In Utero and Lactational 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Exposure: Effects on the Prostate and Its Response to Castration in Senescent C57BL/6J Mice

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In utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure inhibits ventral, dorsolateral, and anterior prostate development in C57BL/6 mice. To determine if prostatic abnormalities persist into senescence, mice born to dams given TCDD (5 µg/kg, po) or vehicle on gestation day 13 were examined at 100 and 510 days of age. Half the mice were castrated ten days prior to necropsy in order to assess androgen dependence, while the remaining mice were sham castrated. Effects of TCDD on the dorsolateral and anterior prostate of senescent sham-castrated mice were relatively subtle, whereas the ventral prostate was rudimentary or absent. Castration of vehicle-exposed mice caused far greater reductions in prostate lobe weights, epithelial cell height, and androgen-dependent gene expression (MP25 and probasin) in young mice than in senescent ones, while cell proliferation was decreased by castration in young mice and increased in senescence. Responses to castration were similar at 100 days of age in vehicle- and TCDD-exposed mice. At 510 days, however, TCDD-exposed mice were substantially more responsive to castration by most indices than vehicle-exposed mice. These results demonstrate that prostatic androgen dependence in mice declines substantially with age in several key ways, and that *in utero* and lactational TCDD exposure protects against this decline. Surprisingly, TCDD increased the incidence of cribriform structures in dorsolateral prostate ducts, from 2–3% in vehicle-exposed senescent mice to 16% in sham-castrated and to 7% in castrated senescent mice. Collectively, these results demonstrate that effects of *in utero* and lactational TCDD exposure on the prostate persist into senescence, and suggest that *in utero* and lactational TCDD exposure retards the aging process in the prostate. However, because cribriform structures are often considered to be associated with prostate carcinogenesis, these results also suggest that TCDD exposure early in development may increase susceptibility to prostate cancer.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) can cause abnormal prostate development in rats and mice (Theobald *et al.*, 2003). In C57BL/6 mice a single maternal dose (5 µg/kg on gestation day [GD] 13) reduces ventral, dorsolateral, and anterior prostate weight, alters androgen-dependent gene expression, and inhibits ductal morphogenesis and branching. These effects are lobe-specific and do not appear to be due to changes in circulating androgen concentrations (Ko *et al.*, 2002; Lin *et al.*, 2002a).

Previous research on the effects of TCDD on prostate development in mice has focused on the ontogeny of these abnormalities, in large part because prostatic vulnerability to TCDD starts before birth (Lin *et al.*, 2002b). Morphologically, prostate development begins when urogenital sinus (UGS) epithelium begins to develop buds in response to inductive signals from the surrounding mesenchyme. These buds project into the UGS mesenchyme, elongate, branch, canalize, and ultimately develop into the various prostate lobes (Cunha *et al.*, 1987; Marker *et al.*, 2003; Raynaud *et al.*, 1942; Timms *et al.*, 1994). We discovered that maternal TCDD treatment delays the formation of anterior and dorsolateral prostatic epithelial buds in mice by about a day, reduces the number of dorsolateral buds by about 25%, and prevents ventral buds from forming (Lin *et al.*, 2003). Effects on prostatic budding can account for many of the prostatic effects of TCDD seen in adult mice.

Research to elucidate the mechanisms by which TCDD inhibits UGS and prostate development is in progress. TCDD appears to inhibit prostatic epithelial bud formation by acting directly on the UGS rather than indirectly via effects on other organs (Lin *et al.*, 2004). The initial site of action was found to be the UGS mesenchyme rather than UGS epithelium (Ko *et al.*, 2004a), and TCDD appears to inhibit prostatic epithelial bud formation by mechanisms other than inhibition of androgen signaling (Ko *et al.*, 2004b).

It is well known that perturbations of early development can have lifelong consequences for the prostate (Coffey, 1988; Prins *et al.*, 2001; Rajfer and Coffey, 1979), and that most prostatic diseases occur later in life (Schulman, 2000). In addition, circulating androgen concentrations in humans and experimental animals tend to decline with advancing age whereas prostate weight tends to increase (Banerjee *et al.*, 1998). This is apparently not due to an enhancement in prostatic sensitivity to androgens with increasing age, at least not in rats. Instead, castration experiments reveal that prostate lobes in rats are substantially more androgen independent in senescence than they are in young adulthood (Banerjee *et al.*, 2000).

Despite the existence of age-dependent differences in prostatic structure and function in adults, little information was available about the possible effects of *in utero* and lactational TCDD exposure on the prostate in middle aged or senescent animals of any species. Gray *et al.* (1995) measured ventral prostate weight and nuclear androgen receptors in 11-month-old rats but neither was significantly affected by *in utero* and lactational TCDD exposure. Similarly, no treatment-related effects were seen in rat ventral prostate weight or histology at 15 months of age (Gray *et al.*, 1997). The only report of effects caused by a similar chemical reveals that *in utero* and lactational 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169) exposure increased the incidence of prostatitis in 600-day-old Long-Evans rats (Gray *et al.*, 1999). Effects on mouse prostate had not been examined past 128 days of age (Theobald and Peterson, 1997).

To address this information gap we investigated the effects of *in utero* and lactational TCDD exposure on the prostate in senescent C57BL/6J mice. The experiments included routine measurements such as organ weight, gene expression, and histology but were also designed to determine if TCDD affects prostatic androgen dependence. Prostates from castrated and sham-castrated mice were examined at 100 days of age (young adulthood) and 510 days of age (senescence). We found that mouse prostate lobes (particularly their weights), like rat prostate lobe weights, are far more androgen independent in senescence than they are in young adulthood. We also discovered that *in utero* and lactational TCDD exposure appears to retard prostatic aging while substantially increasing the incidence of cribriform structures present in senescence. The latter observation suggests that exposure to TCDD and similar chemicals early in development may increase suscepti-bility to prostate cancer.

MATERIALS AND METHODS

Animals and treatments. C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were housed in clear plastic cages with heat-treated chipped aspen bedding, in rooms kept at $24 \pm 1°C$ and lighted from 0600 to 1800 h. Feed (5015 Mouse Diet, PMI Nutrition International, Brentwood, MO) and tap water were available *ad libitum*. All procedures were approved by the University of Wisconsin Animal Care and Use Committee, and conducted in

accordance with the NIH Guide for the Care and Use of Laboratory Animals. To obtain time pregnant dams, females between 90 and 120 days old were paired overnight with males. The next day was considered GD 0. Pregnant mice were given a single po dose of TCDD (5 μg/kg) or vehicle (corn oil, 5 ml/kg) by gavage on GD 13. All pups were weaned on postnatal day 21, at which time they were housed by sex, typically four per cage. At 90 and 500 days of age (±4 days), one male pup from each vehicle- and TCDD-exposed litter was castrated via the scrotal route under isoflurane anesthesia, and euthanized by CO_2 overdose ten days later. The ten-day interval was chosen based on the time course for similar responses to castration in rat prostate (Banerjee *et al.*, 2000). Whenever possible, a single litter-matched male was sham castrated and euthanized at the same ages. Otherwise, males from other litters were used so that litter independence was maintained for each treatment, castration status, and age. Each of the eight experimental groups consisted of 7–9 males. For sham castration, testes were exteriorized via a scrotal incision, then returned prior to wound closure. Ventral prostate, dorsolateral prostate, anterior prostate, and seminal vesicles were identified as described by Sugimura *et al.* (1986), removed, and weighed. Half of each prostate (i.e., the right or left lobe) was frozen in liquid nitrogen for mRNA analysis and the other half was fixed in Bouin's for histological and immunohistochemical analysis.

Histology and immunohistochemical analysis of cell proliferation. After overnight fixation in Bouin's, samples were stored in 70% ethanol, dehydrated in a series of graded ethanol, paraffin embedded, and cut into 5 μm sections. Hematoxylin and eosin staining was used for histologic analysis, with obser-vations confirmed by a certified pathologist (Dr. Weixiong Zhong, University of Wisconsin, Department of Pathology and Laboratory Medicine). The percentage of ducts exhibiting cribriform structures was determined for each mouse, so that a mean incidence of these structures could be determined per treatment group. Epithelial cell height was determined using photomicro-graphs taken near the distal tips of longitudinal sections of prostate ducts. Measurements were made using Adobe Photoshop. Distal prostate regions were characterized by the presence of eosinophilic (dorsolateral and anterior) or pale, serous (ventral) secretions. Twelve areas were measured for each of three separate sections to obtain an average distal epithelial cell height for each prostate lobe in each animal. Cell proliferation was determined on adjacent sections using a proliferating cell nuclear antigen (PCNA) kit (Zymed, South San Francisco, CA) according to manufacturer's instructions. Positive and negatively stained nuclei were counted from three fields of view to establish a mean proliferative incidence per lobe per animal (approximately 500 total cells per lobe).

Testosterone concentrations. Steroids were extracted from serum using diethyl ether. Organic phases from repeat extractions were combined, evapo-rated to dryness, and reconstituted in 100% ethanol. Testosterone concentrations were measured by enzyme immunoassay in duplicate according to the protocol supplied with the kit (Assay Designs, Inc., Ann Arbor, MI). The assay range was 7.8–2000 pg testosterone/ml, sensitivity was 3.8 pg/ml, and the coefficient of variation was about 10% in the sample concentration range. All samples were run in a single assay.

mRNA analysis. mRNA expression for cyclophilin, MP25, and probasin was determined by real-time reverse transcription-polymerase chain reaction mRNA quantification using a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN) as previously described (Lin *et al.*, 2002a).

Statistical analysis. Analyses were conducted with the litter as the experimental unit. Data that satisfied normal distribution and passed Levene's test for homogeneity of variance were analyzed by one-way ANOVA, and individual treatment groups were compared to one another using the Tukey test (SigmaStat; Jandel Scientific, San Rafael, CA). For data that did not pass Levene's test even when transformed, non-parametric Kruskal-Wallis analysis was performed followed by the distribution-free multiple comparison test when appropriate. Differences were considered significant at $p < 0.05$. Results are presented as the mean ± SEM.
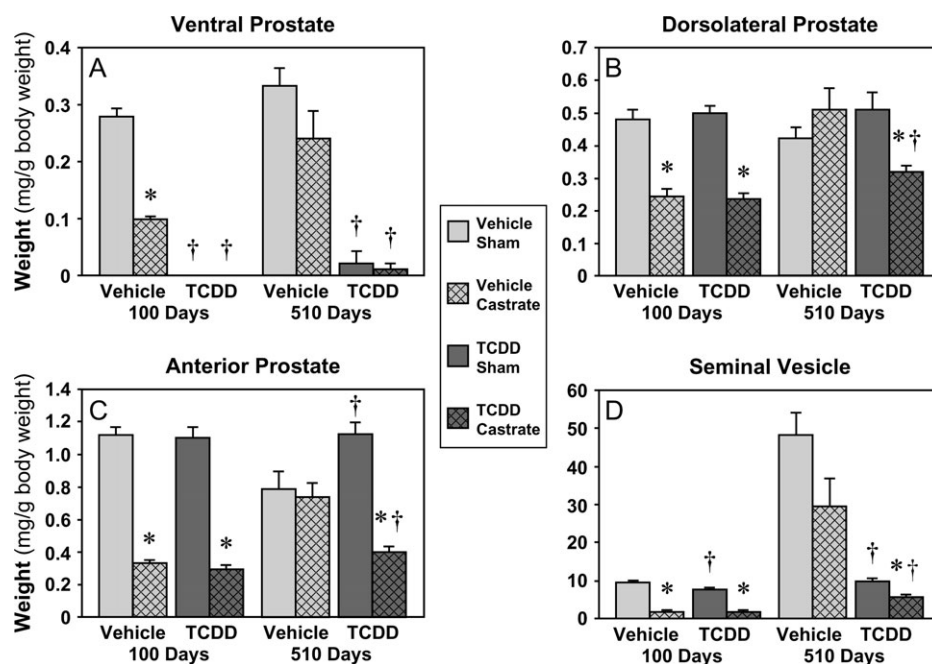
FIG. 1. Effects of *in utero* and lactational TCDD exposure on relative ventral prostate (A), dorsolateral prostate (B), anterior prostate (C), and seminal vesicle (D) weights 10 days after sham castration or castration at 90 or 500 days of age. The mice were born to and nursed by dams given 5 μg TCDD/ kg or corn oil (5 mg/ml) vehicle on GD 13. Bars represent the mean ± SEM for 7–9 males per category. *Significantly different from sham-castrated mice ($p < 0.05$). †Significantly different from vehicle-exposed mice ($p < 0.05$).

RESULTS

Body Weights

Body weights were similar in 100-day-old mice regardless of whether they were exposed to vehicle or TCDD or whether they were sham castrated or castrated (average weight about 26 g, data not shown). Body weights were significantly greater in mice from all groups at 510 days than at 100 days. Vehicle-exposed sham-castrated mice (43.6 ± 1.8 g) were significantly heavier at 510 days than vehicle-exposed castrated (36.1 ± 2.9 g), TCDD-exposed sham-castrated (33.9 ± 1.1 g), and TCDD-exposed castrated mice (35.5 ± 1.1 g).

Prostate Lobe and Seminal Vesicle Weights

Most mice exposed to a single maternal dose of 5 μg TCDD/kg on GD 13 had no detectable ventral prostate. If present, this organ was small and ducts were rudimentary. As a result, TCDD significantly reduced relative ventral prostate weights under all experimental conditions (Fig. 1A). In vehicle-exposed mice ventral prostate weights were reduced 67% by castration in young adulthood but were not significantly reduced in senescence. Effects of castration on the ventral prostate of TCDD-exposed mice, if any, were non-detectable due to the absence of this organ from most animals. Statistical analysis of absolute weights gave the same results as described above for relative weights, except that castration in senescence caused a significant reduction in vehicle-exposed mice.

There were no differences in relative dorsolateral prostate weights between vehicle- and TCDD-exposed sham-castrated mice at either 100 or 510 days of age (Fig. 1B). Castration reduced dorsolateral prostate weights by about 50% when mice were young adults regardless of TCDD exposure, had no significant effect in aged vehicle-exposed mice, and caused a 38% reduction in aged TCDD-exposed mice. TCDD had no significant effect on dorsolateral prostate weights in castrated young adults but significantly reduced these weights in castrated senescent mice. Statistical analysis of absolute weights gave identical results except that the last-named effect was absent.

TCDD had no effect on relative anterior prostate weights in sham-castrated mice at 100 days of age but caused a significant increase in senescence (Fig. 1C). The latter effect was secondary to reduced body weights because absolute anterior prostate weight (not shown) was unchanged. In young adulthood castration reduced relative anterior prostate weights by 70–75% in both vehicle- and TCDD-exposed mice. Castration had no such effect in aged vehicle-exposed mice but caused a 64% reduction in aged TCDD-exposed mice. TCDD had no significant effect on anterior prostate weights in castrated mice when they were young adults but significantly reduced the weight of this organ in senescence.

Relative seminal vesicle weights in vehicle-exposed sham-castrated mice were more than five times greater at 510 days of age than in young adulthood (Fig. 1D). TCDD slightly though significantly reduced relative (though not absolute) weights in
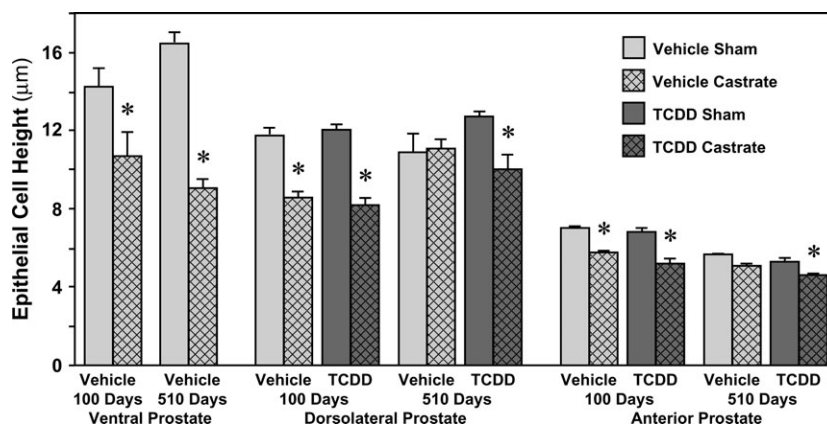
FIG. 2. Effects of *in utero* and lactational TCDD exposure on prostatic epithelial cell height 10 days after sham castration or castration at 90 or 500 days of age. The mice were born to and nursed by dams given 5 µg TCDD/kg or corn oil (5 mg/ml) vehicle on GD 13. Twelve regions per prostate lobe were measured to obtain a mean epithelial cell height for each individual lobe. Bars represent the mean ± SEM for 6–9 mice per category. *Significantly different from sham-castrated mice ($p < 0.05$). †Significantly different from vehicle-exposed mice ($p < 0.05$). Separate statistical analyses were performed for the ventral, dorsolateral, and anterior prostate.

sham-castrated mice in young adulthood and greatly reduced them in senescence. Castration reduced seminal vesicle weights by about 80% in young adults regardless of TCDD exposure. When mice were senescent, castration caused a non-significant weight decrease in vehicle-exposed mice and a significant 43% reduction in TCDD-exposed mice. TCDD had no effect on seminal vesicle weights in castrated mice in young adulthood but greatly reduced these weights in castrated senescent mice.

Prostate Epithelial Cell Morphology

In sham-castrated vehicle-exposed mice, the ventral prostate was composed of predominantly columnar epithelium with minimal infolding into the prostatic lumen. Epithelial cell height in the ventral prostate was comparable in young and old vehicle-exposed mice and was significantly reduced by castration at both ages (Fig. 2). Morphologically, a greater predominance of cuboidal epithelia was seen in castrated mice, with some remaining columnar epithelia and minimal infolding.

The dorsal lobe from all sham-castrated mice, with or without TCDD exposure, was characterized by predominantly columnar luminal cells with a moderate degree of infolding, while lateral lobe epithelium was mostly cuboidal without infolding. Epithelium ranged from cuboidal to columnar in both lobes, and overall epithelial cell height was similar in the dorsal and lateral regions (not shown). Consequently, cell height measurements were combined for analysis. Dorsolateral epithelial cell height was comparable in young, sham-castrated vehicle- and TCDD-exposed mice. In 100-day-old males, dorsolateral prostate cell height was reduced following castration in both vehicle- and TCDD-exposed mice by about 30%. The reductions in cell height were associated with a loss of epithelial infolding and a greater predominance of stroma following castration. In aged males, dorsolateral prostate

epithelial cell height was not significantly affected by TCDD in either sham-castrated or castrated mice. Epithelial cell height was not altered by castration in senescent vehicle-exposed mice but was significantly reduced, by 21%, in TCDD-exposed mice. Conversion from columnar to more cuboidal epithelial morphology with greater stromal composition and loss of infolding observed in young castrates was also observed in the aged dorsolateral prostate of castrated TCDD-exposed mice but not vehicle-exposed castrates.

Anterior prostates contained finger-like projections of columnar epithelia into the luminal space. Anterior prostate epithelial cell height was similar in sham-castrated, 100-day-old mice that were exposed *in utero* and lactationally to vehicle or TCDD. Castration significantly reduced cell height in both vehicle- and TCDD-exposed 100-day-old mice and caused the epithelium to become mostly cuboidal. At 510 days, anterior prostate cell height was the same in both vehicle- and TCDD-exposed sham-castrated mice. Epithelial cell height at 510 days was not reduced by castration in vehicle-exposed mice but was significantly reduced in TCDD-exposed mice. Epithelial cells were predominantly columnar in senescence regardless of TCDD exposure or castration.

Secretory Protein mRNA Expression

In sham-castrated vehicle-exposed mice, mRNA levels for a ventral prostate secretory protein, MP25 (Mills *et al.*, 1987), were 87% lower in 510-day-old males than at 100 days of age (Fig. 3). There was an 89% reduction in MP25 expression at 100 days following castration but no significant reduction ($p = 0.09$) at 510 days.

In the dorsolateral prostate, probasin mRNA expression (Johnson *et al.*, 2000) was similar in both young and aged sham-castrated mice regardless of whether they were exposed
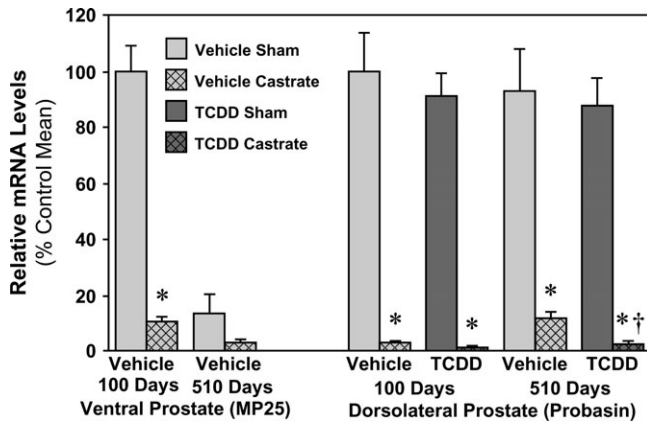
FIG. 3. Effects of *in utero* and lactational TCDD exposure on lobe-specific secretory protein mRNA levels in ventral and dorsolateral prostates 10 days after sham castration or castration at 90 or 500 days of age. The mice were born to and nursed by dams given 5 μg TCDD/kg or corn oil (5 mg/ml) vehicle on GD 13. mRNA expression for MP25 and probasin was determined relative to cyclophilin expression by real-time reverse transcription-polymerase chain reaction mRNA quantification using a LightCycler. Bars represent the mean ± SEM for three samples for 100 day dorsolateral prostates, and four samples per category for 100 day ventral prostates and 510 day dorsolateral and ventral prostates. For each lobe, results are shown as a percentage of the mean value found in 100-day-old vehicle-exposed, sham-castrated mice. *Significantly different from sham-castrated mice ($p < 0.05$). †Significantly different from vehicle-exposed mice ($p < 0.05$). Separate comparisons were made for MP25 and probasin.

to vehicle or TCDD. Probasin mRNA expression was reduced by 97% in dorsolateral prostates from young, vehicle-exposed mice following castration and by 87% in 510 day castrates. It was also reduced by 97–99% by castration in TCDD-exposed mice at 100 and 510 days. The difference in probasin mRNA expression between TCDD- and vehicle-exposed aged, castrated mice was statistically significant.

Prostate Epithelial Cell Proliferation

Proliferative index was characterized by the percentage of epithelial cells that labeled positively for PCNA (Fig. 4). Approximately 5% of the luminal epithelial cells in sham-castrated, vehicle-exposed ventral prostates were PCNA-positive at 100 days of age, while 2.3% were positive at 510 days. Compared to age-matched sham-castrated controls, the PCNA labeling index was significantly reduced in ventral prostates of 100-day-old vehicle-exposed animals following castration but was not significantly altered in castrated animals at 510 days of age.

In the dorsolateral prostate of young sham-castrated mice, PCNA labeling was 4.1% in vehicle-exposed mice and was not significantly altered by TCDD exposure. Following castration at 90 days of age, the PCNA labeling index was reduced in both vehicle and TCDD-exposed animals by more than 70%; the former difference was statistically significant but the latter was not ($p = 0.056$). In senescence, PCNA labeling was not significantly affected by TCDD in sham-castrated mice but was significantly increased several-fold by castration in dorsolateral prostates from both vehicle- and TCDD-exposed mice.

Epithelial cell PCNA labeling was 2–3% in 100 day sham-castrated vehicle- and TCDD-exposed anterior prostates. Staining was reduced by more than 85% in castrated vehicle- and TCDD-exposed mice at 100 days. At 510 days, PCNA labeling in sham castrates was four-times greater in TCDD-exposed
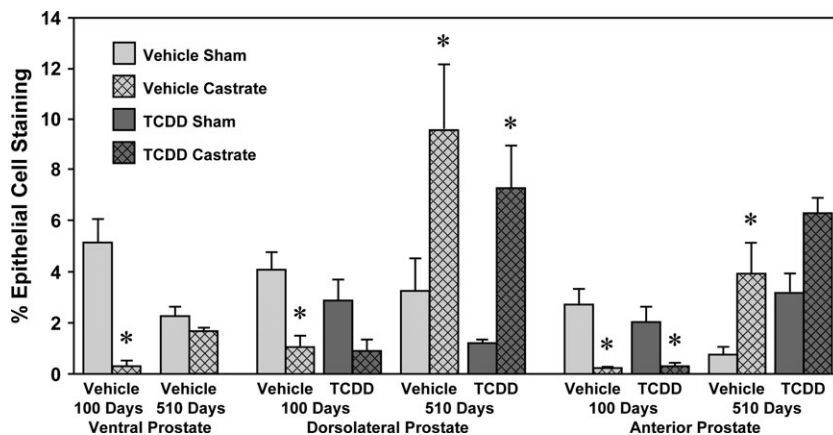
FIG. 4. Effects of *in utero* and lactational TCDD exposure on the percentage of epithelial cells staining for proliferating cell nuclear antigen (PCNA) in ventral, dorsolateral, and anterior prostates 10 days after sham castration or castration at 90 or 500 days of age. The mice were born to and nursed by dams given 5 μg TCDD/kg or corn oil (5 mg/ml) vehicle on GD 13. Epithelial cells staining positively for PCNA were counted on three separate fields of view for each section, with a minimum of 500 total cells counted to obtain a percentage for each individual. Bars represent the mean ± SEM for 6–8 mice per category. *Significantly different from sham-castrated mice ($p < 0.05$). †Significantly different from vehicle-exposed mice ($p < 0.05$). Separate statistical analyses were performed for the ventral, dorsolateral, and anterior prostate.

TABLE 1
Incidence of Cribriform Structures in Prostates of
510-Day-Old Mice

Treatment		Incidence per mouse[a]	Incidence per duct (%)[b]
Vehicle	Sham	4/6 (67)	2.5 ± 1.1
Vehicle	Castrate	4/6 (67)	2.4 ± 1.2
TCDD	Sham	6/6 (100)	16.5 ± 3.8
TCDD	Castrate	5/6 (83)	7.3 ± 2.7

Note. Histomorphological alterations denoting cribriform structures were determined in vehicle and TCDD-exposed mice at 510 days of age, ten days after castration or sham castration. Hematoxylin and eosin staining was used for histologic analysis on paraffin sections, with observations confirmed by a certified pathologist.

[a]Number of mice with cribriform structures divided by the number of mice examined. Percentages are given in parentheses.

[b]Incidence was determined by dividing the number of prostate ducts containing cribriform structures by the total number of ducts observed. At least 20 ducts were examined from each mouse. The percentage for each mouse was used to find the mean incidence for each group.

mice than in vehicle-exposed mice but the difference was not statistically significant ($p = 0.07$). PCNA labeling index was significantly increased by castration in 510-day-old mice exposed to vehicle but the castration-induced increase in TCDD-exposed mice was not statistically significant.

Serum Testosterone Concentrations

Serum testosterone concentrations (not shown) were greatly reduced by castration in all treatment groups. Testosterone concentrations in sham-castrated vehicle-exposed mice were several-fold higher at 100 days of age than in senescence. *In utero* and lactational TCDD exposure had no significant effect on serum testosterone concentrations in either sham-castrated or castrated mice at either time.

Additional Histological Observations

In senescence, most mice had at least one cribriform structure in their dorsolateral prostate (Table 1). Cribriform structures are ducts that contain epithelial stratification with marked thickening and remodeling of the stroma lining the epithelium, resulting in a ''glands within a gland'' appearance (Jones and Young, 1984; see Fig. 5 for examples). The percentage of senescent mice with at least one prostatic cribriform structure was not detectably altered by maternal TCDD treatment or by castration (Table 1), but the number of cribriform structures per dorsolateral prostate was highly treatment-dependent. Prostatic ducts with cribriform structures were infrequent in vehicle-exposed mice regardless of whether they had been sham-castrated or castrated (Table 1). In contrast, the percentage of dorsolateral prostatic ducts with cribriform-like architecture was more than six-fold greater in TCDD-exposed sham-castrated mice and three-fold greater in
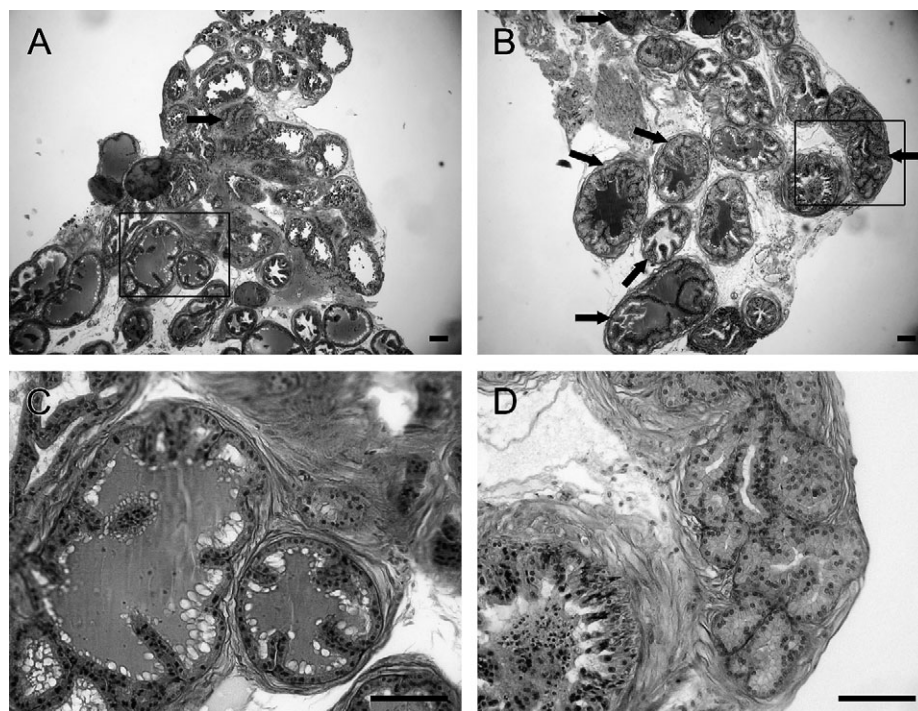
FIG. 5. Effects of *in utero* and lactational TCDD exposure on dorsolateral prostate histology 10 days after sham castration at 500 days of age. The mice were born to and nursed by dams given corn oil (5 mg/ml) vehicle (A and C) or 5 μg TCDD/kg (B and D) on GD 13. Arrows indicate ducts with cribriform structures. Figures C and D are higher magnifications of regions denoted by boxes in Figures A and B, respectively. Bars equal 100 μm.

TCDD-exposed castrated mice than in the corresponding vehicle-exposed mice. Statistically, the effect of TCDD was highly significant, the effect of castration was not, and there no significant interaction between TCDD and castration. Cribriform structures were not observed in ventral or anterior prostates of senescent mice or in any prostate lobe of 100-day-old mice.

There was also increased infiltration and presence of inflammatory cells in the stroma of aging ventral and dorsolateral but not anterior prostates. These effects were not associated with either TCDD treatment or castration (data not shown). No inflammatory infiltration was seen at 100 days of age.

DISCUSSION

Prostate Weights in C57BL/6J Mice Are Far More Androgen Independent in Senescence than in Young Adulthood

Serum testosterone concentrations generally decline with advancing age and prostate weights tend to increase. These observations are consistent with the hypothesis that the prostate becomes increasingly androgen sensitive with advancing age. Yet Banerjee *et al.* (2000) recently demonstrated that ventral, dorsal, lateral, and anterior prostate weights in Brown Norway rats decline only about half as much in response to castration in senescence as they do following castration in young adulthood. Results of the present research reveal that the C57BL/6J mouse prostate, like the Brown Norway rat prostate, is far less androgen dependent in some key ways in senescence than it is in young adulthood. Castration significantly reduced relative weight and cell proliferation (calculated based on PCNA staining) in the ventral prostate in young adulthood but not in senescence. In both the dorsolateral and anterior prostate, absolute weight, relative weight, and epithelial cell height were significantly reduced by castration when mice were young but not in senescence. Our observations of the seminal vesicles were limited to visual inspection and wet weight determination, but here too the reductions in absolute and relative weight caused by castration were statistically significant in young adulthood but not in senescence. To the best of our knowledge these results are the first to demonstrate that androgen dependence of the mouse prostate, most notably prostate lobe weights, declines significantly with age.

In contrast to castration in young adulthood, which caused pronounced reductions in nearly all quantitative measurements, responses to castration in senescence varied substantially from one endpoint to another. Reductions in prostate lobe weights and epithelial cell height were relatively small or absent, reductions in androgen-dependent mRNA expression were much more pronounced, and epithelial cell proliferation was increased in two lobes rather than decreased. Differences among these responses are presumably due to the somewhat different nature of the measurements. Prostate weight depends in part on the balance between production and release of secretory fluid, whereas epithelial cell height is an index of secretory fluid production. Neither declines to zero even if fluid production ceases, and each reflects past as well as present secretory activity. The mRNA measurements, in contrast, are indicators of androgen-dependent gene expression at the time of necropsy and have the greatest potential dynamic range. The gene expression results demonstrate that the decline in prostatic androgen dependence with advancing age does not apply to all aspects of androgen action. The increase in dorsolateral and anterior prostate cell proliferation following castration in senescence appears to be a novel observation whose basis is currently unknown.

Effects of in Utero and Lactational TCDD Exposure on Prostatic Aging

Effects of *in utero* and lactational TCDD exposure on the prostate and seminal vesicles of sham-castrated mice at 100 days of age were generally consistent with previous observations made on intact mice at 90 days of age (Ko *et al.*, 2002; Lin *et al.*, 2002a). The ventral prostate was essentially absent. Dorsolateral prostate weight, epithelial cell height, androgen-dependent mRNA expression, and cell proliferation were not significantly affected by TCDD. Nor were anterior prostate weight, epithelial cell height, or cell proliferation. Seminal vesicle weight was significantly reduced.

Prior to the present study the most advanced age at which effects of *in utero* and lactational TCDD exposure on the prostate had been reported in any strain of mouse was only 128 days of age (Theobald and Peterson, 1997). Our results demonstrate that effects of TCDD on the prostate and seminal vesicles persist into senescence. At 510 days of age the ventral prostate, not surprisingly, was still absent or rudimentary. Absolute weight, relative weight, and androgen-dependent mRNA expression in the dorsolateral prostate were significantly reduced by TCDD in senescent castrated mice, and the incidence of cribriform structures per prostatic duct was greatly increased. Relative anterior prostate weight in sham-castrated mice was significantly increased by TCDD in senescence, while absolute and relative weights in castrated mice were significantly decreased. Absolute and relative seminal vesicle weights were significantly reduced by TCDD in senescent mice regardless of surgical status. Our observation that relative seminal vesicle weights in vehicle-exposed mice were about five times greater in senescent than in young adult mice is consistent with previous reports (Eleftheriou and Lucas, 1974; Finch and Girgis, 1974), but why TCDD prevented this increase is unknown.

The pattern of effects of *in utero* and lactational TCDD exposure on androgen dependence was highly time dependent, although there was no significant effect on serum testosterone concentrations at either time tested. TCDD had little if any effect on androgen dependence in young adult mice, i.e.,

responses to castration were nearly identical in vehicle- and in TCDD-exposed mice. At 510 days of age, however, responses to castration were typically far greater in TCDD-exposed than in vehicle-exposed mice. In the dorsolateral prostate, castration had no significant effect on absolute weight, relative weight, or epithelial cell height in vehicle-exposed senescent mice but significantly reduced relative weight and epithelial cell height in TCDD-exposed mice. In contrast, probasin mRNA expression was reduced and cell proliferation was increased regardless of TCDD exposure. In the anterior prostate, absolute weight, relative weight, and epithelial cell height were not significantly affected by castration in senescent vehicle-exposed mice but each was significantly reduced by castration in senescent TCDD-exposed mice. Cell proliferation was the exception to this pattern: it increased in response to castration in both vehicle- and TCDD-exposed senescent mice and the effect was statistically significant only in vehicle-exposed mice. In the seminal vesicles, reductions in absolute and relative weight seen ten days after castration in senescence were statistically significant only in TCDD-exposed mice.

The results discussed above illustrate three points. First, they demonstrate that effects of *in utero* and lactational TCDD exposure on the prostate and seminal vesicles can persist into senescence. Second, they strongly suggest that *in utero* and lactational TCDD exposure inhibits the normal aging process by which the prostate and seminal vesicles become far more androgen independent by senescence than they were earlier in life, most notably with regard to organ weight. Although there are indications that TCDD may accelerate aging of the female reproductive system (Gray and Ostby, 1995), we are unaware of any previous report that TCDD protects any organ against aging in adulthood. Third, these results demonstrate that effects of *in utero* and lactational TCDD exposure on androgen dependence are not an invariant response to TCDD. Instead, their appearance is developmental stage-dependent. It remains to be determined when in adulthood the TCDD-induced alteration in androgen dependence first develops.

Implications of the Increased Incidence of Cribriform Structures in Dorsolateral Prostates of Senescent TCDD-Exposed Mice

Morphological alterations previously reported in the aging prostate suggest an inverse relationship between circulating androgens and cell proliferation. In senescent rats, prostate epithelial proliferation was greater in the dorsal and to a lesser extent the lateral lobes than it was in young adulthood (Banerjee *et al.*, 2000) despite lower circulating testosterone concentrations in old age (Banerjee *et al.*, 1998). In mice, epithelial cell proliferation in the dorsolateral prostate did not increase with age in sham-castrated mice, but while the response to castration in young adulthood was a reduction in proliferation, the response in senescence was an increase. Consequently, epithelial cell proliferation in the dorsolateral

prostate of castrated mice was many-fold greater in senescence than in young adulthood. These observations reflect a greater proliferative response in a diminished androgen environment in senescence that was not observed in younger animals. More importantly, greater epithelial proliferation in aged rats was accompanied by morphological alterations manifested as lobe-specific hyperplasia (Banerjee *et al.*, 1998). The cribriform structures we observed in the mouse are closely reminiscent of lobe-specific hyperplasia observed in the aging rat. However, the mechanisms involved in formation of cribriform structures, particularly in TCDD-exposed mice, remain to be determined.

Cribriform structures are commonly seen in the early stages of benign prostatic hyperplasia, and have also been reported in aged A $\times$ C rats that develop spontaneous prostate adenocarcinoma (Shain *et al.*, 1975) and in transgenic mice that develop prostate cancer (Kaplan-Lefko *et al.*, 2003). While the presence of cribriform structures does not necessarily mean that overt prostatic disease will develop, some investigators consider these structures in rodents to be precancerous lesions (Kaplan-Lefko *et al.*, 2003; Shain *et al.*, 1975). The fact that *in utero* and lactational TCDD exposure greatly increased the prevalence of cribriform structures in the dorsolateral prostate of mice that are not naturally susceptible to prostate cancer raises the intriguing possibility that exposure to TCDD early in life may increase the incidence and/or severity of prostate cancer in animal strains and species that are susceptible to developing this disease. Experiments to determine whether aryl hydrocarbon receptor signaling pathway activation affects prostate cancer development in the TRAMP mouse prostate cancer model are in progress.

The potential relevance of the increased incidence of cribriform structures in TCDD-exposed mice to human prostate health is unknown. No experimental evidence directly addresses the question of whether TCDD causes morphological alterations in the human prostate, but epidemiological studies suggest that TCDD exposure is associated with altered prostate pathology. The Institute of Medicine (2005) has found "limited or suggestive evidence" of an association between exposure to Agent Orange (an herbicide contaminated with TCDD) and human prostate cancer, and U.S. Air Force veterans with the greatest serum dioxin concentrations were found to have the greatest prostate cancer risk (Akhtar *et al.*, 2004). It remains to be determined whether the increased incidence of cribriform structures in TCDD-exposed the mice is indicative of possible effects of TCDD on prostate disease in humans.

REFERENCES

Akhtar, F. Z., Garabrant, D. H., Ketchum, N. S., and Michalek, J. E. (2004). Cancer in US Air Force veterans of the Vietnam War. *J. Occup. Environ. Med.* **46,** 123–136.

Banerjee, S., Banerjee, P. P., and Brown, T. R. (2000). Castration-induced apoptotic cell death in the Brown Norway rat prostate decreases as a function of age. *Endocrinology* **141,** 821–832.

Banerjee, P. P., Banerjee, S., Lai, J. M., Strandberg, J. D., Zirkin, B. R., and Brown T. R. (1998). Age-dependent and lobe-specific spontaneous hyperplasia in the Brown Norway rat prostate. *Biol. Reprod.* **59,** 1163–1170.

Coffey, D. S. (1988). Androgen action and the sex accessory tissues. In *The Physiology of Reproduction* (E. Knobil and J. D. Neill, Eds.), pp. 1081–1119. Raven Press, New York.

Cunha, G. R., Donjacour, A. A., Cooke, P. S., Mee, S., Bigsby, R. M., Higgins, S. J., and Sugimura, Y. (1987). The endocrinology and developmental biology of the prostate. *Endocr. Rev.* **8,** 338–362.

Eleftheriou, B. E., and Lucas, L. A. (1974). Age-related changes in testes, seminal vesicles and plasma testosterone levels in male mice. *Gerontologia* **20,** 231–238.

Finch, C. E., and Girgis, F. G. (1974). Enlarged seminal vesicles of senescent C57BL/6J mice. *J. Gerontol.* **29,** 134–138.

Gray, L. E., Jr., Kelce, W. R., Monosson, E., Ostby, J. S., and Birnbaum, L. S. (1995). Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: Reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol. Appl. Pharmacol.* **131,** 108–118.

Gray, L. E., Jr., and Ostby, J. S. (1995). *In utero* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol. Appl. Pharmacol.* **133,** 285–294.

Gray, L. E., Jr., Ostby, J. S., and Kelce, W.R. (1997). A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans Hooded rat offspring. *Toxicol. Appl. Pharmacol.* **146,** 11–20.

Gray, L. E., Jr., Wolf, C., Lambright, C., Mann, P., Price, M., Cooper, R. L., and Ostby, J. (1999). Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p′*-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol. Ind. Health* **15,** 94–118.

Institute of Medicine. (2005). Veterans and Agent Orange: Update 2004 pg. 7. National Academies Press, Washington, DC.

Johnson, M. A., Hernandez, I., Wei, Y., and Greenberg, N. (2000). Isolation and characterization of mouse probasin: An androgen-regulated protein specifically expressed in the differentiated prostate. *Prostate* **43,** 255–262.

Jones, E. C., and Young, R. H. (1994). The differential diagnosis of prostatic carcinoma. Its distinction from premalignant and pseudocarcinomatous lesions of the prostate gland. *Am. J. Clin. Pathol.* **101,** 48–64.

Kaplan-Lefko, P. J., Chen, T.-M., Ittman, M. M., Barrios, R. J., Ayala, G. E., Huss, W. J., Maddison, L. A., Foster, B. A., and Greenberg, N. M. (2003). Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. *Prostate* **55,** 219–237.

Ko, K., Moore, R. W., and Peterson, R. E. (2004a). Aryl hydrocarbon receptors in urogenital sinus mesenchyme mediate the inhibition of prostatic epithelial bud formation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* **196,** 149–155.

Ko, K., Theobald, H. M., and Peterson, R. E. (2002). *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the C57BL/6J mouse prostate: Lobe-specific effects on branching morphogenesis. *Toxicol. Sci.* **70,** 227–237.

Ko, K., Theobald, H. M., Moore, R. W., and Peterson, R. E. (2004b). Evidence that inhibited prostatic epithelial bud formation in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-exposed C57BL/6J fetal mice is not due to interruption of androgen signaling in the urogenital sinus. *Toxicol. Sci.* **79,** 360–369.

Lin, T.-M., Ko, K., Moore, R. W., Simanainen, U., Oberley, T. D., and Peterson, R. E. (2002a). Effects of aryl hydrocarbon receptor null mutation and *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on prostate and seminal vesicle development in C57BL/6 mice. *Toxicol. Sci.* **68,** 479–487.

Lin, T.-M., Rasmussen, N. T., Moore, R. W., Albrecht, R. M., and Peterson, R. E. (2003). Region-specific inhibition of prostatic epithelial bud formation in the urogenital sinus of C57BL/6 mice exposed *in utero* to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Sci.* **76,** 171–181.

Lin, T.-M., Rasmussen, N. T., Moore, R. W., Albrecht, R. M., and Peterson, R. E. (2004). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibits prostatic epithelial bud formation by acting directly on the urogenital sinus. *J. Urol.* **172,** 365–368.

Lin, T.-M., Simanainen, U., Moore, R. W., and Peterson, R. E. (2002b). Critical windows of vulnerability for effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on prostate and seminal vesicle development in C57BL/6 mice. *Toxicol. Sci.* **69,** 202–209.

Marker, P. C., Donjacour, A. A., Dahiya, R., and Cunha, G. R. (2003). Hormonal, cellular, and molecular control of prostatic development. *Dev. Biol.* **253,** 165–174.

Mills, J. S., Needham, M., Thompson, T. C., and Parker, M. G. (1987). Androgen-regulated expression of secretory protein synthesis in mouse ventral prostate. *Mol. Cell. Endocrinol.* **53,** 111–118.

Prins, G. S., Birch, L., Couse, J. F., Choi, I., Katzenellenbogen, B., and Korach, K. S. (2001). Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor α: Studies with αERKO and βERKO mice. *Cancer Res.* **61,** 6089–6097.

Rajfer, J., and Coffey, D. S. (1979). Effects of neonatal steroids on male sex tissues. *Invest. Urol.* **17,** 3–8.

Raynaud, A. (1942). Recherches embryologiques et histologiques sur la différenciation sexuelle normale de la souris. *Bull. Biol. Fr. Belg.* **29**(Suppl.), 1–114.

Schulman, C. C. (2000). The aging male: A challenge for urologists. *Curr. Opin. Urol.* **10,** 337–342.

Shain, S. A., McCullough, B., and Segaloff, A. (1975). Spontaneous adenocarcinomas of the ventral prostate of aged A × C rats. *J. Natl. Cancer Inst.* **55,** 177–180.

Sugimura, Y., Cunha, G. R., and Donjacour, A. A. (1986). Morphogenesis of ductal networks in the mouse prostate. *Biol. Reprod.* **34,** 961–971.

Theobald, H. M., Kimmel, G. L., and Peterson, R. E. (2003). Developmental and reproductive toxicity of dioxins and related chemicals. In *Dioxins and Health,* 2nd ed. (A. Schecter and T. A. Gasiewicz, Eds.), pp. 329–431. John Wiley and Sons, Hoboken, NJ.

Theobald, H. M., and Peterson, R. E. (1997). *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Effects on development of the male and female reproductive system of the mouse. *Toxicol. Appl. Pharmacol.* **145,** 124–135.

Timms, B. G., Mohs, T. J., and Didio, L. J. (1994). Ductal budding and branching patterns in the developing prostate. *J. Urol.* **151,** 1427–1432.