12 Prostate Development: Mechanisms for Opposite Effects of Low and High Doses of Estrogenic Chemicals

Catherine A. Richter, Barry G. Timms, and Frederick S. vom Saal

CONTENTS

12.1 Introduction ................................................................. 380
12.2 Historical Overview of the Prostate ................................... 381
12.3 Prostate Anatomy and Homology Among Species .................. 382
  12.3.1 Prostate Development ............................................. 382
  12.3.2 Evidence for Homology of the Rodent Dorsolateral and
  Human Prostate ......................................................... 384
12.4 Regulation of Prostate Development .................................. 387
  12.4.1 Testosterone and 5α-Dihydrotestosterone (DHT) .............. 387
  12.4.2 Growth Factor Signaling Between Mesenchyme and
  Epithelium .............................................................. 388
12.5 Estrogen Modulates Prostate Development ......................... 390
  12.5.1 Inhibitory Effects of High, Pharmacological Doses
  of Estrogen ............................................................ 390
  12.5.2 Stimulatory Effects of Low, Physiological Doses of Estrogen .... 392
  12.5.3 Mechanisms of Effects of Low Doses of Estrogen .............. 393
12.6 Environmental Endocrine-Disrupting Estrogenic Chemicals Alter
  Prostate Development .................................................. 396
12.7 Estrogen and Adult Prostate Pathology .............................. 401
12.8 Summary ................................................................. 402
Acknowledgments .................................................................. 403
References ............................................................................ 403
12.1 INTRODUCTION

The prostate is one of the male accessory glands that contributes to seminal fluid. In male mice, removal of this organ reduces fertility. There has been speculation for some time that estrogen plays a role in normal development as well as subsequent disease of the prostate. One basis for this speculation is that embryologists recognized that the region of the developing urogenital sinus (UGS) just caudal to the bladder, from which the prostatic ducts emerge during fetal life, develops into a portion of the vagina in females. It thus seemed reasonable to speculate that, since the vagina is an estrogen-responsive organ, portions of the prostate might also be responsive to estrogen. This led to speculation that estrogen might play a role in regulating prostate development and subsequent function, as well as diseases associated with aging.

In contrast to the above prediction that estrogen might play a role in normal prostate development, there are numerous reports that prenatal or neonatal exposure to high, pharmacological doses of estradiol or the estrogenic drug diethylstilbestrol (DES) dramatically interfere with prostate development in mice and rats. More recently there have been studies concerning the effects of endogenous estradiol as well as very low doses of estrogenic chemicals on prostate development. Many questions have been raised regarding the potential for environmental chemicals with estrogenic activity to alter prostate development at concentrations encountered in the environment (referred to as environmentally relevant doses of chemicals). It is likely that very high doses of DES do not serve as a model for potential effects of low doses of these chemicals. This conclusion is based on the generally greater potency of DES relative to most “environmental estrogens” and, most importantly, on the fact that high doses of a hormone can lead to “down-regulation” of the capacity for tissues to respond to the hormone, while low doses of the same hormone can stimulate or “up-regulate” response capacity. The idea that dose is important is not a new concept in endocrinology, toxicology, or pharmacology. However, prior to recent findings based on manipulating estrogen levels within a physiological range in fetal mice, physiologically relevant low doses of estrogen had simply not been examined. One reason for this was the difficulty associated with measuring estradiol in very small volumes of serum from fetal and neonatal rats and mice, in which levels of other lipids are very high and the free, biologically active fraction (2.0 to 0.3% of total serum estradiol) is extremely low; total estradiol measured by radioimmunoassay consists of free estradiol as well as estradiol bound to plasma proteins.

In humans, the prostate is notable for its vulnerability to disease. Prostate cancer is one of the most common cancers in men in the United States. Benign prostatic hyperplasia (BPH) is a common condition beginning in middle age in men. Nearly half of middle-aged men can expect to develop urinary problems associated with BPH during their lifetime. We review in vivo and in vitro studies showing that exposure to a very small increase in circulating estradiol or to very low doses of estrogenic chemicals present in the environment during fetal life in male mice can lead to differences in prostate differentiation that persist into adulthood. The potential for environmentally relevant concentrations of environmental estrogens to impact human prostate pathogenesis is of immense importance as a public health issue.

However, there are no human data at this time to assess whether any aspect of prostate disease in men is related to developmental exposure to manmade estrogenic chemicals present in the environment. The marked similarity in effects in mice and humans of exposure during sexual differentiation to high doses of DES, and the conclusion from this literature that the mouse is the best animal model for predicting the effects of developmental exposure to estrogen in humans is cause for concern, based on the effects on the mouse prostate that we describe below.

12.2 HISTORICAL OVERVIEW OF THE PROSTATE

Pathology of the prostate was attributed in 1685 by Samuel Collins to “indulgence in venery” (the pursuit of sexual pleasure). Collins recognized that prostate enlargement was important with regard to urethral obstruction; this was also discussed in 1769 by Giambattista Morgagni. Franks noted that Morgagni identified the site of origin of hyperplasia within the prostate and also examined the prevalence of this disease in old men.

John Hunter observed in 1786 that the prostate (and other accessory reproductive organs) underwent involution following castration. Zuckerman comments on the remarkable fact that even though the implication of this observation would suggest that castration might serve as a treatment to relieve the effects of prostatic enlargement on urethral obstruction, which results in death due to uremic poisoning if untreated, the implication of this observation was not grasped until almost a hundred years later. Castration as a treatment for enlargement of the prostate was finally proposed in 1893, after which it became the method of treating this disease. However, this approach was soon abandoned, because at that time, mortality associated with surgery was unacceptably high. In addition, although it might seem surprising today, at the end of the nineteenth century there was still considerable controversy concerning the role of the testes in accessory reproductive organ function. This controversy is interesting in that it had been reported in the middle of the nineteenth century that testicular grafts reversed the effects of castration in cockerels, which led to attempts to reverse impotence in aging men by means of grafting animal testicular tissue. At this time it was believed that any effects of the testes on organs such as the prostate were probably mediated by nerves, not secreted substances. However, in 1927 methods for extracting gonadal steroids were described, and testosterone was finally identified as the most potent of the testicular hormones in 1935. Prostate growth in castrated rats was being routinely used as a bioassay for potency of testicular hormones by this time.

Our current understanding of prostate development and anatomy appears to have progressed steadily during the past 60 years, but has been partially hindered by reliance on old anatomical descriptions. Lowley's description of prostatic lobes has been replaced by the now widely accepted concept that the human prostate is better described as consisting of zones. The recent advancement in computer technology now allows organ structure to be visualized by three-dimensional (3-D) reconstruction, from digitized serial sections. Three-dimensional reconstruction requires tracing, digitizing and axial alignment of identified objects (anatomical structures) within each section. This provides a powerful tool for examining anatomy,
including that of the prostate. This technique has been particularly useful in understanding the complex pattern of ductal morphogenesis, a feature that is extremely difficult to grasp when viewing two-dimensional histological sections in a microscope.\textsuperscript{16,19} Also, the digitized information used to reconstruct the prostate provides the basis for making quantitative comparisons of experimental manipulations on the developing prostate.\textsuperscript{8,19}

12.3 PROSTATE ANATOMY AND HOMOLOGY AMONG SPECIES

12.3.1 Prostate Development

The prostate ducts begin forming as epithelial buds at about 10 weeks of gestation in humans and gestation day 17 in mice.\textsuperscript{20} These outgrowths begin as solid epithelial buds that branch during late fetal life in humans, forming a compound tubulo-alveolar gland structure.\textsuperscript{21} In mice, birth occurs within 2 days of the beginning of prostate differentiation, and extensive ductal branching occurs throughout infancy and adolescence. The adult structure is not achieved until approximately postnatal day 50.\textsuperscript{22}

In humans, the pubertal reawakening of androgen secretion by the testes results in the prostatic glandular ducts forming a patent lumen within the terminal acini. The epithelial lining becomes highly differentiated, after which androgen-dependent secretory activity begins.

As shown in Figure 12.1, the anatomy of the human prostate is now described in terms of zones.\textsuperscript{18} The transition zone is composed of short glandular ducts and surrounds the urethra above the intersection of the ejaculatory ducts; the central zone surrounds the transition zone just under the bladder and is traversed by the ejaculatory ducts; and the peripheral zone lies outside the posterior of the central zone and extends along the urethra below the intersection of the ejaculatory ducts.\textsuperscript{23} The morphology of the mouse prostate, which is divided into dorsolateral and ventral lobes, has been described in a series of papers by Sugimura.\textsuperscript{22,24,25} Individual prostatic glandular ducts extend from the urethra and branch into terminal ducts that are lined with pseudostratified columnar epithelium. In humans, a continuous layer of basal cells underlies the epithelium, while in rodents basal cells are dispersed along the epithelial basement membrane.\textsuperscript{26} The epithelial glandular ducts of the prostate are surrounded by a layer of smooth muscle cells. In humans, this layer of smooth muscle is much thicker than in rodents.\textsuperscript{26}

The ejaculatory ducts form from the embryonic Wolffian ducts. The ejaculatory ducts enter the prostatic urethra caudal and lateral to the site of the urethra, which is the remnant of the Müllerian ducts as they merge and enter the posterior UGS (Figure 12.2). The urethra becomes enclosed in the central zone of the human prostate. Each ejaculatory duct (vas deferens) merges with the ipsilateral seminal vesicle duct, which differentiates during the 13th week of embryonic life in humans from the Wolffian duct proximal to the UGS. The ejaculatory ducts lead into the prostatic urethra next to an enlarged portion of the urethral crest, the verumontanum (also referred to as the colliculus seminalis) in the posterior wall of the urethra (Figure 12.1).

\textbf{FIGURE 12.1} Diagrams of frontal and sagittal sections of the male urogenital complex showing the anatomical position of the adult prostate and associated structures. The prostatic zones are: central zone (CZ), peripheral zone (PZ), and transition zone (TZ). The anterior fibromuscular stroma (AFS) is also shown. From Reference 16.

The prostatic urethra is divided into a proximal segment (from bladder neck to verumontanum) and distal segment (from verumontanum to external sphincter), forming a 35 to 45° angle from the horizontal at the verumontanum. The proximal urethra is surrounded by circular smooth muscle, which is referred to as the preprostatic sphincter and functions to stop retrograde ejaculation into the bladder. In the proximal portion of the urethra in humans closest to the bladder neck, short prostatic ducts that mingle with sphincteric stroma have been proposed as the potential site for pathogenesis of BPH.\textsuperscript{27} Hyperplasia of the short ducts in the transition zone during development of BPH impinges on the urethra and can lead to obstruction.

McNeal introduced the hypothesis of a reawakening of embryonic inductive interactions to describe the inappropriate new ductal budding that occurs during the onset of BPH in old age. This was thought to result from non-prostatic stroma (the proximal urethral sphincter) inducing adjacent transition zone ducts to begin new ductal formation in areas of stromal proliferation. Tissue recombination studies have confirmed that adult human prostatic epithelium is capable of undergoing proliferation and differentiation in response to stromal signals.\textsuperscript{28} A very interesting aspect of prostate carcinoma is that it is predominantly found in glands originating from the central and posterior region of the prostatic urethra. In contrast to the short glands in the transition zone that are predominantly the site of BPH, these are long ducts that branch and extend in the peripheral zone of the prostate in men. The portion of the embryonic UGS from which these different prostate ducts originate is thus highly predictive of the type of pathology observed in the ducts during aging.
42-DAY-OLD HUMAN EMBRYO

FIGURE 12.2 Genital ducts prior to differentiation. Drawing of a human embryo at about 42 days of age with the upper half and left body wall cut away to demonstrate the gonads, associated Wolffian (mesonephric) and Müllerian (paramesonephric) ducts, and urogenital sinus (UGS). The prostate differentiates from the cranial UGS. The gut and its mesentery have been removed. Modified from Reference 140.

12.3.2 Evidence for Homology of the Rodent Dorsolateral and Human Prostate

It has been proposed that the variety of interspecies differences observed in the structure of the adult prostate gland reflects a diversity that makes it difficult to find a suitable animal model for the study of human prostatic disease.²⁷ Using a computer-assisted three-dimensional approach to visualize the microanatomy of prostate development, Timms¹⁹ has compared the ductal budding patterns during prostate morphogenesis in rat, mouse, and human (Figure 12.3 and Figure 12.4). The three-dimensional reconstruction procedure revealed marked similarities among rodents and human prostate gland genesis.

When different species share similar regulatory systems and a common pattern of development of a structure, this is taken as evidence that the structure in the different species is homologous, even though the final form in the adult might appear markedly different (the classic example is the wing of a bat, hand of a human, and fin of a dolphin). Timms proposed that the prostatic ducts that originate from similar regions of the UGS in rats, mice, and humans are homologous structures.¹⁹ First, the ducts that develop into the dorsolateral lobe in rodents show a pattern of budding

FIGURE 12.3 Serial section reconstructions of the rat urogenital complex on gestation day (GD) 18, 19, 20, and 21, illustrating the stages of early prostate development. Prostate morphogenesis begins at GD 18 and by GD 19 the dorsal, ventral, lateral, and dorsocranial (coagulating gland) buds are visible. On GD 21 (the day before birth), prostate budding from the UGS is essentially complete. U: urethra; DP: dorsal prostate; LP: lateral prostate; VP: ventral prostate; CG: conglobating [dorsocranial] gland; SV: seminal vesicle; VD: vas deferens. From Reference 141.
absent. The glandular ducts that form the zones of the prostate prone to BPH in men, the transition and central zones, are thus absent in dogs. Homology also involves functional similarities, and a focus of research is on regulatory factors that mediate development in humans and animal models. However, mesenchyme from the mouse prostate has been shown to produce the appropriate regulatory factors that induced differentiation of human bladder epithelium into epithelium characteristic of the human prostate, providing evidence for the similarity of regulatory factors required to identify homologous structures. As lobe-specific or zone-specific molecular determinants of development emerge, further insight into homology between species will be realized.

12.4 REGULATION OF PROSTATE DEVELOPMENT

12.4.1 TESTOSTERONE AND 5α-DIHYDROTESTOSTERONE (DHT)

Between the seventh and eighth week of gestation in humans, and around gestation day 12 in mice, Leydig cells in the developing testes begin production of androgens, with testosterone being the major androgen secreted throughout sexual differentiation. Testosterone secreted by each testis mediates differentiation of the ipsilateral Wolffian (mesonephric) duct system. Testosterone in the circulation mediates development of the UGS and external genitalia (Figure 12.2). Secretion of Müllerian-inhibiting hormone (MIH) by the Sertoli cells, which line the seminiferous tubules, suppresses the development of the Müllerian (paramesonephric) duct ipsilateral to each testis. Estrogen antagonizes the action of MIH, while testosterone facilitates the action of MIH.

Testosterone produced by the testes is delivered to target tissues through the blood. Within some androgen-target organs, testosterone is converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase. Testosterone and DHT are both ligands for the androgen receptor. DHT has a higher affinity than testosterone for the androgen receptor, thus enabling it to induce the same response as testosterone at a lower concentration. Expression of 5α-reductase in the Wolffian ducts occurs after sexual differentiation in most species, thus providing one basis for high levels of testosterone achieved by diffusion from the ipsilateral testis being required for development of each Wolffian duct into the epididymis, vas deferens, and seminal vesicle. Production of DHT by 5α-reductase in mesenchymal tissue is required for normal masculinization of the cranial UGS into the prostate as well as development of the penis and scrotum. This is revealed by studies in which testosterone levels in the fetal blood are in a normal range and androgen receptor numbers in fetal tissues are normal, but 5α-reductase activity is inhibited by administration of drugs such as finasteride. Genetic defects may also produce a deficiency in the capacity to produce DHT, and in this condition normal masculinization of the prostate and external genitalia does not occur. There is some evidence that testosterone and DHT have distinct roles in regulation of gene expression in the adult prostate.
12.4.2 Growth Factor Signaling Between Mesenchyme and Epithelium

Development of the prostate begins with outgrowths (glandular buds) of the urothelium lining the lumen of the UGS. The first detectable molecular event in prostate development is the expression of the homeobox gene Nkx3.1 in UGS epithelium, which precedes formation of epithelial buds. Nkx3.1 expression is dependent on activity of the sonic hedgehog (Shh) gene. The homeobox transcription factors HoxA10, HoxA13, and HoxD13 are also required for prostate development.  

Tissue recombination studies have shown that the developing prostatic epithelium is dependent on androgen-induced paracrine secretions from the mesenchyme.  

In turn, the epithelium influences the architecture of the mesenchyme. DHT binds to androgen receptor expressed in UGS mesenchyme, which induces the initial formation and development of epithelial buds from the urethra. The epithelial buds show little capacity to bind androgen, and functional androgen receptor in the epithelium is not required for initial prostate development. Since the interactions of the mesenchyme and epithelium are hormone dependent and are central to the development of the prostate, we discuss below evidence that they represent a point of vulnerability to perturbation by chemicals in the environment referred to as "endocrine disruptors."

An important and as-yet-unanswered question in prostate development is the identity of the paracrine factor(s), or andromedins, that induce epithelial development. The simplest model of andromedin action is that transcription of a single growth factor expressed only in prostate mesenchyme is directly up-regulated by liganded androgen receptors, and the andromedin acts directly on receptors in epithelial cells to induce proliferation and budding. The expected properties of an andromedin is thus that it is produced by mesenchyme cells in an androgen-dependent manner, that it induces proliferation of epithelial cells, and that it is able to induce prostate epithelial development in epithelium lacking androgen receptors. No single factor with all these properties has yet been found. In fact, a more complex picture, involving an array of permissive and restrictive signals, is emerging. The controls on epithelial duct development in the prostate appear to be closely related to developmental signals in other organs derived from branched epithelial structures, including the pancreas, salivary gland, lung, kidney, and mammary gland. A potential mechanism of feedback of growth factor signaling on androgen receptor activity is phosphorylation of androgen receptors. Specifically, epidermal growth factor (EGF) induces phosphorylation of androgen receptors at ser-650. EGF can enhance the transcriptional activity of androgen receptors in response to androgen.

The family of fibroblast growth factors (FGFs) offers several members with andromedin-like properties. FGF-7, also known as keratinocyte growth factor (KGF), is expressed in mesenchyme and its receptor, FGFR2(IIIb), is expressed in epithelium. FGF-7 stimulates epithelial development. However, FGF-7 mRNA is not induced by testosterone in vivo, and stimulation of epithelial development by FGF-7 can be blocked by an antiandrogen. Another andromedin candidate, FGF-10, is expressed by prostate mesenchyme and is necessary for prostate development, as revealed by the lack of a prostate in FGF-10 knockout mice. However, FGF-10 is not directly regulated by androgen receptors, and addition of FGF-10 is not sufficient to stimulate formation of epithelial buds in the absence of testosterone. These results suggest that an additional, androgen-dependent factor is required to support stimulation of epithelial growth by FGF-10. The available data support the hypothesis that FGF-10 has distinct roles in the initial process of formation of epithelial buds, which requires both FGF-10 and androgen, and in further growth and branching of the epithelial ducts, which can be stimulated by FGF-10 alone. FGF-10 is highly expressed in the ventral mesenchymal pad (VMP) of the developing UGS in both males and females. As epithelial buds grow into the VMP, they undergo extensive growth and branching to form the ventral prostate.

In insulin-like growth factor-1 (IGF-1)-deficient mice develop prostates with reduced size, reduced number of duct tips, and reduced branch points. The observation that initial formation of prostatic buds occurs in IGF-1 knockout animals suggests that IGF-1 stimulates prostate development at a later point in development than FGF-10. A discontinuous smooth muscle layer that differentiates within the UGS mesenchyme separates the outer layer of mesenchyme, including the VMP, from the epithelium early in development. In females, this smooth muscle layer thickens and becomes continuous, potentially blocking the epithelium from receiving proliferative signals from the mesenchyme. Testosterone induces thinning of the smooth muscle layer, and thus may function to maintain communication between the mesenchyme and epithelium; this would allow the action of mesenchymal growth factors such as FGF-7 and FGF-10 on the epithelium.

Epidermal growth factor and transforming growth factor α (TGF-α) both bind the EGF receptor, and are both expressed in developing prostate in humans and rodents. Androgens do not directly regulate TGF-α, but EGF treatment up-regulates TGF-α in mesenchyme cells. Disruption of the EGF gene in mice did not alter formation of prostatic buds. However, disruption of both EGF and TGF-α resulted in significantly fewer buds in the dorsolateral region, and disruption of TGF-α alone resulted in significantly more buds in the dorsolateral region. These results suggest a partially redundant and partially antagonistic relationship between EGF and TGF-α in regulation of formation of prostatic buds.

Epithelial buds extending from different regions of the urethra, organized as lobes in mice and zones in humans, form distinct regional architectures. These changes in organization of the epithelium are produced by different balances of branching, proliferation, and differentiation, which are controlled by different regions of mesenchyme surrounding the UGS. One molecule that may contribute to some of these differences is fucosyltransferase 1, an enzyme that synthesizes the H antigenic determinant carbohydrate structure on certain proteins and lipids. Fucosyltransferase 1 supports epithelial proliferation and is found in a restricted distribution within the developing prostatic epithelium. Proliferation signals in the developing prostate epithelium must be balanced by signals that limit inappropriate proliferation. Members of the transforming growth factor β (TGF-β) family, including TGF-β's, activins, and bone morphogenetic proteins (bmp's) are negative regulators of epithelial growth. Bmp-4 is expressed in prostate mesenchyme immediately adjacent to the epithelial ducts. Bmp-4 expression is attenuated at the tips of epithelial ducts. During growth and branching of
12.5 ESTROGEN MODULATES PROSTATE DEVELOPMENT

12.5.1 Inhibitory Effects of High, Pharmacological Doses of Estrogen

An extensive literature relating to the effects of exposure to synthetic estrogens during differentiation of the prostate and other accessory reproductive organs in rodents consistently has shown inhibitory effects of very high, pharmacological levels of estrogen on prostate function. For example, exposure to a high dose of DES or estradiol caused abnormal development and lesions throughout the reproductive system in males. Administration of high, supra-physiological levels of androgen during sexual differentiation has similar effects. High doses of estrogens act directly on the developing prostate to inhibit epithelial proliferation and branching, and disrupt differentiation of the stroma and epithelium, and androgen receptor expression is permanently suppressed. Squamous metaplasia of prostatic and coagulating gland (dorsocranial prostate) ductal epithelium in male mice and rats has been reported after exposure to exogenous estrogen during early life. Similar effects of high doses of estrogen on rat and mouse prostate in primary culture have been reported.

Administration of the high (200 μg/kg/day) dose of DES to pregnant mice completely inhibited the formation of ducts in the dorsal and lateral prostate in male fetuses. Relative to the negative controls, the high dose of DES caused a very different pattern of budding in the ventral UGS, with numerous abnormal short buds being apparent throughout the entire length of the urethra that we examined.

The literature using high doses of DES was stimulated by the finding that similar high doses of DES resulted in a rare vaginal cancer in the female offspring of women treated with DES during pregnancy. This led to extensive research on the DES daughters, as well as on both male and female rats and mice exposed to DES during sexual differentiation. Unfortunately, studies of DES sons have been much smaller and not of sufficient power to adequately assess the possibility of abnormalities of the prostate. Epidemiological studies of DES daughters and experimental evidence concerning DES-exposed female mice has revealed that there is over 90% concordance for effects. Prostate abnormalities would thus be expected in DES sons given the findings that tumors (albeit at a low frequency) occur in mid-life following developmental exposure to DES in male rats and mice.

FIGURE 12.5 These computer-assisted, serial-section reconstructions show the dorsal portion of the prostate from two mouse fetuses. The top prostate (Panel A) is reconstructed from a male fetus exposed to 0.32 pg/ml free serum estradiol. The prostate from an untreated male with 0.21 pg/ml free serum estradiol is shown below in Panel B. Glandular buds that form into the dorsocranial (DC) and dorsal (D) glands in the adult prostate can be seen as outgrowths of the fetal urogenital sinus (ventral buds are not visible). The utriculus (Ut) is the remnant of the regressing embryonic female reproductive tract (Müllerian ducts). Compared to controls, estradiol significantly increased the number and size of prostatic glandular buds and caused a reduction in the size of the lumen of the urethra, which passes through the prostate. From Reference 8.
**12.5.2 Stimulatory Effects of Low, Physiological Doses of Estrogen**

In contrast to the early studies of responses to high doses of estrogens that led to the initial view that estrogen inhibited prostate development, we found that male mouse and rat fetuses exposed in utero to the highest levels of endogenous estradiol (i.e., within a physiological range) showed an increase in prostate size, associated with an increase in prostatic androgen receptors.\(^{53,56}\) We subsequently administered increasing doses of both estradiol (via Silastic capsule) and DES (via feeding) to pregnant female mice and examined the prostate in male offspring in adulthood. Following fetal exposure to both estradiol and DES, we found an inverted-U dose-response relationship for adult prostate weight.\(^*\) Specifically, as serum estradiol concentrations were increased in male mouse fetuses via maternal Silastic implants from 50 to 800% relative to controls, first an increase and then a decrease in adult prostate weight was observed in male offspring.

The lowest dose of estradiol that we administered to pregnant mice via Silastic capsule resulted in a 50% increase in free serum estradiol in male mouse fetuses from 0.2 pg/ml (in controls) to 0.3 pg/ml (measured on gestation day 18). This 0.1 pg/ml increase in free serum estradiol was associated with an increase in total serum estradiol of 52 pg/ml (from 94 pg/ml in controls to 146 pg/ml); the percentage free estradiol in fetal mouse serum is 0.2%. The 0.1 pg/ml increase in free serum estradiol increased the number of developing prostate glands (by 40%) based on three-dimensional reconstruction of the prostate collected from male fetuses on gestation day 18, 1 day after initiation of fetal prostate development (Figure 12.5). The developing prostatic glandular ducts in the dorsal region of the UGS were also enlarged in estrogen-treated males relative to control males. This effect on the prostate was permanent. In adulthood, males exposed to the 50% increase in estradiol during fetal life had enlarged prostates (by 40%) that showed a sixfold increase in prostatic androgen receptors relative to prenatally untreated males.\(^8\)

There is also a significant enlargement of the urethra in male mouse fetuses caused by the 0.1 pg/ml increase in free serum estradiol.\(^8\) It is well known that elevated levels of estrogen inhibit regression of the Müllerian ducts. For example, treatment of pregnant females with DES interferes with the action of Müllerian-inhibiting hormone on Müllerian duct regression in mice\(^{30}\) and humans.\(^{71}\) The urethra is the Müllerian duct remnant that persists within the central zone of the human prostate,\(^{77}\) and the size of this area of the prostate in men may thus correlate with fetal estradiol exposure; this portion of the Müllerian duct differentiates into the dorsocranial portion of the vagina in females.\(^{38}\)

Similar to low doses of estradiol, feeding pregnant mice DES at doses of 0.02, 0.2 and 2 μg/kg/day body weight/day permanently increased prostate weight in male offspring (Figure 12.6). A DES dose of 20 μg/kg/day led to prostate weight that did not differ significantly from control males, while 200 μg/kg/day significantly decreased adult prostate weight.\(^8\) Taken together, the above findings provide evidence that with regard to prostate development, effects seen in response to high, pharmacological/toxicological doses of natural or manmade estrogens are opposite to effects seen with low doses within the normal physiological range of estrogenic activity.\(^5\)\(^7\) \(^8\)

**FIGURE 12.6** Mean (±SEM) prostate weight (mg) in 8-month-old CF-1 male mice produced by females fed different doses of DES from day 11 - 17 of pregnancy. Group means that differed significantly from controls are indicated by an asterisk. From Reference 8.

Using computer assisted 3-D reconstruction, we have also found that feeding pregnant mice a low, 0.1 μg/kg/day dose of DES stimulated additional prostatic duct formation as well as an increase in the size of the ducts when male mouse fetuses were examined on gestation day 19 just prior to parturition. In contrast, a high 200 μg/kg/day dose of DES completely inhibited the development of ducts in the dorsolateral prostate.\(^{79}\) Gupta reported virtually identical findings based on feeding pregnant mice the same low and high doses of DES. Gupta also conducted an in vitro experiment with fetal UGS in primary culture. She held the level of testosterone constant, and addition of 0.1 μg/ml DES stimulated an increase in prostate ducts, confirming the very high sensitivity of the developing mouse prostate to estrogen.\(^5\)

**12.5.3 Mechanisms of Effects of Low Doses of Estrogen**

There is considerable evidence for estrogen responsiveness (the presence of estrogen receptors) of the prostate in rodents and other mammals.\(^{40,43}\) The discovery of two types of estrogen receptor (ERα and ERβ), and their differential localization in prostatic epithelium and stroma, has led to speculation about different biological effects. This is particularly relevant when comparing effects of natural and environmental estrogens. For example, bisphenol A leads to different responses with ERβ present or ERα present.\(^{83}\) In addition, the ligand-activation properties of ERα and ERβ differ in response to estrogenic chemicals present in plants such as soy, flax, clover, etc., such that ERβ is fully induced by some phytoestrogens that are only partial agonists for ERα.\(^{86}\) The high-dose effects of estrogens on the mouse prostate, including histological changes and down-regulation of AR, appears to be...
mediated by ERα, which is expressed in mesenchyme, and not ERβ, which is expressed in epithelium in the mouse. Our findings also show that during prostate development in fetal rats (on gestation day 20), UGS mesenchyme strongly expresses mRNA for ERα, while ERs mRNA in UGS epithelium is at background levels.

In the developing human, ERα protein is not detectable in mesenchyme or epithelium during prenatal development. ERβ protein is not present in the human UGS during the initial formation of epithelial buds. However, ERβ protein is strongly expressed in epithelial basal cells beginning in mid-gestation, when prostate proliferation is most active, leading these authors to suggest that ERβ might mediate the effects of estrogen on epithelial cell proliferation in the prostate of human fetuses. Since ERβ is the only estrogen receptor present in the human UGS during development, and since ERβ is more strongly activated by phytoestrogens and xenoestrogens than ERα, studies of xenoestrogen effects in the mouse, which expresses ERα in the UGS mesenchyme during development, may actually underestimate effects on the prostate in human fetuses.

In addition to the studies conducted by Gupta reviewed above, there are a number of other studies that have shown a stimulating effect of estrogen on the prostate. For example, estradiol (10 pM) stimulated androgen receptor-mediated transcriptional activity induced by dihydrotestosterone (DHT). This was demonstrated in vitro using UGS cells co-transfected with estrogen receptor and androgen receptor expression vectors. In addition, estrogen and androgen have been shown to have a synergistic interaction in stimulating stromal cells obtained from hyperplastic human prostates, and estradiol can stimulate androgen receptor transcriptional activity in the presence of the co-activator ARAP. We have investigated the effects of estradiol on fetal mouse UGS mesenchyme cells in primary culture. Our results partially recapitulate the effects of estradiol observed in vivo. We have shown that at low, physiological doses, estradiol acts directly on cells of the UGS mesenchyme to up-regulate expression of androgen receptor mRNA. This up-regulation of AR mRNA was observed over a wide dose range, from 1 pM to 10 μM estradiol. A dose of 100 nM estradiol induced the maximum AR expression. Although the dose-response curve for AR mRNA induced by estradiol was inverted-U shaped, as expected from in vivo results, the dose range resulting in up-regulation of AR mRNA was much greater than expected. AR mRNA was up-regulated at both physiological and pharmacological doses. In addition, down-regulation of AR mRNA relative to the control was not observed, in contrast to in vivo studies, which consistently show down-regulation of AR at pharmacological doses.

Thus, the down-regulation of AR mRNA at high doses of estradiol must come about through a separate mechanism of action, distinct from the low-dose up-regulation of AR mRNA in response to estradiol. Indeed, a recent study has shown that neonatal exposure to high doses of estrogens permanently increases the rate of proteosomal degradation of androgen receptor protein in the prostate. Based on these results, enhanced transcription of AR mRNA may contribute to the increase in androgen receptor activity observed at low doses of estrogen, and degradation of AR protein by the proteosome may contribute to the decrease in androgen receptor activity observed at high doses of estrogen. The report that estrogen only stimulates TGF-α at supra-physiological doses in MCF-7 breast cancer cells, and that a markedly different array of other genes is turned off and on as the doses of estrogen increase from the physiological range to the supra-physiological range, makes it likely that multiple mechanisms will be found to mediate inverted-U dose-response curves for estrogen in different tissues in vivo.

The changes in AR mRNA levels that we observed in cells exposed to physiological levels of estradiol were relatively modest, between 20% and 50%. However, androgen binding measurements both in vivo and in vitro have revealed consistently greater changes, from twofold to sevenfold, in response to physiological doses of estrogens. Gene array analyses of gene expression patterns often use a cut-off of twofold or threefold changes when determining which genes are regulated by a treatment. However, the magnitude of change in mRNA levels is not always indicative of the magnitude of change in the physiology of the tissue or organ under study. A further complication is that different genes have different sensitivities, different-shaped dose-response curves, and different time courses. Therefore, investigations of gene expression patterns at high doses of estrogens may not be relevant to the physiological responses observed at low doses of estrogens. The inverted-U-shaped dose-response curve observed for prostate size in mice exposed to estrogens is likely to reflect interplay between systemic and local responses. At the tissue level, each of hundreds of changes in expression of genes could contribute to dose-related differences in phenotype.

Estradiol can directly bind and activate androgen receptor (AR) in the presence of the co-activator ARAP. In the LNCaP prostate cancer cell line, either estrogen or androgen can activate formation of a complex of AR, estrogen receptor (ER), and Src, and thus induce cell proliferation through the Src-Ras-Erk pathway. AR activates PAK6 kinase activity, and PAK6 inhibits transcriptional activation by AR and ER. Estradiol receptor alpha (ERα) can directly bind AR and alter transcriptional activation by AR. Finally, estrogen alters AR expression levels in a tissue-specific manner. Analysis of gene expression patterns in adult human prostate stroma cells in response to a high dose of estradiol revealed hundreds of estrogen-regulated genes. Estradiol treatment thus has pleiotropic effects, both in vivo and in vitro. Many “housekeeping” genes are up-regulated by estradiol, including the ribosomal protein RBP and the cytoskeletal protein vimentin.

There is evidence that EGF and IGF-1 may be required to mediate the effects of estrogens on prostate epithelial proliferation. EGF can mimic effects of estrogens by activating the estrogen receptor in female mice. EGF is required for DES-induced growth and branching of mouse prostate organ cultures, while IGF-1 is required only for DES-induced branching.

In dogs, estradiol synergizes with dihydrotestosterone to increase androgen binding in prostatic cells and thus increases prostate growth. Studies have also shown that estradiol influences hypothalamic androgen receptors in adult male rats. In addition, estradiol regulates the expression of receptors for a number of hormones, such as uterine oxytocin receptors and both uterine and brain progesterone receptors. Taken together, these findings show that the physiological effects of exposure to estrogen can include changes in the functioning of a variety of tissues due to changes in the receptors for other hormones that regulate these tissues.
Importantly, when exposure to estrogen occurs during critical periods in development, effects on tissue function are permanent. Interestingly, elevation of testosterone levels during development appears to have similar effects compared to elevation of estrogen levels. Aromatase knockout mice are unable to produce estrogen, and males exhibit increased testosterone and DHT levels in serum and tissues. These males also have enlarged prostates. Thus, both an increase in serum androgen levels caused by deficient aromatase activity, and an increase in prostatic androgen receptor levels induced by elevated estrogen exposure, can lead to stimulation of prostate growth.

12.6 ENVIRONMENTAL ENDOCRINE-DISRUPTING ESTROGENIC CHEMICALS ALTER PROSTATE DEVELOPMENT

Studies now identify that many chemicals have the capacity to disrupt the functioning of the endocrine system, either by binding to endogenous hormone receptors, by interfering with enzyme activity, or via other mechanisms, such as interfering with plasma transport of hormones. Thus, there are chemicals being used in common household products that, prior to being used to manufacture these products, were not tested for the possibility that they might be able to bind to receptors for natural steroids, such as estrogen and androgen. Because development of all organs is coordinated by endocrine signals, the disruption of endocrine signals during critical periods in organ development can lead to permanent effects on organ function. Functional effects might not be noticed based only on examination for gross malformations, which, along with cancer, has been the focus of toxicological testing.

Chemicals used as pesticides, such as methoxychlor, stimulate enlargement of the prostate as a result of exposure to very low, environmentally relevant doses during development. Interestingly, the organochlorine hexachlorobenzene (HCB) enhances androgen signaling in the prostate at low doses and represses androgen signaling at high doses. We recently examined the effects of fetal exposure to bisphenol A, an estrogen-mimicking chemical. Bisphenol A is used to make polycarbonate plastic (for example, baby-feeding bottles are made from polycarbonate). Bisphenol A is also a component of the resin lining of food and beverage cans, in dental sealants, and many other plastic products. Approximately 2 billion pounds of bisphenol A are used per year, and another 100 million pounds of brominated bisphenol A are used as flame retardants in a wide variety of products.

We used a screening assay involving human breast cancer cells (MCF-7) to assess the estrogenic potency of bisphenol A. This assay revealed that the plasma binding proteins that result in a very low free, bioavailable fraction of estradiol in fetal blood show only limited binding to bisphenol A. The proportion of the unconjugated bisphenol A in blood that is bioactive is thus high relative to estradiol. Our findings suggested that developing mouse fetuses would respond to doses of bisphenol A within the range that humans are exposed to this chemical, such as through the use of polycarbonate to store food, eating canned products, and having dental sealant applied to protect teeth.

Based on predictions from our in vitro assay, we fed pregnant mice 2 or 20 μg/kg/day bisphenol A per gram body weight per day for 7 days from gestation day 11 to 17, prior to and during the initial period of prostate development. We observed numerous effects in male offspring, including permanent enlargement of the prostate and preputial glands, a decrease in testicular sperm production, and a decrease in seminal vesicle and epididymal size. In female offspring, we observed abnormal body growth and an early onset of puberty. Many other effects of very low doses of bisphenol A have been reported in over 60 peer-reviewed publications in mollusks, insects, fish, frogs, rats, and mice.

The Wolffian ducts and UGS express estrogen receptors during prenatal development in the mouse. Therefore, these organs can potentially be directly affected by compounds that bind to estrogen receptors, such as bisphenol A. The decrease in the size of the epididymis and seminal vesicles suggests that bisphenol A interfered with the normal development of the Wolffian ducts as well as the testes. In contrast, bisphenol A significantly increased the size of the preputial glands and prostate relative to untreated males. The finding that an elevation in an estrogenic chemical during fetal life decreased seminal vesicle size in adulthood is consistent with our prior findings. Specifically, male mice that developed in utero between two female fetuses (2F males), and were thus exposed to elevated estradiol via diffusion from the adjacent females, had smaller seminal vesicles in adulthood than their siblings who developed in utero between two male fetuses (2M males); in contrast, 2F males had larger prostates.

Subsequent studies have suggested that this effect was mediated by a permanent “imprinted” decrease in seminal vesicle 5α-reductase activity in 2F males relative to 2M males (unpublished observation). However, the larger seminal vesicles found in 2M male mice were initially thought to be due solely to the supplement in testosterone that 2M males received due to being positioned in utero between male fetuses. The finding that a low dose of an estrogenic chemical during fetal life can permanently decrease seminal vesicle and epididymis size provides additional evidence that suggests the elevated estradiol in 2F males may have contributed to the development of small seminal vesicles in these males. It had previously been reported that estrogen exerts an inhibitory effect on 5α-reductase activity in accessory reproductive organs.

In contrast to findings regarding organs that differentiate from Wolffian ducts, adult 2F male mice, as well as male mice exposed experimentally as fetuses to a 50% increase in serum estradiol, exhibited enlargement of the prostate that was associated with a permanent increase in prostatic androgen receptors. As mentioned above, the prostate develops from the UGS, while seminal vesicles develop from a different embryonic tissue, the Wolffian ducts, under different hormonal control. Taken together, these findings provide evidence that during fetal life, the specific genes influenced by estrogen are different in the Wolffian ducts and UGS. Thus, what appeared initially as contradictory findings, with some organs increasing in size and others decreasing in size, associated with a small increase in serum
estradiol during fetal life, now has proven to be a consistent outcome following administration of estrogenic chemicals during fetal life.

An interesting finding is that bisphenol A stimulated proliferation of human prostate cancer (LNCaP) cells. There was an inverted-U dose-response curve, with maximum stimulation at 1 nM (~230 parts per trillion, or ppt) and lower stimulation at doses tenfold lower (23 ppt) or tenfold higher (2.3 ppt), and no stimulation at either 2.3 ppt (NOAEL) or 23 ppb. Of considerable importance is that the 23 ppb dose of bisphenol A would have been erroneously thought to be the no adverse effect level (NOAEL), if this had been the lowest dose tested in a study that had only examined higher but not lower doses.117

There is a mutant form of the androgen receptor in LNCaP cells that appears to show a higher binding to bisphenol A than the wild-type androgen receptor. This raises the question as to the potential for bisphenol A to exhibit significant binding to other members of the nuclear receptor superfamily, such as androgen receptors. In fact, there is a report that bisphenol A can bind to androgen receptors. Specifically, bisphenol A had an efficacy similar to the antiandrogenic drug Flutamide in inhibiting binding of DHT to androgen receptors in a yeast reporter assay. But, at the concentrations detected in human blood, there should not be significant binding of bisphenol A to wild-type androgen receptors. In sharp contrast, the concentration of bisphenol A that stimulated LNCaP prostate cells with the mutant form of the androgen receptor was directly within the range of bisphenol A found in human blood.

Gupta2 reported that in CD-1 mice, oral administration of bisphenol A to pregnant mice at a dose of 50 µg/kg/day from gestation day 14 to 18 resulted in a permanent increase in prostate size and prostate androgen receptors. Bisphenol A also caused a decrease in the size of the epididymis. In this study by Gupta, male mice were examined at 3, 21, and 60 days of age. The finding that fetal exposure increased prostate androgen receptors is virtually identical to the significant increase in prostate androgen receptors produced by a small increase in fetal estradiol8 or a maternal dose of 0.1 µg/kg/day diethylstilbestrol (DES), and also exactly replicated our findings112 of an increase in prostate size and a decrease in epididymis size in male mice using 2 and 20 µg/kg/day bisphenol administered to pregnant cf-1 mice.

A novel finding in the study by Gupta is that the 50 µg/kg/day dose of bisphenol A resulted in an increase in the length of the space between the anus and genital papilla (that becomes the scrotum) on postnatal days 3 and 21, similar to the increase in the size of the prostate. It is well known that the UGS (from which the prostate differentiates) and the external genitants are similar in the hormonal and enzyme activity (specifically 5α-reductase) requirements for normal differentiation during fetal life. In contrast, as described above, the development of the seminal vesicles and epididymis from the Wolffian ducts showed marked differences from the UGS in the hormonal requirements and intracellular enzymes that mediate the early period of differentiation. It is thus consistent with other findings89 that exposure during fetal life to low doses of bisphenol A increases the size of the prostate and the anogenital distance measure, yet decreases the size of the seminal vesicles and epididymis. It is important that in these same studies Gupta found that a high dose of DES (200 µg/kg/day) administered to pregnant mice had opposite effects than a low dose (0.1 µg/kg/day); the high dose both inhibited prostate development and decreased the anogenital distance measure, consistent with many prior findings in rats and mice.6,8

To determine whether effects of bisphenol A were directly on the prostate, Gupta5,102 placed the fetal mouse prostate in primary culture. A 50 µg/ml (50 ppt) dose of bisphenol A stimulated prostate growth and gland formation, as well as androgen receptors, while a dose of 5 ppt bisphenol A did not produce a significant stimulatory effect.12 The effect of 50 ppt bisphenol A was similar to the effect of 0.5 ppt DES examined in the same experiment, demonstrating that bisphenol A is about 100-fold less potent relative to DES. We will review other studies below also showing effects of bisphenol A at doses of 50 ppt in invertebrates.

Based on initial findings from experiments in which we observed permanent enlargement of the prostate in male offspring as a result of administering pregnant mice doses of bisphenol A,7 DES,8 and ethynyl estradiol,119 as well as the findings reported by Gupta,2 we directly compared the effects of these three estrogenic chemicals on the fetal prostate in mice using 3-D reconstruction. Figure 12.6 shows that relative to controls, DES and ethynyl estradiol at a dose of 0.1 µg/kg/day and bisphenol A at a dose of 10 µg/kg/day stimulated the formation of additional prostate ducts and epithelial hyperplasia. Epithelial hyperplasia was revealed by more than a 50% increase in staining for proliferating cell nuclear antigen (PCNA) by each of the three estrogenic chemicals based on staining of sections containing prostate buds from the 3-D reconstruction. The pattern of PCNA staining overlapped with staining for mouse keratin 5 (MK 5), a basal cell marker.

Based on the above findings, in Figure 12.7 we propose a model of potential stages and tissues in early ductal development in the UGS that are influenced by exposure to estrogenic chemicals.79 Our findings show that proliferation of UGS epithelial cells in the dorsolateral prostate by estrogenic chemicals promotes ductal growth from the base to the distal tip through increased stimulation of cell proliferation at the proximal end of the duct. Basal cells are a subset of epithelial cells found in the undifferentiated UGS and then in the developing prostate ducts,120 and our findings suggest that basal cells provide the proliferative pool during the initial formation of ducts. The cords of ductal cells appear to be pushed out from the UGS into the surrounding mesenchyme as a result of proliferation of the basal cells. Estrogenic chemical stimulated the formation of additional ducts and also increased the rate of epithelial proliferation in the dorsal and lateral region of the UGS, while little effect of estrogen was observed in the ventral UGS. Estrogen may act to stimulate a larger proportion of basal cells into the proliferating pool in the dorsal and lateral UGS, revealing a regional effect of estrogen within the developing UGS, which is known to express estrogen receptors at this time in development.6,114 An interesting aspect of these findings is that once branching of the ducts begins, proliferation occurs at the ductal tip.21

An interesting additional observation is that these estrogenic chemicals also resulted in a significant decrease in the size of the urethra at the bladder neck, as well as a gross malformation in the region of the colliculus (Figure 12.4). These findings show that bisphenol A is approximately 100-fold less potent relative to DES, which is consistent with findings by Gupta based on both in vivo and in vitro
An important aspect of these findings is that the blood levels of bisphenol A in fetal mice throughout the 24 hours after maternal administration of bisphenol A are significantly lower than mean blood levels of unconjugated bisphenol A in human fetuses.\textsuperscript{118,121}

Finally, in primary cultures of UGS mesenchyme cells, bisphenol A also significantly increased AR mRNA levels at the lowest dose (1 nM) so far examined.\textsuperscript{122} These findings confirm two different studies by Gupta et al.\textsuperscript{106} that bisphenol A increases androgen receptor protein in the fetal prostate in primary organ culture.

Takanashi et al. are consistent in showing virtually identical effects of both bisphenol A and DES on the prostate in vitro and in vivo in outbred mice (cf. -1 and CD-1). In contrast, in inbred mice (C57BL/6J), bisphenol A was reported to not alter testis, epididymis, or seminal vesicle weight at doses of 2, 20, or 200 μg/kg/day administered at different life stages.\textsuperscript{123} This finding is interesting in that we have found that C57BL/6J males are 1000-fold less responsive to the stimulatory effects of fetal DES exposure on prostate size relative to either -1 or CD-1 male fetuses, while the effects of DES on the uterus of the female siblings of these males showed an identical response to DES (unpublished observation). Our findings, and that of Nagao et al., thus are in contrast to the findings of Spearow,\textsuperscript{114} who reported that peripubertal administration of estradiol to C57 mice had a greater suppressing effect on testis relative to CD-1 mice. Studies to compare the response to different estrogenic chemicals at different life stages in different rat and mouse strains are needed to clarify these diverse findings.

In a study by Ramos et al.\textsuperscript{125} on gestation day 8 pregnant Wistar rats were implanted with Alza osmotic pumps that released bisphenol A at doses of 25 and 250 μg/kg/day. Prenatal exposure to both doses of bisphenol A increased the size of the area occupied by fibroblasts but decreased the size of the area occupied by smooth muscle in the periductal stroma of the ventral prostate of males examined when 30 days old. These changes in the cytoarchitecture of the ventral prostate were associated with a decrease in the proportion of periductal stroma cells that were positive for androgen receptors in males exposed to both doses of bisphenol A. These findings are thus different from those observed in the mouse prostate as a result of exposure during fetal life to low doses of bisphenol A. However, our findings have suggested that the ventral region of the rat and mouse prostate has a different sensitivity to estrogenic effects relative to the dorsolateral prostate.\textsuperscript{76,79}

12.7 ESTROGEN AND ADULT PROSTATE PATHOLOGY

Exposure to supplemental estrogen (in combination with androgen) in adulthood has been related to hyperplasia of the prostate in dogs\textsuperscript{126} and dysplasia and neoplasia in Noble rats.\textsuperscript{127} In mice, elevation of either androgens or estrogens alone fails to produce dysplasia, but treatment with androgens and estrogens in combination resulted in prostatic dysplasia.\textsuperscript{128} In Noble rats, neoplastic tumors can be induced to form in the dorsolateral prostatic lobes, while Sprague-Dawley rats typically do not develop tumors.\textsuperscript{127,129-131} Although fewer than 1% of Noble rats spontaneously develop adenocarcinoma of the prostate, treatment with a combination of low doses
of testosterone and estradiol-17b (via Silastic capsules) for 4 months leads to multifocal epithelial dysplasia,13,14 and longer treatment (about 10 months) results in the transition from dysplasia to neoplastic tumors in about 20% of treated males. Histological examination of prostate tumors in Noble rats treated with androgen and estrogen showed that they primarily involved glandular epithelium, and metastases after transplantation into hosts revealed differentiated epithelial components.15 Neoplastic development occurs in specific regions of the peripheral zone of the human prostate gland.16 Dysplasia in the dorsolateral lobe of testosterone and estradiol treated Noble rats is almost identical to the premalignant lesans described in the human gland.

12.8 SUMMARY

There is little or no information concerning the issue of whether prostate enlargement in men might be related to exposure during fetal life to estrogenic chemicals. However, there has been a doubling of the incidence of abnormal development of the penile urethra (hydrospadias) in male babies over the past 20 years in the U.S., suggesting that an environmental factor is involved.17 There is historical evidence that male sperm counts have declined by 50% over the past 50 years, while the incidence of testicular and prostate cancer has increased; there are regional differences in sperm counts as well as prostate and testicular cancer rates. These findings suggest that environmental factors are mediating these effects, which is supported by recent evidence correlating herbicide levels in men with sperm density.18 Prospective studies in humans (the Children’s Health Initiative) that will include examination of the relationship of exposure to chemicals during fetal life via the mother (as well as many other factors), and consequences to health, are being planned based on findings from animal studies.

At this time there have been no published human studies to raise awareness within the medical community or the Food and Drug Administration (FDA) concerning fetal exposure to bisphenol A from polycarbonate plastic food and beverage containers, tin cans, and dental sealants or in drinking water. The focus of the relatively few studies of exposure of human fetuses to ethynylestradiol during the critical period of reproductive organ development has only been on externally visible malformations at birth. Based upon generally negative findings of grossly observable external malformations at birth, DES was considered safe for administration to millions of women during pregnancy for over 2 decades, but later DES was found to result in serious long-term harm to offspring. This tragic lesson appears to have been forgotten with regard to conclusions being drawn from similar studies of ethynylestradiol. The current assumption is that the amounts of ethynylestradiol or bisphenol A to which human fetuses are exposed are safe. We propose that the data from this and other animal studies regarding the potential for ethynylestradiol and bisphenol A to be considered as risk factors during fetal development at current exposure levels is sufficient, and together with the similarity to effects of low doses of DES, warrant a thorough reevaluation of this assumption.

ACKNOWLEDGMENTS

We thank Dr. Ellen Shapiro for providing the human tissue. Funding was provided by grants to CAR from NIEHS (ES-11549), BGT from EPA R-827403, and FVS from NIEHS (ES11283).

REFERENCES

63. Lung, B. and Cunha, G.R., Development of seminal vesicles and coagulating glands in neonatal mice. I. the morphogenetic effects of various hormonal conditions, The Anatomical Record, 199, 73, 1981.


13 Metal Ions as Endocrine Disruptors: Implications for Prostate Cancer

Shuk-Mei Ho

CONTENTS

13.1 Abstract ............................................................................................................ 411
13.2 Introduction ..................................................................................................... 412
13.3 Zinc .................................................................................................................. 413
   13.3.1 Mechanisms of Zinc Action in the Prostate ................................................. 413
   13.3.2 Prostatic Zinc Content and Prostate Carcinogenesis ............................... 413
   13.3.3 Dietary Zinc and Prostate Cancer Risk .................................................. 414
13.4 Cadmium ......................................................................................................... 415
   13.4.1 Cadmium Is a Significant and Growing Environmental Contaminant ..... 415
   13.4.2 Cd Is a Suspected Carcinogen for the Human Prostate ........................... 415
   13.4.3 Cadmium Is a Proven Carcinogen for the Rat Prostate ............................ 416
   13.4.4 Mechanisms of Cadmium-Induced Carcinogenesis ............................... 417
   13.4.5 Cadmium is an Estrogenic/Androgenic Endocrine Disruptor —
       Ramifications in Prostate Carcinogenesis ................................................... 418
   13.4.6 Interplay between Cadmium and Other Heavy Metals in
       Prostate Carcinogenesis .............................................................................. 418
   13.4.7 Metallothioneins and Cadmium-Induced Carcinogenicity ....................... 419
13.5 Copper, Nickel, and Arsenic ............................................................................. 420
13.6 Summary ......................................................................................................... 421

References ............................................................................................................. 421

13.1 ABSTRACT

Metal ions are significant contaminants of the environment. Yet, their impacts on normal and malignant prostatic functions are poorly understood. Studies implicating metal ions as environmental risk factors for the prostate are limited and have been focused mainly in the area of prostate cancer. Information on heavy-metal–ion influences on the other major prostatic diseases, such as benign prostatic hyperplasia