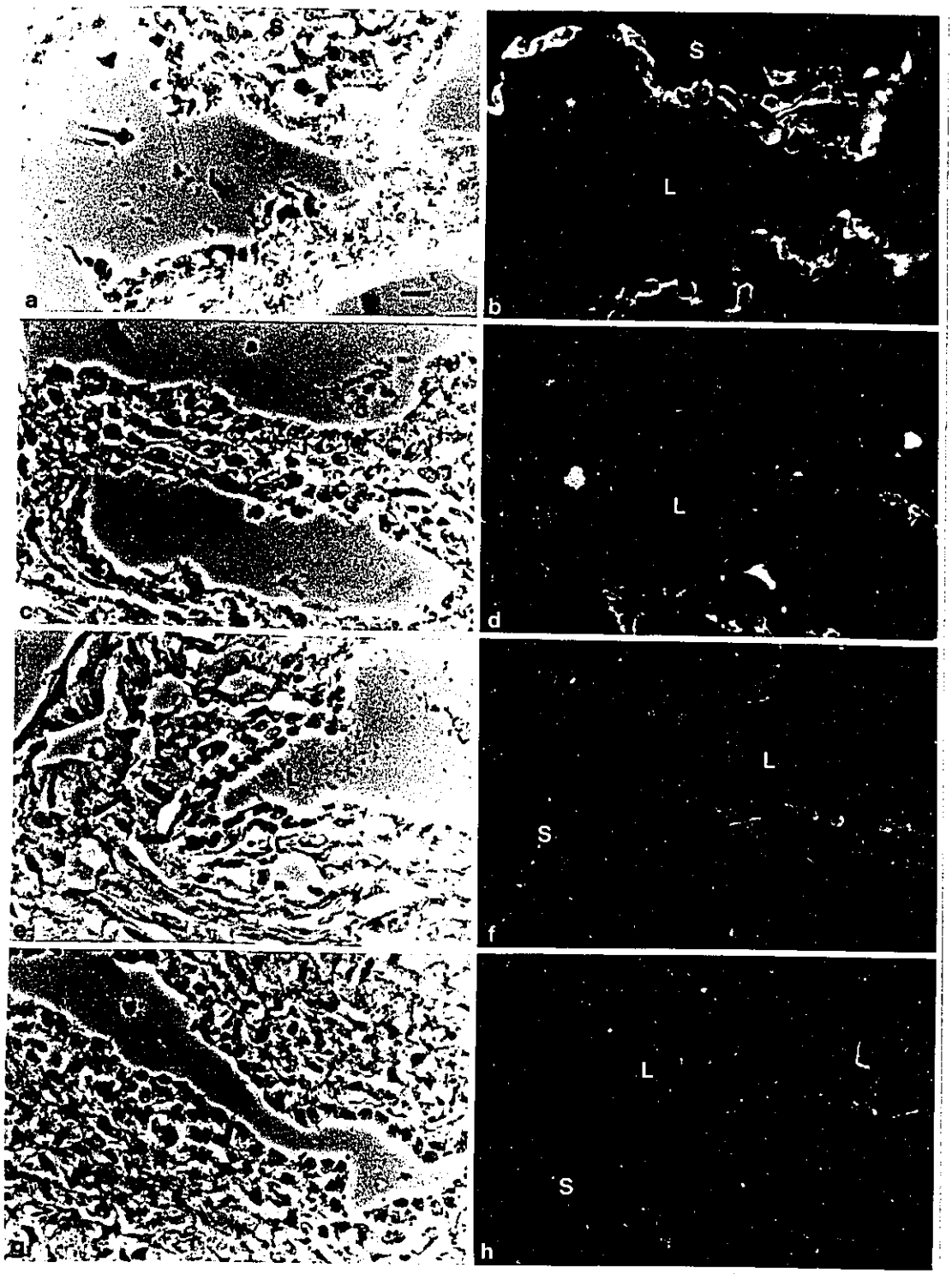
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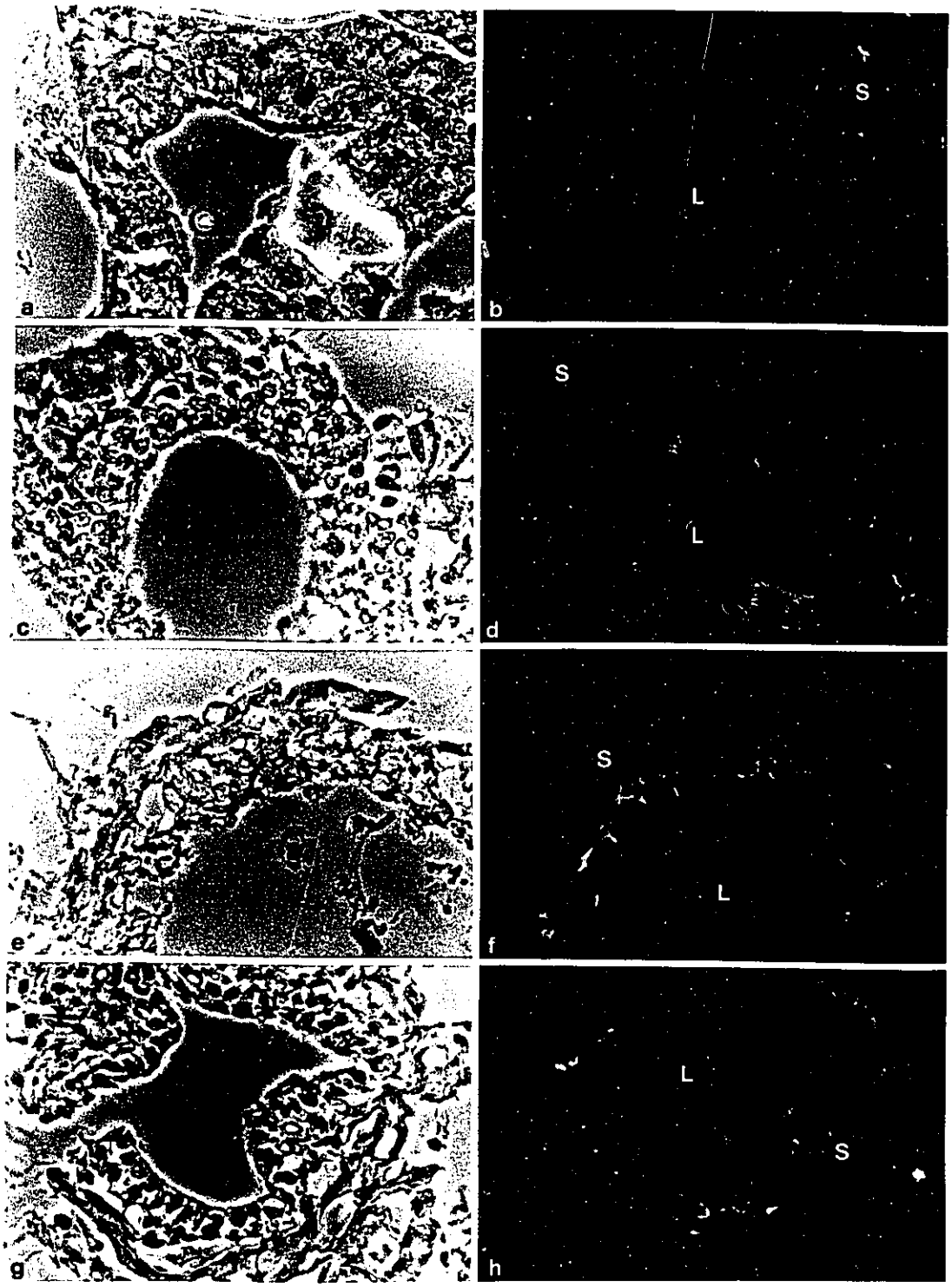
Figure 22: Immunofluorescent staining with a basal cell specific antibody of longitudinal sections of micro-dissected ventral prostates of rats at various times after castration. B. Distal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after castration
Panels c and d: 4 days after castration
Panels e and f: 6 days after castration
Panels g and h: 8 days after castration

Scale bar: 30 μm

L: Lumen; S: Stroma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antibodies that the proximal region of the prostatic duct is not affected by the androgen ablation. In contrast, the distal region undergoes major changes since the luminal cell size and number decrease dramatically and the ratio of basal to luminal epithelial cells increases greatly as a result.

7. Effects of Androgen Ablation on Desmosomal Content :

Staining of the micro-dissected prostatic ducts from control rats (Fig. 19) has suggested that desmosomes in the distal tips have a higher content of desmoplakin I than those in the proximal region. Desmoplakin I staining of proximal and distal regions of the ducts from castrated rats shows that the heterogeneity between the two regions persists after castration (Fig. 23A and B). The proximal region is lightly stained by the anti-desmoplakin I antibody but this staining intensity and distribution remains identical after castration (Fig. 23A, Panels b, d, f and h). Similarly, the staining intensity does not alter significantly in the distal region of the ducts following androgen ablation (Fig. 23B, Panels b, d, f and h). However, the pattern of staining differs slightly in the distal region from day two to day eight after castration. While in intact rats desmosomes appear to be regularly spaced from one cell junction to the next (Fig. 19), this organized arrangement is disrupted after castration and is best visualized in rats castrated eight days previously (Fig. 23B, Panel h). The desmoplakin I appears highly disorganized, which contrasts with the regular staining pattern seen in the proximal region. Again, it appears that the absence of androgens specifically affects the distal region of the prostatic ducts, rather than the proximal regions.

8. Effect of Androgen Administration on the Rat Ventral Prostate:

The previous sections have described the heterogeneity of the ventral prostate from castrated rats at a number of different levels. Luminal epithelial cells in the distal regions of the prostatic ducts are clearly the most affected by androgen ablation. Under these conditions,

Figure 23: Immunofluorescent staining with anti-desmoplakin I antibody of longitudinal sections of micro-dissected ventral prostates of rats at various times after castration. A. Proximal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after castration
Panels c and d: 4 days after castration
Panels e and f: 6 days after castration
Panels g and h: 8 days after castration

Some desmosomes are indicated by *arrows* in Panels b, d, f and h.

Scale bar: 30 μm

L: Lumen; S: Stroma

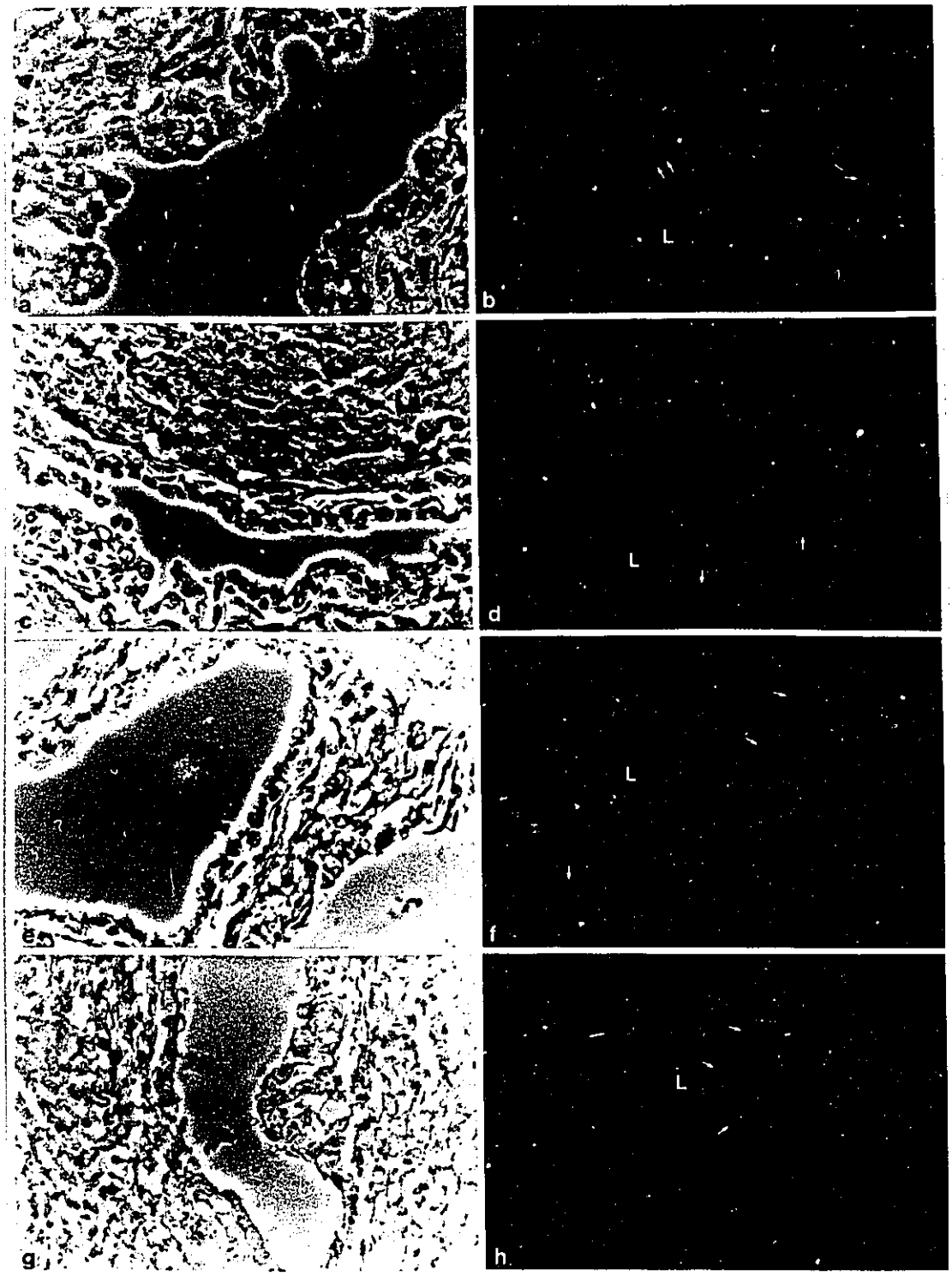
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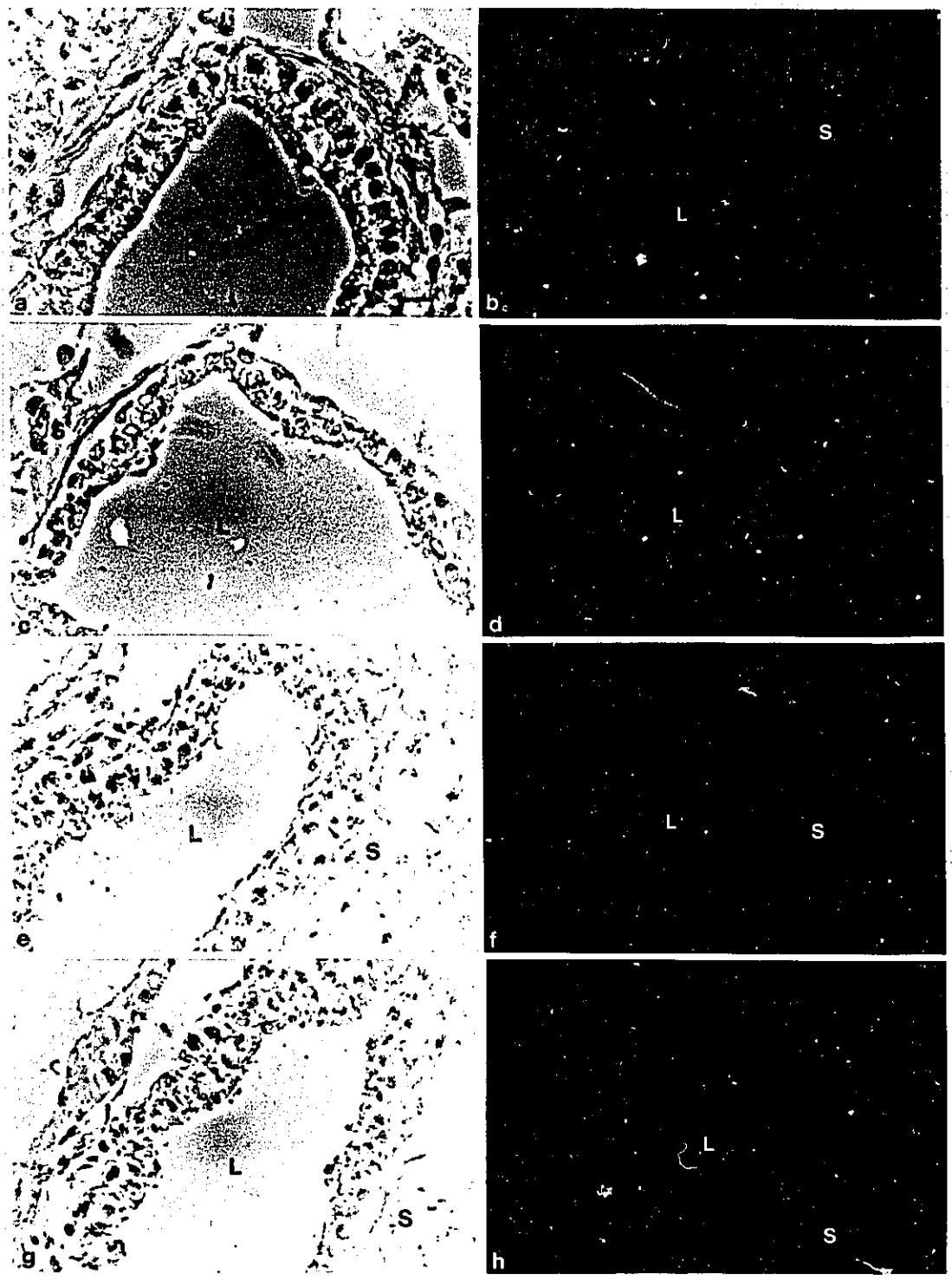
Figure 23: Immunofluorescent staining with anti-desmoplakin I antibody of longitudinal sections of micro-dissected ventral prostates of rats at various times after castration. B. Distal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after castration
Panels c and d: 4 days after castration
Panels e and f: 6 days after castration
Panels g and h: 8 days after castration

Scale bar: 30 μm

L: Lumen; S: Stroma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the epithelial cells are reduced in size, the desmoplakin I distribution is perturbed and it is likely that a significant portion of these cells die as demonstrated by the increased ratio of basal to luminal epithelial cells. As mentioned in the introduction, injection of exogenous testosterone to the castrated rat restores both prostate size and function. It was of interest to evaluate if this is also true of the structural components of the distal epithelial cells such as desmoplakin I and to determine if the distal region is the only region to respond to androgen administration.

The re-growth of the rat ventral prostate expressed as the OW to BW ratio is presented in Fig. 20, and clearly shows that after eight days of androgen administration, the OW/BW ratio almost returned to 1, which is the lower end of the normal range for animals of this size (Jackson *et al.*, 1977).

As androgen ablation, re-administration of androgens to castrated rats did not alter the basal and luminal cell numbers in the proximal region of the prostatic ducts (Fig. 24A, Panels b, d, f and h). In contrast, the cellular content of the distal region is modified and restored by androgen re-administration (Fig. 24B, Panels b, d, f and h). Staining with basal cell specific antibodies demonstrates that two days of injection are not sufficient to produce any significant change in the cell distribution in the distal region (Panel b). However, by day four, there is a marked decrease in the number of basal cells in the distal region (Panel d) which is further accentuated by day eight (Panel h). The ratio of basal to luminal epithelial cells returns to normal between day four and day six of injection (Panels d and f).

The shape of luminal epithelial cells also returns to the tall columnar form characteristic of the secretory cells in the distal region of the control ducts as soon as four days after injection. These changes occur without alterations in the content of rat CKs 8, 18 and 19 (not shown). In contrast, the luminal epithelial cells in the proximal region

Figure 24: Immunofluorescent staining with a basal cell specific antibody of longitudinal sections of micro-dissected ventral prostates of castrated rats at various times after androgen re-administration. A. Proximal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after androgen administration
Panels c and d: 4 days after androgen administration
Panels e and f: 6 days after androgen administration
Panels g and h: 8 days after androgen administration

Scale bar: 30 μm

L: Lumen; S: Stroma

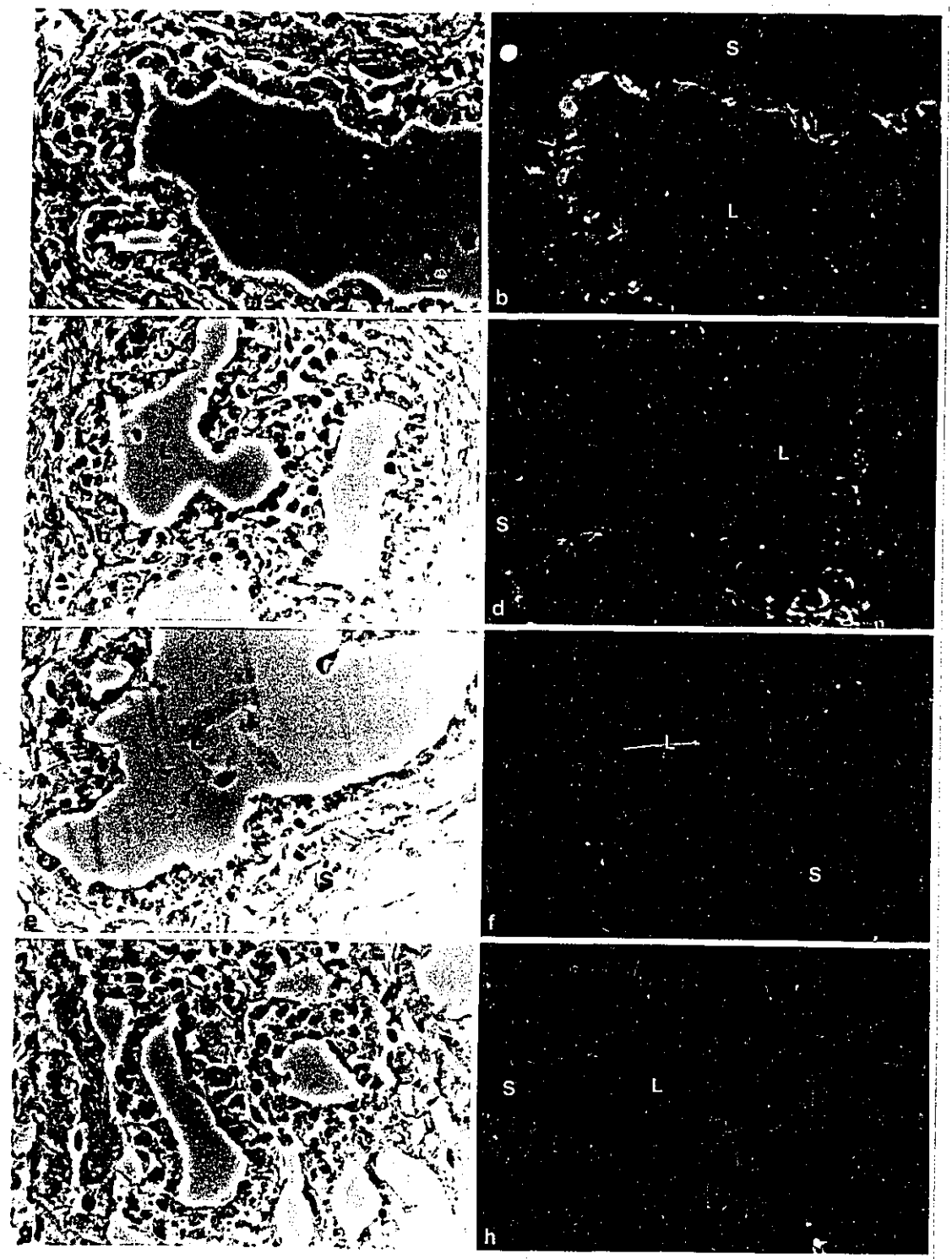
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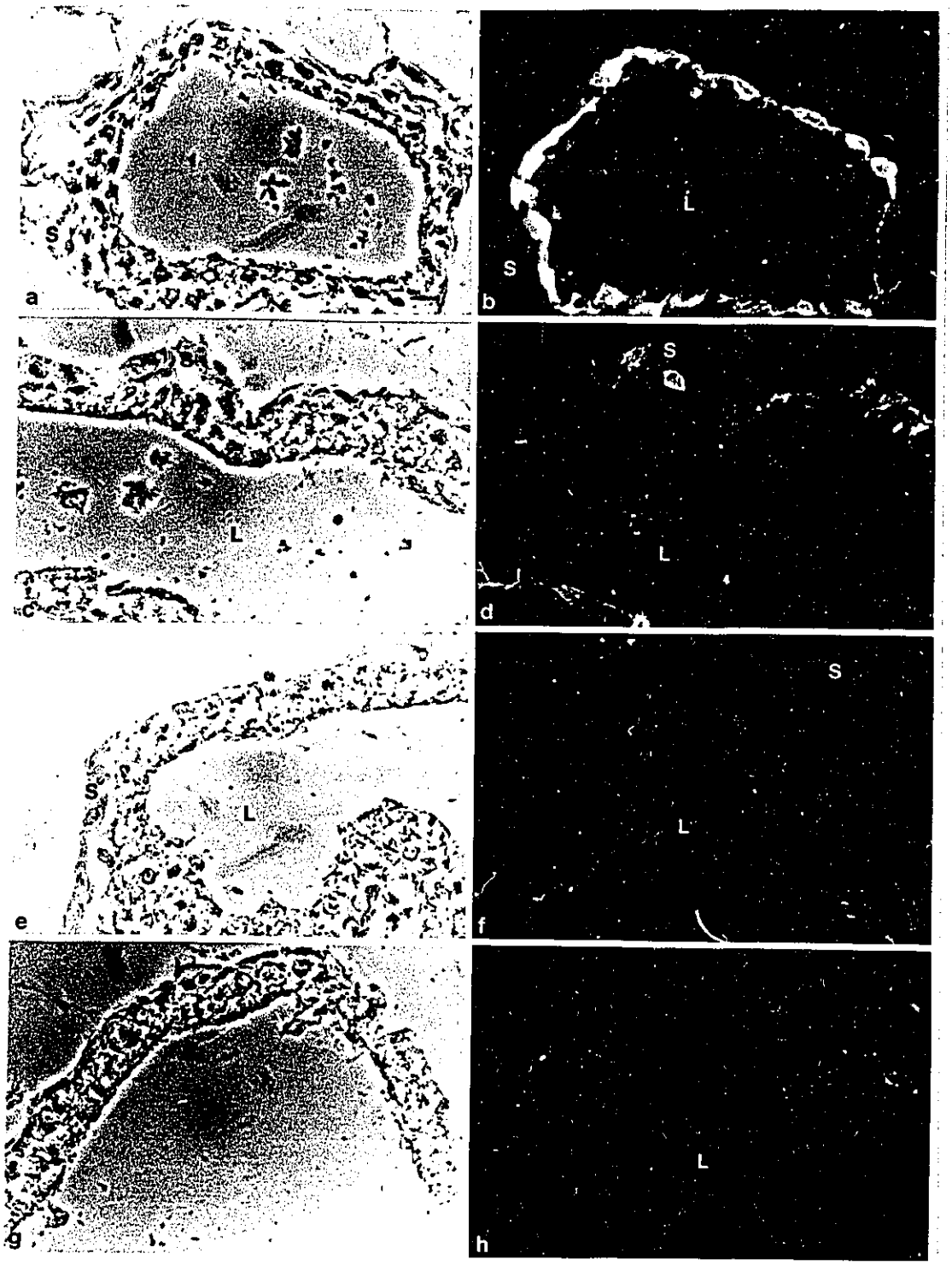
Figure 24: Immunofluorescent staining with a basal cell specific antibody of longitudinal sections of micro-dissected ventral prostates of castrated rats at various times after androgen re-administration. B. Distal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after androgen administration
Panels c and d: 4 days after androgen administration
Panels e and f: 6 days after androgen administration
Panels g and h: 8 days after androgen administration

Scale bar: 30 μm

L: Lumen; S: Stroma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do not undergo any detectable changes of shape or cytokeratin content.

Stained micro-dissected sections of the ducts from the animals receiving androgen replacement with anti-desmoplakin I show that as soon as two days after injection, the desmosomes return to their normal appearance, in the distal region of ducts, showing a regular array of staining at the cell-cell interface and around the nucleus (Fig. 25B, Panel b). The differential staining intensity of desmoplakin I between the proximal and distal regions of the ducts, which remains after castration is similarly observed after androgen re-administration (Fig. 25 A and B, Panels b, d, f and h).

In summary, it appeared clear that testosterone administration induces the restoration of normal cellular contents and cytoskeletal structures such as desmoplakin I, in the distal regions of the prostatic ducts.

Figure 25: Immunofluorescent staining with anti-desmoplakin I antibody of longitudinal sections of micro-dissected ventral prostates of castrated rats at various times after androgen re-administration. A. Proximal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after androgen administration
Panels c and d: 4 days after androgen administration
Panels e and f: 6 days after androgen administration
Panels g and h: 8 days after androgen administration

Some desmosomes are indicated by *arrows* in Panels f and h.

Scale bar: 30 μm

L: Lumen; S: Stroma

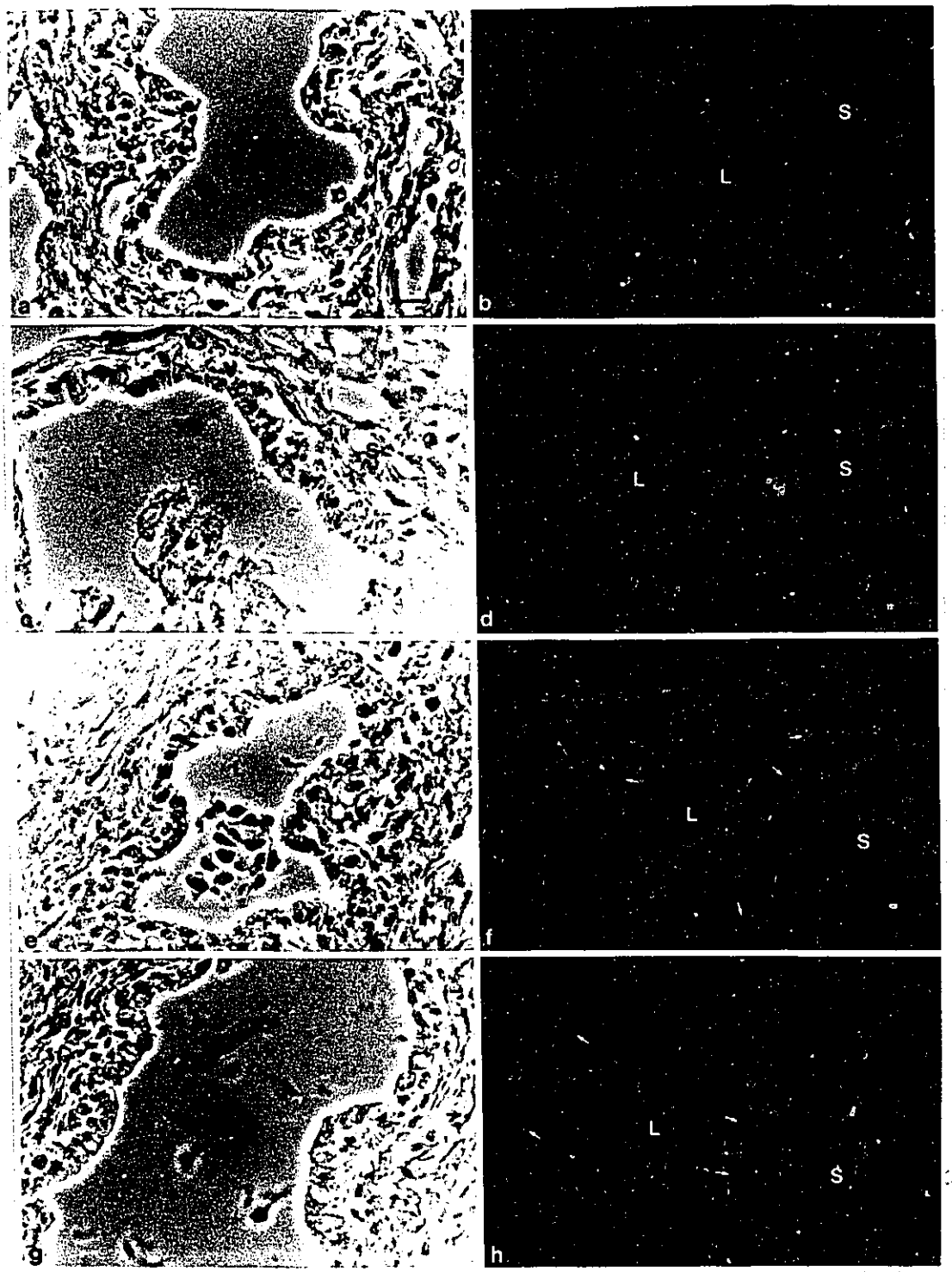
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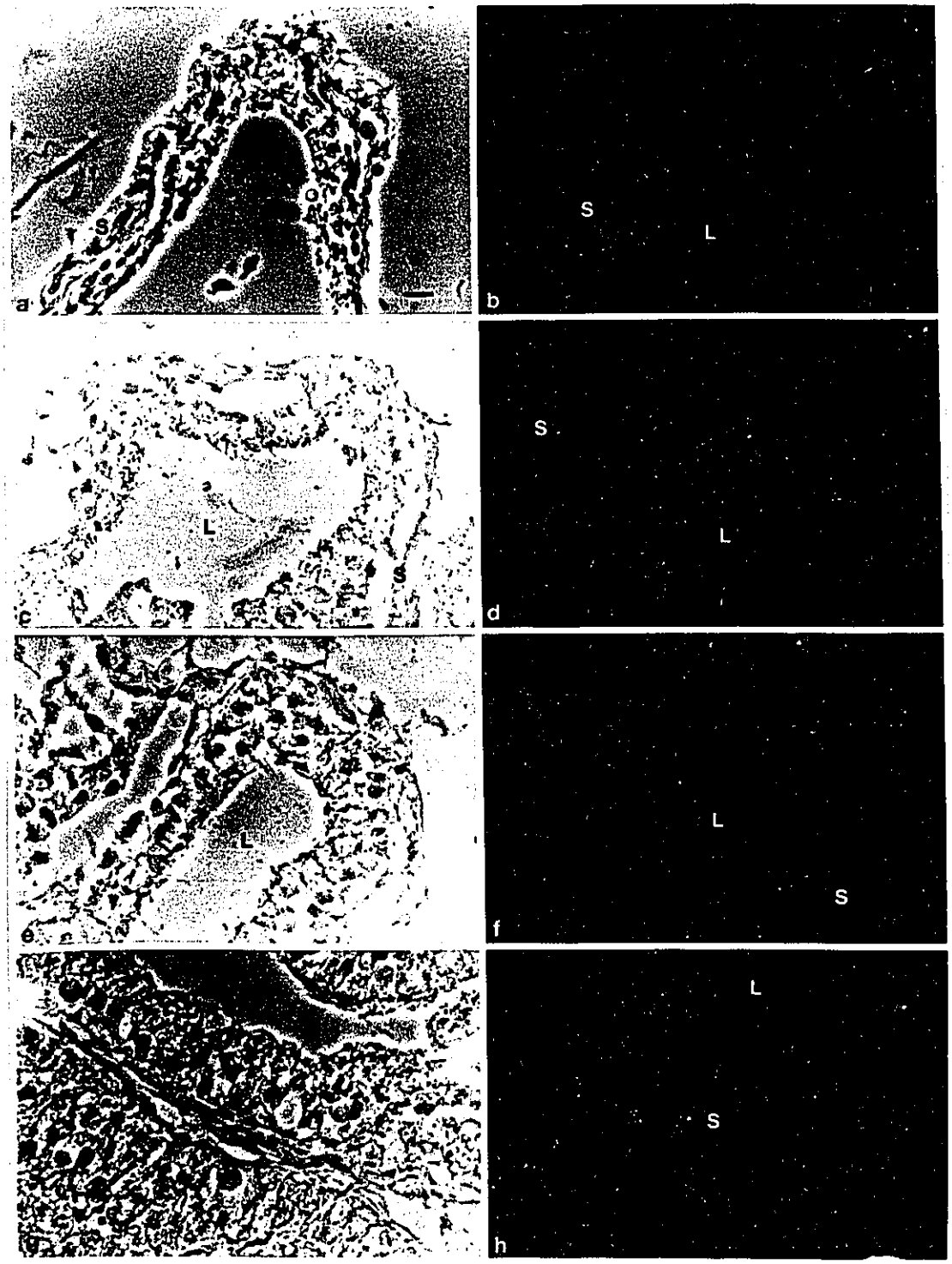
Figure 25: Immunofluorescent staining with anti-desmoplakin I antibody of longitudinal sections of micro-dissected ventral prostates of castrated rats at various times after androgen re-administration. B. Distal region

Panels a, c, e and g: Phase contrast photomicrographs
Panels b, d, f and h: Immunofluorescent photomicrographs

Panels a and b: 2 days after androgen administration
Panels c and d: 4 days after androgen administration
Panels e and f: 6 days after androgen administration
Panels g and h: 8 days after androgen administration

Scale bar: 30 μm

L: Lumen; S: Stroma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DISCUSSION

The facts that the prostate is composed of two cellular compartments, the epithelial cells and the stromal cells, and that this heterogeneity extends to the ductal location of the epithelial cells along the prostatic ducts (Sugimura *et al.*, 1986c) have often been ignored by the prostate biochemists. To understand more clearly the role of androgens on the prostate and the function of the gland, it is necessary to consider the prostate as a complex structure where different components play a specific role and interact with each other to maintain its integrity. The aim of the research presented in this thesis was to expand our knowledge of the heterogeneity in the gland by characterizing the content and the organization of the intermediate filaments in the rat ventral prostate and their sensitivity to androgens.

1. Cytoskeletal Components of the Rat Ventral Prostate Epithelial Cells:

Characterization of the CK content of the intact adult rat ventral prostate indicated a quasi similar content in the rat and human gland. The rat CKs 8 and 18, identified both biochemically and immunologically, are found in the simple luminal epithelial cells, lining the prostatic ducts, and are absent from basal cells and stromal elements, as was observed in human prostate (Nagle *et al.*, 1985; Wernert *et al.*, 1987; Achtstätter *et al.*, 1985). Verhagen *et al.* (1988) have also reported similar results for the rat ventral prostate. The very similar amounts of rat CKs 8 and 18 suggests that the two proteins are co-ordinately regulated in the epithelial cells of the rat ventral prostate as has been demonstrated in other tissues (Domenjoud *et al.*, 1988; Kulesh and Oshima, 1988).

The basal epithelial cells of the prostate react strongly with EAB 903 (raised against human CKs 1, 5, 10 and 11) and 312C8-1 (raised against human CK 14). In contrast, PKK1 (raised against human CKs 8, 18 and 19), PKK3 (raised against human CK 18) and CK 8.60 (raised against human CKs 10/11 and 1) do not immunoreact with these cells. Therefore, their CK content appears to be specific and different from the luminal epithelial cells. Traces of CK 5 have been found previously in the human epithelium

(Achtstätter *et al.*, 1985). Specific staining of human prostatic basal cells has been reported with an antibody that recognizes CKs 4, 5 and 6 (Nagle *et al.*, 1987). Both epithelial cell types in the rat ventral prostate (basal and secretory) react with an antibody that is specific for CKs 5 and 18 (Verhagen *et al.*, 1988). Therefore, the rat CK 5 seems to be a hallmark of basal cells of the rat ventral prostate. Unfortunately, EAB 903 is unreactive toward the cytokeratins extracted from the rat ventral prostate and blotted to nitrocellulose. This lack of reactivity is probably due to a denatured epitope or to the absence of the recognized epitope in the rat CKs. Verhagen *et al.* (1988) were similarly unable to obtain any reactivity between prostatic cytoskeletal extract and another basal cell specific antibody, RCK 103, on Western blots. The presence of CK 14 in basal cells, although never reported in the human or rat prostate, is indicated by the strong immunocytochemical reaction of 312C8-1 with basal cells. According to the catalog of human CKs (Moll *et al.*, 1982; O'Guin *et al.*, 1987), CKs 5 and 14 form a cytokeratin pair whose expression is coupled and is specific to keratinocytes. The inability of others to detect CK 14 is most likely due to the minute amounts of this cytokeratin in extracts (CK 5 has been reported to be in trace amounts only).

The PKK2 antibody, which recognizes CKs 7, 16, 17 and 19 stained only the basal cells in the intact rat ventral prostate. Therefore, the basal cells, in addition to the CK pair 5/14, presumably contain one or more additional cytokeratins. Wernert *et al.* (1987) have reported the presence, although in very low amount, of CKs 7 and 19 in the basal cells of human prostate. Unfortunately, PKK2 is also unreactive to CKs on nitrocellulose and it was therefore not possible to conclusively identify the CKs recognized.

Table II summarizes the different findings on the cytokeratin content of the intact rat ventral prostate discussed above.

Desmoplakin I, an obligatory constituent of desmosomes, is located

Table II: Specific cytokeratin immunoreactivity and expression by the two epithelial cell types of the rat ventral prostate.

	PKK1	PKK2	PKK3	EAB 903	5	7	8	14	18	19
luminal	+	+/-	+	-	-	(-)	+	-	+	(-)
basal	-	+	-	+	+	(+)	-	+	-	(+)

+: Abundant cytokeratin staining

(+): Cytokeratin present in small amounts

+/-: Cytokeratin detected after castration only

(-): not detected in this study but detected by others (see text)

-: not detected

at the junction of secretory cells, at their apical side, in intact rat ventral prostate. None are detected at the basal side, suggesting that junctions between these cells are not of the desmosomal type, which is surprising as they have been morphologically described as so by Brandes (1966). However, due to the low staining intensity, the detection of desmoplakin I in that region may require higher antibody concentration. The differential intensity of desmosomes staining (by desmoplakin I antibody) along the prostatic duct was surprising. It may be caused by a lower amount of desmoplakin I in the desmosomes of the proximal region but may also be due to fewer desmosomes in that region.

Actin subunits were immunologically localized surrounding prostatic ducts, which is similar to the staining pattern reported by Verhagen *et al.* (1988) for desmin, the major IF of smooth muscle cells. It suggests that actin filaments are for the most part located in these cells, which are involved in the mechanics of secretion of prostatic products. It was not possible to study the variations in actin levels along prostatic ducts since the micro-dissection of the ducts disrupts the connective tissue to allow the spreading of the ductal-acinar network. However, actin staining was detected at the apical side of luminal epithelial cells, indicating that it could be involved in the secretory activity of the cells themselves.

2. Effects of Androgens on the Cytoskeletal Proteins:

Castration is well known to induce glandular involution and cell death (Kerr and Searle, 1973; Sandford *et al.*, 1984) and is accompanied by significant alteration in the types of cells present in the rat ventral prostate, and in the arrays of CKs present in the tissue. Immunofluorescence staining of ventral prostate from rats castrated eight days previously indicated that the luminal epithelial cells are especially affected by the absence of androgens since their shape is significantly altered. It would appear that CKs 8 and 18 content of the luminal epithelial cells are not qualitatively or quantitatively affected by

androgens. However, the decreased size of cells results in a crowding of the CK filaments stained by PKK1.

The detection of a new cytokeratin by biochemical analysis of CK extracts from rats castrated eight days previously suggests that CK 19 is also present after castration in the luminal cells. However, the analysis of the cytoskeletal changes by biochemical techniques is limited since only relative changes are observed. It is possible that CK 19 is present in the cytoskeletal extracts from the control rats but is masked by dilution due to the presence of other more abundant CKs, such as CK 8 and 18. Since CK 19 may also be found in basal epithelial cells, a decrease in luminal cell relative number, following castration, and the resultant loss of CK 8 and 18 would allow the detection of CK 19 in extracts from castrated rat prostate. This, however, does not rule out the possibility that the increase in CK 19 levels is due to an induction or enhancement of the expression of the CK 19 gene, increased stability of CK 19 mRNA and/or protein, or increased solubility of CK 19. To answer this question, *in situ* hybridizations with DNA probes specific for CKs 8, 18 and 19 would be necessary.

An analysis of the staining pattern of desmoplakin I in ventral prostate of rats castrated eight days previously indicates that the intensity of the immunofluorescent signal decreases relative to that seen in intact animals and resembles that of the proximal region of an intact rat prostatic duct. On the other hand, the desmosomes in distal regions of ducts after castration present an irregular pattern of distribution while staining intensity remains. This suggests an involvement of desmosomal plaques in androgen response. Although only speculations can be made, it suggests that cell-cell interactions become weaker after androgen ablation. Kartenbeck, Schmid, Franke and Geiger (1982) have shown that separation of cells in culture is accompanied by a rapid internalization of desmosomal plaque proteins followed by a decrease in the relative number of desmosomes. After castration, the disruption and decrease in desmosomes in the rat ventral prostate may be the result of

apoptosis process, since one of the early event of this mode of cell death is the detachment of the dying cell from neighbouring cells which involves disruption of cell junctions (Wyllie, 1987). Paranko and Virtanen (1986) have also observed a decrease in desmosomal plaque proteins in the regressing rat paramesonephric duct during male development.

After castration, a relative increase in actin subunits, clearly seen by biochemical analysis of castrated rat ventral prostate cytoskeletal extracts, is paralleled by the "crowding" of actin staining around the smaller prostatic acini of the castrated rat, as observed in random sections. It therefore appears that smooth muscle cells are not affected in their actin content and distribution by androgen ablation.

In summary, androgen ablation does not appear to cause dramatic changes in the type of cytoskeletal proteins present in the prostate. Changes in the amount of the different cytokeratins observed by Western analysis appear to be more a factor of cell survival than of new synthesis.

3. Effects of Androgens on the Cell Populations of the Rat Ventral Prostate:

The basal to luminal epithelial cell ratio decreases from the proximal region to the distal tips of intact rat prostatic ducts. This may be compared to the ratio of basal to luminal cells before and after castration monitored in random sections. In the proximal region, basal to luminal cell ratio is about equivalent to that in the prostate of a rat castrated eight days previously. In the distal region, this ratio resembles that of normal rat. These observations suggest that the tall columnar epithelial cells in the distal region of the ducts are androgen dependent for their size and function, and die after castration. The cuboidal epithelial cells in the proximal region and the basal epithelial cells survive after castration, resulting in an increase in the basal to luminal epithelial cell ratio. This further suggests that basal cells are

androgen independent, an hypothesis which is supported by the results of English *et al.*, (1987) who have shown that only luminal cell number is significantly reduced after castration in the rat ventral prostate. Furthermore, Verhagen *et al.* (1988) demonstrated that there is no significant change in basal cell number after castration and therefore termed them androgen-independent. Taken together, these results demonstrate that the distal tips of the prostatic ducts contain the secretory epithelial cells that are androgen dependent.

The staining pattern obtained with PKK2 further supports the above hypothesis. PKK2 staining of the random sections from the ventral prostates of intact rats reveals the presence of two subpopulations of luminal cell: The majority of the luminal epithelial cells are tall columnar and are not stained by PKK2. However, a few ducts are lined by small cuboidal cells that were strongly stained by that antibody. These ducts bear a strong resemblance to the luminal cells found along the prostatic ducts after castration and to the cells in the proximal regions of the ducts of intact rats, both in terms of their morphology and cytoskeletal components. Since the proximal region recognized by PKK2 antibody is very limited (that is from urethra to first branch point of prostatic duct) (See Fig. 15), most of the cells seen in random section of normal rat ventral prostate are of the "distal region" type. This suggests that cells left after castration are the ones found in the proximal region of the intact rat ventral prostate. This is supported by the observation that a loss of 35% of ductal tips and branch points occurs after castration (Sugimura *et al.*, 1986c). The changes in the cell population along the prostatic ducts after castration, as mentioned above, provide further evidence for this concept since the cytoskeletal components of the cells in the proximal region are unaffected by androgen ablation while the distal region of the ducts shows significant changes in the same components and alters to resemble the proximal region.

Cell dynamics during prostate regression and development have been compared on the basis of the basal to luminal cell ratios. It has been

reported that during postnatal development the proportion of basal cells in the prostate declines from 10% to less that 2-3% (Evans and Chandler, 1987a). The basal cells initially form a complete peripheral layer around glandular elements, but they become increasingly dispersed with the gland maturation. After castration, it appears that the reverse process takes place. The sparse distribution of basal cells of the intact rat ventral prostate becomes an almost continuous layer of basal cells over luminal cuboidal cells in animals castrated eight days previously. It is known that the development and regeneration of the prostate occurs from the proximal region towards the distal region and that the proliferative cells are almost exclusively located at the distal tips (Sugimura *et al.*, 1986c). In this study, androgen administration to castrated rats produces a gradual reduction of the basal to luminal cell ratio in the distal region of prostatic ducts suggesting that the growth occurs in the distal region while the proximal region remains unaffected by androgens.

Recent *in situ* hybridization experiments have shown that the expression of specific mRNA sequences is also regionally localized and restricted to the distal region of the gland (Rouleau, M., Léger, J. and Tenniswood, M., submitted). It therefore appears that cell shape (Cunha *et al.*, 1987, this thesis), cell proliferation (Sugimura *et al.*, 1986), cell content, gene expression and androgen dependence (Rouleau, M., Léger, J. and Tenniswood, submitted) are all dependent of the ductal location. These results suggest that epithelial cell heterogeneity in the rat ventral prostate is not limited to basal epithelial versus luminal epithelial cells but also includes at least two different luminal epithelial cell types, each of which has a specific ductal location and a particular shape that correspond to a specific cytokeratin content and a clearly defined sensitivity to androgens.

The regional regulation that androgen seems to exert along prostatic ducts is intriguing, as how it may be achieved. Androgens have been hypothesized to act through the stromal fraction (Cunha, Sugimura and

Bigsby, 1985; Tenniswood, 1986). Therefore, the stromal signals would differ from one region to another along the duct. From the observations presented in this thesis, one may hypothesize that cytoskeletal elements are involved in signal transduction and, therefore, that the different cytokeratin contents transduce a variety of signals to stromal cells. The key role that intermediate filaments may play in signal transduction has often been pointed out. Their binding to the nuclear matrix, and more particularly to lamin B (Georgatos and Blobel, 1987) suggests that they are indirectly attached to some chromatin segments and, therefore, are able to regulate transcription. The finding that androgen receptors are located on the nuclear matrix (Barrack and Coffey, 1982) increases the complexity of the regulatory controls that may exist between androgens and cytoskeleton. Different degree of polymerization, attachment of intermediate filament associated proteins (IFAPs) to IFs, interactions with other cytoskeletal components may all modulate tension in the IF-lamin B interaction, which may in turn open potential binding sites for androgens or for the initiation of transcription.

The importance of cell morphology in regulating gene expression has recently been pointed out (Ben Ze'ev *et al.*, 1988). It appears that, in the prostate, cell shape correlates with cytokeratin content, as observed by the differential staining of luminal cells of the prostate by PKK2. It has recently been shown that the synthesis of PSBP occurs exclusively in tall columnar luminal epithelial cells (Rouleau, M., Léger, J. and Tenniswood, submitted), indicating that protein synthesis may be closely related to shape and cytokeratin content.

Indeed, polyribosomes have been found in association with the cytoskeleton (Lenk *et al.*, 1977; Ornelles *et al.*, 1986) and the polymerization characteristics of the cytokeratins or IFAPs binding may modulate polyribosome attachment and therefore control the translation of specific mRNA species. Desmoplakins, characterized as IFAPs as well, may also trigger CK polymerization. A lower amount of desmoplakin I may indirectly control transcription and translation processes. Evidence for

all that comes from the close relationship existing between cell shape, presence of PKK2 stained CKs, secretory activity and androgen dependence. The order in which control processes are achieved, i.e. which factor is affected first and regulates the other ones, is not known. However, a regulative role may be assigned to basal cells, as controller of signals between stroma and luminal epithelium. The increased relative number of basal cells in the proximal region, forming a continuous layer over luminal epithelial cells may block stromal signals from reaching the luminal epithelium while in the distal region, luminal cells are in a more direct contact with stromal cells, through the ECM. The role played by the ECM is still under intensive investigation in several systems, but there is clear evidence that they are involved in the control of gene expression (Lee *et al.*, 1984; Lee *et al.*, 1985; Blum and Wicha, 1988). Therefore, it may appear that ECM components such as laminin, fibronectin, collagen, etc., varies along the prostatic duct and modulates androgen effects.

4. Stem Cells in the Rat Ventral Prostate:

As mentioned previously, cell proliferation occurs mostly at the distal region of prostatic ducts. Cells in the more proximal region are therefore "older" than more distal cells, that is, they arose earlier in the development of the prostate. The aging of luminal cells may be accompanied by a change in CK pattern of expression, which is frequently observed in differentiating tissues. Several line of evidence have suggested that the induction of specific mRNA synthesis occurs as epidermal epithelial cells undergo terminal differentiation (Fuchs *et al.*, 1987). Similar results were demonstrated in the vaginal epithelium differentiation induced by estrogens (Roop, 1987). In the prostate, the cellular differentiation and changes in CK contents may result in a loss of androgen responsiveness which will cause losses in secretory protein synthesis and secretion, in cell proliferative activity and in the expression of androgen repressed genes such as TRPM-2 (its protein product being involved in apoptosis) (J. Léger, personal communication), with the concomitant loss of the potential to undergo programmed cell death in the

absence of androgens.

PKK2 staining revealed that small cuboidal cells, decorated by PKK2, extended only as far as the first branch point of the prostatic ducts. After castration, the prostatic ductal-acinar network, although much reduced in size and complexity, still displays some branch points. Therefore, it seems unlikely that the limited length of the "proximal region" in normal animals alone could account for the number of small cuboidal cells that are present in the castrated animals. In addition to the small cuboidal cells already existing in the intact rat ventral prostate, a subset of tall columnar cells may undergo a specific pattern of gene expression and shape modifications to become similar to the small cuboidal cells, and thus, be protected from apoptosis (programmed cell death). This model, presented in Figure 26, has been expanded from a model of the control of cell proliferation and death by Isaacs (1987). He hypothesized that the epithelial cells remaining after castration can be divided into two types on the basis of their proliferative activity: Stem cells, which possess extensive proliferative potential and amplifying cells which are of limited potential of proliferation. Several studies have ruled out the possibility that basal cells are able to act as stem cells or amplifying cells for prostate growth, regeneration, or for prostate homeostasis (Evans and Chandler, 1987a, 1987b; English *et al.*, 1987). Stem cells and amplifying cells may therefore be located among the luminal epithelial cells. These cells have so far not been identified, probably due to the lack of a suitable marker as indicated in Figure 26 by a series of hatched lines. The shape and cytokeratin content of amplifying cells may be similar to tall columnar cells under androgen stimulation. After castration, these amplifying cells return to a quiescent state which may be controlled by their shape and cytokeratin content. The tall columnar amplifying cells may therefore become cuboidal, partially modify their cytokeratin content and in this way survive the absence of androgens (indicated as cells containing both hatched lines and dots on Fig. 26). However, on re-administration of androgens, these cells are capable of returning into luminal tall columnar cells and in that

Figure 26: Model of cell proliferation and cell death in the ventral prostate of intact and castrated rats.

Panel a: Schematic view of a prostatic duct from an intact rat. Luminal epithelial cells in the proximal region are cuboidal, stained by PKK1 and PKK2 antibodies and very few possess a proliferative potential in that region. The luminal epithelial cells in the distal region of the prostatic duct are columnar, recognized by PKK1 but not by PKK2 and, especially at the ductal tips, amplifying cells are found.

Panel b: Schematic view of a prostatic duct from a castrated rat. The cells in the proximal region are similar to that of an intact rat prostatic duct. However, amplifying cell are more numerous, due to the regression of the duct starting at the distal tips. The distal region resembles the proximal region in terms of luminal cell shape and basal cell number.

L: Lumen; S: Stroma; P: Proximal region; D: Distal region.

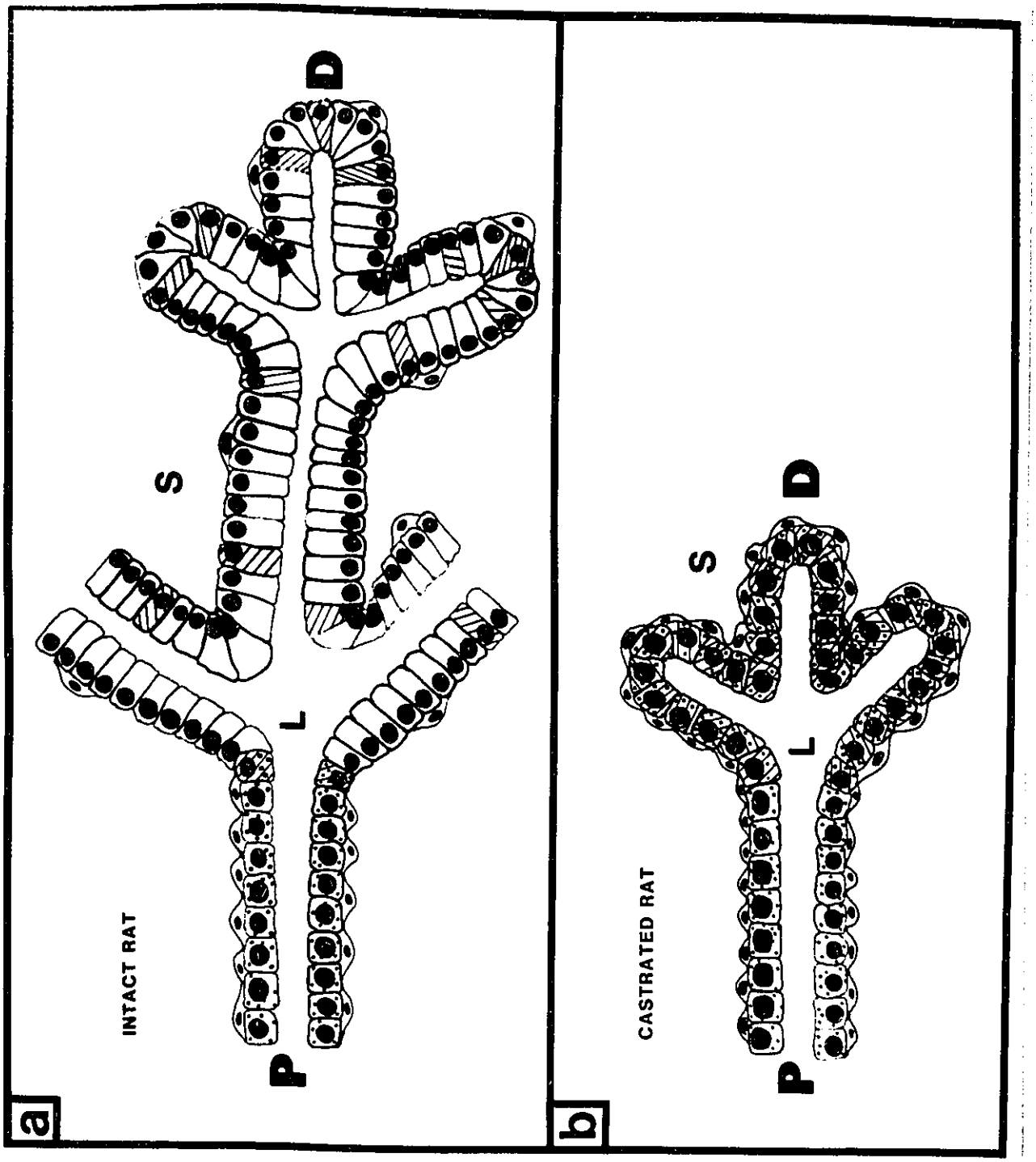
 : unknown marker of amplifying cells

 : proteins recognized by PKK2 antibody

 : amplifying cells which contain cytokeratins recognized by PKK2. These cells have a high proliferative potential.

 : amplifying cells which do not contain cytokeratins recognized by PKK2. These cells have a proliferative potential limited relative to the cuboidal amplifying cells but may regain this high potential after androgen ablation.

 : basal cells


a
INTACT RAT
S
D
L
P
b
CASTRATED RAT
S
D
L
P

manner regenerate the prostatic ductal network. PKK2 may be a marker for these cells. When amplifying cells are in an "active" state, they display similar behaviour as luminal tall columnar cells and are not stained by PKK2. However, in periods of androgen ablation, these cells display the shape and staining pattern with PKK2 similar to that of small cuboidal cells that are in the very proximal region of prostatic duct. This hypothesis requires further investigation, to determine if other markers can be found to discriminate between "true proximal luminal cells" and "amplifying quiescent cells" in the atrophied prostate after castration and between "true tall columnar secretory cells" and "active amplifying cells" in the ventral prostate of intact animals.

5. Conclusions:

It is found that the cytokeratin content of the basal and luminal epithelial cells are characteristic for each cell type and constitute good markers for these cell types. The ratio basal to luminal epithelial cells is shown to vary along the prostatic duct in the ventral prostate of intact rats and uniquely in the distal region of prostatic ducts after castration, suggesting a preferential loss of luminal epithelial cells in absence of androgens. It appears that a correlation exists between the ductal location, cell shape, cytokeratin content and androgen dependence.

In order to fully understand androgen action on the rat ventral prostate, it is now necessary to consider the heterogeneity that exists along prostatic ducts. The results presented in this thesis demonstrate that each discrete region must be studied separately in order to have a clear picture of the mechanism of action of androgens. It is now clear that the use of prostate homogenates or random sections are not suitable in view of the complexity of the gland.

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Appendix 1:

ABBREVIATIONS

A: Acidic

B: Basic

BLE: Bladder epithelium

BW: Body weight

CK: Cytokeratin

CMF: Calcium Magnesium free

DNA: Desoxyribonucleic acid

DHT: 5α-dihydrotestosterone

ECM: Extracellular matrix

EDGF: Epithelially derived growth factor

EDIF: Epithelially derived inhibiting factor

EDTA: Ethylenediaminetetra-acetic acid

FITC: Fluorescein isothiocyanate

g: gram

h: hour

hnRNA: heterogeneous nuclear RNA

IF: Intermediate filament

IFAP: Intermediate filament associated protein

mRNA: messenger RNA

PAGE: Polyacrylamide gel electrophoresis

PSBP: Prostate steroid binding protein

PBS: Phosphate buffered saline

PMSF: Phenylmethylsulfonylfluoride

OW: Organ weight

RER: Rough endoplasmic reticulum

RITC: Rhodamin isothiocyanate

RNA: Ribonucleic acid

SDGF: Stromally derived growth factor

SDS: Sodium dodecyl sulfate

SM: Skin mesenchyme

TEMED: N, N, N', N' tetramethylethylenediamine

Tfm: Testicular feminization

tRNA: transfer RNA

TRPM-2: testosterone repressed prostate messenger-2

UGE: Urogenital epithelium

UGM: Urogenital mesenchyme

UGS: Urogenital sinus

WT: Weight

Appendix 2:

MATERIAL

Male Sprague-Dawley rats were purchased from Charles River Inc. (Montréal, Qué.). 5α-androstan-17ß-ol-3-one propionate was supplied by Steraloids (Wilton, NH). The fine electron microscopy forceps were purchased at J.B.EM Services Inc. (Montréal, Qué.). The embedding medium (Tissue Tek O.C.T. compound), microscope slides and coverslips were obtained from Fisher Scientific Co. Ltd. (Nepean, Ont.). Monoclonal antibodies against cytokeratins were purchased from the following companies: PKK1, PKK2, PKK3 were purchased from Ortho Diagnostics Systems (Don Mills, Ont.); EAB 903 was supplied by Enzo Biochem. Inc. (New-York, NY); 312C8-1 was kindly supplied by S.H. Dairkee (Oakland, CA); CK 8.60, C4 and DP-2.17 were purchased from ICN Immunobiologicals (Montréal, Qué.). Goat anti-mouse IgG and IgM antibodies conjugated to FITC, RITC or to horseradish peroxidase were obtained from Organon Teknika (Scarborough, Ont.). The Mini-Protean II Electrophoretic cell, Mini-Trans-Blot Electrophoretic transfer cell, nitrocellulose membranes and molecular weight standards were from Bio-Rad Laboratories (Mississauga, Ont.). Collagenase type IV, rabbit muscle actin, Ponceau red S and 4-chloronaphtol were obtained from Sigma Chemical Co. (St-Louis, MO.). Vimentin was purchased from Boehringer Mannheim (Dorval, Qué.). Skim milk powder was obtained from Difco (Detroit, Mich.). Acrylamide, bis-acrylamide, Tris, SDS and TEMED for gel electophoresis were of "ultra pure reagent" quality from International Biotechnologies, Inc. (Toronto, Ont.). Ilford films, Kodak paper and other dark room chemicals were obtained from Bill's Camera Craft (Ottawa, Ont.). All other chemicals were of "reagent" grade and were purchased from BDH (Toronto, Ont.) or Fisher Scientific Co. Ltd. (Nepean, Ont.).

Appendix 3:

CURRICULUM VITAE

PERSONAL INFORMATION

Name: **MICHELE ROULEAU**

Date of Birth: 28 September 1965

Place of Birth: Sherbrooke, Canada

Marital Status: Single

Address (Home): 19 Eastwood Place #4
Vanier, Ontario,
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(Work): Faculty of Health Sciences
Department of Biochemistry
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EDUCATION

Secondary School Education (1977 - 1982)	Ecole Secondaire Les Compagnons de Cartier Ste-Foy (Québec)
College Education (1982 - 1984)	CEGEP de Ste-Foy, Ste-Foy (Québec) Degree conferred: Collegial Studies Diploma (D.E.C.) Sciences 1984
University Education (1984 - 1987)	Université Laval, Ste-Foy (Québec) Degree conferred: B.Sc. Biochemistry, 1987
(1987 - present)	University of Ottawa, Ottawa (Ontario) Degree expected: M.Sc. Biochemistry, October 1989 Title of the thesis: Cellular and Cytoskeletal Heterogeneity Along the Rat Ventral Prostatic Duct (Supervisor: Dr Martin Tenniswood)

TEACHING EXPERIENCE

1. UNIVERSITY OF OTTAWA, Department of Biochemistry
   (September 1988 - December 1988)
   Demonstrator in the Teaching Laboratory :
   BCH 3946, Introduction to Experimental Biochemistry

RESEARCH EXPERIENCE

1. LAVAL UNIVERSITY, Department of Biochemistry
   Laboratory of Dr Jacques Lapointe
   (June 1987 - August 1987)
   Research Assistant

2. LAVAL UNIVERSITY, Department of Biochemistry
   Laboratory of Dr Jacques Lapointe
   (May 1986 - August 1986)
   Research Assistant

METHODOLOGIES ACQUIRED

1. The Master's project involved the set up and the use of the following techniques:
   1. SDS-Polyacrylamide Gel Electrophoresis
   2. Western Analysis
   3. Micro-dissection of the rat ventral prostate
   4. Immunofluorescence

2. The research assistant positions required the use of the following techniques:
   1. Cloning in the vector pBR322
   2. Subcloning in the phage M13
   3. Generation of deletion clones with the enzyme Bal 31
   4. DNA sequencing by the dideoxy nucleotide chain termination reaction

COMPETITIVE AWARDS

Graduate

1. MRC, Research Scholarship	January 1990-January 1991
2. Fonds FCAR, Research Scholarship	September 1987-May 1989
2. University of Ottawa, Entrance Scolarship	September 1987-August 1989

Undergraduate

1. N.S.E.R.C., Summer Scholarship	May 1986-August 1986

PUBLICATIONS

1. Tenniswood, M., Montpetit, M.L., Léger, J.G., Pineault, J. and Rouleau, M. (1988) Epithelial-stromal interactions and cell death in the prostate. In: The Prostate as an Endocrine Gland?, Eds.: R.J. Ablin and W.E. Farnsworth, CRC Press Boca Raton, Florida.

2. Rouleau, M. and Tenniswood, M. (1989) Desmosomes in the rat ventral prostate: heterogeneity along the prostatic duct and possible involvement in programmed cell death. (in preparation).

3. Rouleau, M., Léger, J.G. and Tenniswood, M. (1989) Ductal heterogeneity of cytokeratins, gene expression and cell death in the rat ventral prostate. J. Cell Biol. (submitted)

ABSTRACTS

1. Rouleau, M., Léger, J.G. and Tenniswood, M. Cytokeratin composition and androgen dependence of the epithelial cells in the rat ventral prostate (Poster presented at the 80th Annual Meeting of the American Association for Cancer Research, San Fransisco, May 1989).

2. Rouleau, M. and Tenniswood, M. Variations in the cytoskeletal components of the rat ventral prostate (Poster presented at the 4th International Congress of Cellular Biology, Montréal, August 1988).

3. Rouleau, M. and Tenniswood, M. The cytokeratins of the rat ventral prostate (Poster presented at the Canadian Federation of Biological Sciences Congress, Québec city, June 1988).

4. Rouleau, M. and Tenniswood, M. Cytosquelette de la prostate ventrale du rat (Poster presented at the ACFAS Congress, Moncton, May 1988)