Induction of Dorsolateral Prostate Adenocarcinomas and Other Accessory Sex Gland Lesions in Male Wistar Rats by a Single Administration of N-Methyl-N-nitrosourea, 7,12-Dimethylbenz(a)anthracene, and 3,2′-Dimethyl-4-aminobiphenyl after Sequential Treatment with Cyproterone Acetate and Testosterone Propionate

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ABSTRACT

Groups of 20–25 male Wistar rats (Cpb:WU), nine groups of 4-week-old rats, and nine groups of 8-week-old rats, were given cyproterone acetate (CA) s.c. or by gavage daily for 18 days at a dose of 50 mg/kg/day. Directly following CA treatment, the rats received 3 daily s.c. acetate (CA) s.c. or by gavage daily for 18 days at a dose of 50 mg/kg/old rats, and nine groups of 8-week-old rats, were given cyproterone acetate (CA) s.c. or by gavage daily for 18 days at a dose of 50 mg/kg/day. On the day after the last TP administration, a single dose of one of the following carcinogens was given to 3 groups: N-methyl-N-nitrosourea (MNU), 50 mg/kg i.v.; 7,12-dimethylbenz(a)anthracene, 30 mg/kg i.v.; 3,2′-dimethyl-4-aminobiphenyl, 250 mg/kg s.c. Three other groups received the same carcinogen treatments after 7 days of recovery from the CA administration. The last 3 groups received carcinogen without TP treatment, but immediately after CA pretreatment was stopped. A 25% incidence of invasively growing, metastasizing adenocarcinomas was found in the dorsolateral prostate region of 8-week-old rats that had received MNU after treatment with CA plus TP. In addition, this group had a 5% incidence of carcinoma in situ and a 5% incidence of atypical hyperplasia in the dorsolateral prostate. Lower incidences of adenocarcinoma of the dorsolateral prostate region and of carcinoma in situ and atypical hyperplasia of the dorsolateral prostate were found in other groups that were treated with MNU or 7,12-dimethylbenz(a)anthracene after pretreatment with CA, followed by TP or recovery, but never in rats that had been treated with CA only. In the groups treated with 3,2′-dimethyl-4-aminobiphenyl, which is slowly metabolized, these lesions were also found in groups that were pretreated with only CA. The carcinomas seemed to originate from the dorsolateral prostate and their average latency time was approximately 61 weeks. The 8-week-old rat given a MNU injection after sequential treatment with CA and TP may provide a relevant animal model for human prostatic cancer.

INTRODUCTION

Prostate cancer is one of the main human cancers in the Western world. The etiology of this neoplastic disease is essentially unknown (1–3). Research on prostatic carcinogenesis has been impeded by a lack of adequate and reliable animal models. Growth of prostatic carcinomas beyond the phase of progression to invasively growing carcinoma can be studied in a few transplantable systems (4–6). However, certain factors influencing the transition of early noninvasive latent carcinomas into invasive adenocarcinomas are thought to be decisive for prostatic cancer risk in humans (1, 7, 8); specifically a role for diet and sexual factors has been suggested in this respect (1–3, 9, 10). Therefore, it is essential that appropriate animal models of prostatic carcinogenesis possess identifiable early neoplastic stages that allow the study of such factors.

Spontaneous prostatic adenocarcinomas have been reported in some rat strains in aging animals (11–14). In most strains, these lesions develop in the ventral prostate lobes (11–13), for which there is no human homologue (15, 16). Furthermore, they occur only at a very old age (12–14) and sometimes require germ-free conditions (14). These features, site, long latency, and germ-free status, are not desirable for a practical and appropriate animal model for prostatic carcinogenesis. Hormonal induction of prostatic cancer reported in some rat strains (5, 17) involves lasting, profound changes in the endocrine milieu, notably long-term treatment with androgens at high doses. Since at least some modifying factors of prostatic carcinogenesis are believed to act by affecting relevant hormonal systems (1–3), hormonal induction of prostatic cancer is not a very appropriate model for studying prostatic carcinogenesis and modification thereof.

Fingerhut and Veenema (18) reported carcinomas induced by DMBA3 in gonadectomized animals having atrophied prostates, but this has not been reproducible (19). Recently, hyperplastic epithelial changes of the rat prostate were described after repeated systemic administration of N-nitrosobis(2-oxopropyl)amine (20). However, these hyperplasias occurred in the ventral prostate and developed into squamous cell carcinomas and not adenocarcinomas. Repeated parenteral injection of DMABP resulted in epithelial proliferative lesions of the ventral prostate classified as adenocarcinoma in situ in a 33% incidence (19, 21).

A well-known phenomenon in chemical carcinogenesis is the enhancing effect of cell proliferation in the target tissue during treatment with carcinogens leading to fixation of promutagenic DNA lesions (22, 23). In the rodent ventral prostate, but also in other accessory sex glands in males, cell proliferation can be induced by daily administration of androgens to animals that are castrated 7–30 days previously (24, 25). In this system, the rate of cell proliferation usually reaches a peak on the fourth day of androgen administration (24). We applied temporary chemical castration rather than surgical castration and gave a single carcinogen administration after three daily testosterone injections following cessation of the chemical castration. This ensured an intact status of the animals during the promotion and progression stages of prostatic carcinogenesis. In addition, the effects of this treatment on rats that were prepubertal at the start of the CA treatment and that had juvenile prostates were compared with those on young adult rats with actively secreting prostates. The carcinogens arbitrarily selected for this study are

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3 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; CA, cyproterone acetate; DMABP, 3,2′-dimethyl-4-aminobiphenyl; MNU, N-methyl-N-nitrosourea; TP, testosterone propionate.

4 M. C. Bosland, unpublished observations.
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Table 1 Summary of experimental protocols in Experiments A, B, and C

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at start of experiment (wk)</th>
<th>Treatment</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Days 1-18: CA</td>
<td>Days 19-21: TP</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Days 1-18: CA</td>
<td>Days 19-21: TP</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Days 1-18: CA</td>
<td>Days 19-25: RC</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>Days 1-18: CA</td>
<td>Days 19-25: RC</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Days 1-18: CA</td>
<td>Day 19: Carcinogen</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Days 1-18: CA</td>
<td>Day 19: Carcinogen</td>
</tr>
</tbody>
</table>

* N = 20 (Experiments A and B), or 25 (Experiment C).

* CA, 50 mg/kg/day, by s.c. injection, dissolved in oil (Experiments A and B), or by gavage, suspended in water (Experiment C); TP, 100 mg/kg/day, by s.c. injection, dissolved in oil; carcinogen: Experiment A, MNU, 50 mg/kg i.v., in saline (pH 5); Experiment B, DMBA, 30 mg/kg i.v., in fat emulsion; Experiment C, DMABP, 250 mg/kg s.c., in oil.

* RC, recovery (no treatment) after 18 days of CA exposure.

known pluripotent carcinogens in rats, belonging to three categories of chemical carcinogens: MNU, DMBA, and DMABP. DMABP has also been claimed to induce in situ adenocarcinomas of the ventral prostate in F344 rats (19). The effects of MNU in 8-week-old rats have been published previously (26); the data from that paper have been reevaluated and are incorporated in the present report.

MATERIALS AND METHODS

Animals. Male random-bred specific-pathogen-free Wistar rats (Cpb:WU) were obtained from the Central Institute for the Breeding of Laboratory Animals (CPB-TNO), Zeist, The Netherlands, and were used after at least 1 week of adaptation. They were housed under conventional conditions, 5 to a cage, in stainless steel wire mesh cages, in a well-ventilated room, at an ambient temperature of 23 ± 1°C and humidity of Laboratory Animals (CPB-TNO), Zeist, The Netherlands, and were given. A 15% fat emulsion containing (CAS 57-85-2) solution in oil (50 mg/ml; Neohombreol) was obtained as Androcur tablets (50 mg CA/tablet) dispersed in tap water. A TP (of Germany) was either used as the pure compound, dissolved in benzyl alcohol, or in oil (Experiments A and B), or by gavage, suspended in water (Experiment C); TP, 100 mg/kg/day, by s.c. injection, dissolved in oil; carcinogen: Experiment A, MNU, 50 mg/kg i.v., in saline (pH 5); Experiment B, DMBA, 30 mg/kg i.v., in fat emulsion; Experiment C, DMABP, 250 mg/kg s.c., in oil.

Experimental Design. A summary of the experimental protocol is given in Table 1. At 4 or 8 weeks of age, the rats were divided into 6 groups of 20 (Experiments A and B) or 25 animals (Experiment C) each. They were given CA daily for 18 days, at a dose of 50 mg/kg. CA was administered by s.c. injection in oil in Experiments A and B. In Experiment C, CA was administered by gavage a suspension of CA-containing tablets dispersed in water. The suspension was continuously stirred and the gavage syringe was swirled until just before gavage. The reason for using CA in tablet form was the unavailability of the pure compound in sufficiently large amounts at the time of Experiment C. Directly after CA treatment, Groups 1 and 2 of each experiment were given TP, 100 mg/kg, daily, on days 19, 20, and 21 of the experiment between 8 and 12 a.m. On day 22, carcinoen was administered as detailed below. Groups 3 and 4 were allowed to recover from CA treatment from Day 19 till Day 25 and received carcinogen on day 26. Groups 5 and 6 were given carcigenin directly after the CA pretreatment, on Day 19.

The dosages of CA and TP and the duration of the treatment and the recovery period for Groups 3 and 4 of each experiment were chosen on the basis of data from pilot experiments on the effects of CA and TP on accessory sex gland and body weights (see “Results” and Ref. 28). The time of carcinogen administration was based on studies by Coffey et al. (24), who showed that on Day 4 of androgen treatment of surgically castrated rats there is a maximum in cell proliferation of the prostate.

Animals were checked daily and killed when moribund. Surviving rats were killed at 81 (Experiment A), 79 (Experiment B), or 78 weeks (Experiment C) after carcigenin treatment.

Carcinogen Treatment. On Day 21 (Groups 1 and 2), Day 26 (Groups 3 and 4) or Day 19 (Groups 5 and 6), the rats were transferred to a carcigenin containment area in the same animal facility and housed, 5 to cage, in disposable plastic cages with filter tops (Scanbur, Koge, Denmark) containing sterilized sawdust bedding. In Experiment A, the animals received a single i.v. injection in the tail vein of MNU at a dose of 50 mg/kg, immediately after the MNU solution was prepared. In Experiment B, a single tail vein injection of DMBA, 30 mg/kg, was given. In Experiment C, a single s.c. DMABP injection was given at a dose of 250 mg/kg. Carcinogen was given between 10 a.m. and 1 p.m. in all experiments. The dosages used were based on the lowest lethal dose described in the literature (MNU, Ref. 29) or found in preliminary experiments (DMBA and DMABP) and were aimed at a maximal (sub)acute mortality of 5% or less. Extensive safety measures were taken during carcigenin exposure and a period thereafter to protect workers (Tyvek jumpsuits plus hoods/shoe covers; double latex gloves; NIOSH-approved toxic particle respirators; working in pairs; carcigenin injection in anesthetized (ether) animals; showering after disposal of protective gear) and to prevent contamination of the environment (carcigenin administration and animal housing in an area negative pressure, fitted with airlocks and HEPA-filtered air exhaust; double-bagged waste stored in airtight closed, incinerable plastic barrels; incineration of all waste at 1200°C). After a period of 2 weeks (MNU), 4 weeks (DMBA), or 8 weeks (DMABP), considered safe with regard to excetration of the carcigenin, MNU (30), DMBA,5 or DMABP (31), the animals were returned to a conventional room and wire mesh cages.

Pathology. The animals were killed by exsanguination via cannulation of the abdominal aorta while under ether anesthesia. Each rat was subjected to a complete autopsy, and all observed gross lesions were recorded. The following tissues were preserved in a 4% aqueous, neutral, phosphate buffered formaldehyde solution: accessory sex glands (dorsolateral and ventral prostate lobes, ampullary glands, coagulating glands, and seminal vesicles) and urinary bladder in toto; pituitary; thyroids plus parathyroids; adrenals; lungs; liver; spleen; and all gross lesions. Tissues were embedded in a paraffin wax, and 5-μm sections were prepared and stained with hematoxylin and eosin. Sections of pituitary, thyroids, and adrenals, made at 3 levels, and of liver, spleen, and all gross tumor-like lesions were examined microscopically. Lungs were histologically examined to detect metastases. In the pilot experiments, ventral and dorsolateral prostate were quickly dissected, weighed, and fixed. In the tumor induction experiments, the accessory sex glands were dissected after fixation as follows (see also Ref. 32).

5 M. C. Bosland and A. Schouten, unpublished observation.

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Urinary bladder was trimmed away, leaving the prostatic part of the urethra in place. Then, the ventral prostate lobes and seminal vesicle/coagulating gland structures were cut away from the dorsolateral prostate complex, and embedded separately. The dorsolateral prostate complex (with ampullary glands and prostatic urethra) was cut in halves, transversely at a right angle to the urethra. A detailed histological examination of all accessory sex glands was carried out to search for preneoplastic lesions and clues for the site of origin of the prostatic carcinomas found. Twelve- to 15-step sections were made approximately 200 μm distance of the ventral prostate lobes, of the dorsolateral prostate lobes, including the ampullary glands, and of seminal vesicles and coagulating glands. All tissues were examined by the same pathologist (M. C. B.). Some accessory sex gland tumors were sampled for electron microscopy (see Ref. 33).

**RESULTS**

Effects of Pretreatment with CA and TP on Body Weight and Accessory Sex Gland Weights and Morphology

In preliminary experiments (data not shown; see Ref. 28), p.o. administration of 50 mg/kg/day of CA for 18 days appeared to be maximally effective in reducing body and prostatic weights and about equally effective as the same dose administered by s.c. injection. A 3-day period of treatment with TP (100 mg/kg/day) following CA treatment caused drastic increases in relative prostate weights and a slight increase in body weight. These increases were comparable to those observed after a 7-day recovery following CA treatment. Morphologically, CA treatment resulted in smaller acini and decreased epithelial height. TP treatment for 3 days, following CA administration, greatly increased epithelial cell height. Mitotic figures were distinctly more frequent in the prostate of TP treated rats than in control prostates, in which mitotic activity was rare, and no mitoses were present in the prostate of animals treated with only CA.

Survival

The average survival in the various treatment groups ranged from 58 to 74 weeks but did not differ considerably among the 3 experiments (Tables 2, 4, and 6). Survival was similar in Experiments A and B (average survival, 63 and 60 weeks, respectively) and somewhat longer in DMABP treated rats (Experiment C, average of 69 weeks). Within each experiment, there were, with some exceptions, no significant differences in survival among the 6 groups (Tables 2, 4, and 6).

Adenocarcinomas of the Dorsolateral Prostate

In all three experiments, adenocarcinomas located in the dorsolateral region of the prostate were found in several groups (Tables 2, 4, and 6). In the groups that were given injections of MNU, following pretreatment with CA and TP, the combined incidence of adenocarcinomas and carcinomas in situ of the dorsolateral prostate was 30% in Group A-2 (rats treated from 8 weeks of age) and 10% in Group A-1 (rats treated from 4 weeks of age).
weeks of age). In other groups and experiments, the incidence of carcinomas in the dorsolateral prostate was low (approximately 5%).

In Experiments A and B, carcinomas and carcinomas in situ were found only in rats that had been treated with TP or that were allowed to recover after CA administration before carcinogen was given (Tables 2 and 4). No carcinomas were found in animals that were given carcinogen directly after pretreatment with CA only in these experiments. In Experiment C, however, carcinomas were not found in rats pretreated with CA and TP, but only in animals given CA with or without recovery (Table 6).

The adenocarcinomas always involved the dorsolateral prostate, ampullary glands, and the base of seminal vesicles and coagulating glands, whereas the ventral prostate lobes and the apical parts of the seminal vesicle-coagulating gland complex were always free of these malignant tumors. Even the smaller tumors involved more than one structure, and thus their exact site of origin was not clear. However, lesions classified as carcinomas in situ were found only in the dorsal or lateral lobes of the prostate, and not in any of the other accessory sex glands (Tables 2, 4, and 6). An exception was an animal in Group B-6 that was killed at 42 weeks after DMBA injection following pretreatment with TP for 3 days, or by a 7-day recovery (Table 6).

The survival of animals with adenocarcinomas of the dorsolateral prostate region was similar to the mean survival of other groups and experiments (mean, 61 weeks). Carcinomas in situ of the dorsolateral prostate were observed only in animals that were sacrificed at the end of the experiment, or close to that moment (Tables 2, 4, and 6). In a separate paper (33), several characters of these carcinomas (in situ), including their morphology and metastasizing capacity, are described in detail.

The survival of animals with adenocarcinomas of the dorsolateral prostate region was similar to the mean survival of other rats in the same groups (Tables 2, 4, and 6). It ranged from 46 to 80 weeks after carcinogen treatment in the various groups and experiments (mean, 61 weeks). Carcinomas in situ of the dorsolateral prostate were observed only in animals that were sacrificed at the end of the experiment, or close to that moment (Tables 2, 4, and 6). An exception was an animal in Group B-2 that was killed at 42 weeks after DMBA injection following recovery from CA, while moribund due to a generalized lym-
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#### Experiment C: incidence and types of tumors of the accessory sex glands in rats given a single s.c. injection of DMABP, following pretreatment with C4 alone for 18 days, or with C4 followed by TP for 3 days, or a 7-day recovery

| Group | Treatment | Survival of rats with primary tumors in the accessory sex glands | Survival of rats with carcinomas of the accessory sex glands
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>CA + TP + DMABP</td>
<td>23</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>C2</td>
<td>CA + RC + DMABP</td>
<td>22</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>C3</td>
<td>CA + TP + DMABP</td>
<td>25</td>
<td>70 ± 3</td>
</tr>
<tr>
<td>C4</td>
<td>CA + RC + DMABP</td>
<td>23</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>C5</td>
<td>CA + TP + DMABP</td>
<td>25</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>C6</td>
<td>CA + RC + DMABP</td>
<td>23</td>
<td>68 ± 2</td>
</tr>
</tbody>
</table>

1. Survival of rats with primary tumors in the accessory sex glands
2. Survival of rats with carcinomas of the accessory sex glands
3. Adenocarcinoma
4. Survival rate of rats with carcinomas of the accessory sex glands

#### Sarcomas of the Dorsolateral Prostate

In Experiment A, two sarcomas located in the dorsolateral prostate region were found (versus 9 carcinomas; Table 2). In Experiment C (DMABP; Table 6), and particularly Experiment B (DMBA; Table 4), there were more sarcomas in the dorsolateral prostate region: respectively, 3 (versus 3 carcinomas) and 7 (versus 2 carcinomas). The sarcomas occurred randomly in the various groups (Tables 2, 4, and 6). A few tumors had light and electron microscopic characteristics of neurogenic sarcomas or leiomyosarcomas. The great majority of the tumors were diagnosed as histiocytic sarcomas, based on their light and electron microscopic characteristics (34–36), which are described elsewhere (28).

#### Tumors of Other Accessory Sex Glands (Tables 2, 4, and 6)

In Experiment A, a benign tumor of the ventral prostate was found in Group 2. This microscopic tumor was classified as a cystadenoma; its morphological appearance, which differed from ventral prostate tumors described by others (11–13, 19, 21), is described elsewhere (28, 37).

In Experiment B, no tumors were observed in accessory sex glands other than the dorsolateral prostate. However, in Experiment C (DMABP), two adenocarcinomas were found in Group C-6 that clearly originated from the seminal vesicle, and one distinct coagulating gland adenocarcinoma was seen in a rat from Group C-4 (see Ref. 38 for description of their morphology). These tumors were all found at the terminal kill and were of microscopic size.

#### Nonneoplastic Proliferative Lesions of the Accessory Sex Glands

**Dorsolateral Prostate.** Lesions, classified as focal atypical hyperplasia, were found in Experiment A (MNU) in Groups 1, 2, and 3 in a low incidence (Table 3). No such lesions were observed in Experiment B (DMBA; Table 5), while a few atypical hyperplasias occurred in Groups 1 and 5 of Experiment C (DMABP; Table 7). The morphology of these lesions is described in detail in a separate paper (33).

Reactive hyperplasia associated with acute and chronic inflammatory processes was common in the dorsolateral prostate in all groups in all experiments; there was no association between the occurrence of this lesion and the treatments (data not shown). This type of hyperplasia was distinctly different from atypical hyperplasia (see Ref. 32). The diagnosis of atypical hyperplasia was never attached to a lesion with a distinct inflammatory component.

Focal squamous metaplasia was occasionally observed. It was never associated with atypical or reactive hyperplasia (Tables 3, 5, and 7). There was no apparent relationship between the occurrence of this change and the various treatments.

**Ventral Prostate.** Atypical hyperplasia of the ventral prostate was observed in low incidence (0–16%) in all experiments. There was no association between the occurrence of this lesion and any of the treatments (Tables 3, 5, and 7). The morphology of this lesion is described in detail elsewhere (32) and was very similar to that described by others for spontaneous and chemically induced atypical hyperplasia of the rat ventral prostate (11–13, 19, 21).

Reactive hyperplasia occurred in lower frequency than in the dorsolateral prostate; there was no apparent relationship between the incidence of this lesion and the various treatments (data not shown).
Table 7. Experiment C: incidence and types of hyperplastic and metaplastic lesions of the accessory sex glands in rats given a single s.c. injection of DMABP, following pretreatment with CA alone for 18 days, or with CA followed by TP for 3 days, or by a 7-day recovery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Age at start of experiment (wk)</th>
<th>No. (%) of rats with lesions in</th>
<th>Dorsolateral prostate</th>
<th>Ventral prostate</th>
<th>Seminal vesicles</th>
<th>Ampullary glands</th>
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<tbody>
<tr>
<td>C-1</td>
<td>CA + TP + DMABP</td>
<td>4</td>
<td>19</td>
<td>2 (11)</td>
<td>— —</td>
<td>20</td>
<td>1 (5)</td>
</tr>
<tr>
<td>C-2</td>
<td>CA + TP + DMABP</td>
<td>8</td>
<td>17</td>
<td>— —</td>
<td>— —</td>
<td>20</td>
<td>— —</td>
</tr>
<tr>
<td>C-3</td>
<td>CA + RC + DMABP</td>
<td>4</td>
<td>23</td>
<td>— —</td>
<td>1 (4)</td>
<td>24</td>
<td>— —</td>
</tr>
<tr>
<td>C-4</td>
<td>CA + RC + DMABP</td>
<td>8</td>
<td>21</td>
<td>— —</td>
<td>— —</td>
<td>24</td>
<td>1 (4)</td>
</tr>
<tr>
<td>C-5</td>
<td>CA + DMABP</td>
<td>4</td>
<td>24</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>25</td>
<td>1 (4)</td>
</tr>
<tr>
<td>C-6</td>
<td>CA + DMABP</td>
<td>8</td>
<td>24</td>
<td>— —</td>
<td>— —</td>
<td>25</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

* — no lesions observed. No hyperplastic or metaplastic lesions were observed in the coagulating glands.
* N, number of rats from which tissue was evaluated; only tissues without signs of autolysis are included.
\[ P < 0.01 \] for difference with Group C-4.

Seminal Vesicles. The most common proliferative lesion in these glands was a focal change, best characterized as a combination of focal hyperplasia and cellular hypertrophy. Increase of cell size was generally much more pronounced than the apparent increase in the number of cells (Fig. 1). There was no distinct cellular atypia and the lesion was usually well demarcated (Fig. 2), while it occasionally seemed to compress surrounding normal epithelium. The lesion was highly variable in size and seemed capable of secretory activity. Focal dysplastic areas were occasionally present within these lesions (see below). There was no structural association between the areas of hypertrophy-hyperplasia or dysplasia and the two seminal vesicle adenocarcinomas observed in Group 6 of Experiment C. The incidence of hypertrophy-hyperplasia was variable (Tables 3, 5, and 7). The lesion occurred less frequently in Experiment C than in Experiments A and B. Its incidence was distinctly lower than average in Group 6 in all experiments. In the other groups, there was no apparent association with treatment.

In a total of three animals in Experiment A (Group 3) and Experiment B (Group 4) a focal lesion was seen that was classified as atypical hyperplasia (Tables 3 and 5). These dysplastic lesions consisted of focal hyperplastic areas with cellular atypia and loss of cellular polarity (Fig. 3). The lesion occurred both inside and outside areas of hypertrophy-hyperplasia.

Occasionally, reactive hyperplasia was encountered, usually in association with severe inflammation in the other accessory sex glands; there was no correlation between the occurrence of this lesion and the various treatments (data not shown).

Coagulating Glands. Reactive hyperplasia was observed in a few rats in each of the three experiments (data not shown). One animal in Group A-3 showed a focal hypertrophy/hyperplasia that was very similar to that found in the seminal vesicles (Table 3).

Ampullary Glands. Focal reactive hyperplasia (Fig. 4) occurred in variable frequency. The lesion was almost invariably associated with the presence of sperm in the ampullary gland.
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Fig. 3. Dysplasia of the seminal vesicle epithelium (L, lumen). H & E, x 120.

alveoli involved, which may have been the cause of the reaction. There was no apparent relationship between incidence and treatment in the various groups and experiments (data not shown).

Tumors in Organs Other Than the Accessory Sex Glands

In all three experiments a wide spectrum of tumor types and sites was found. The incidence and latency times of these tumors are described elsewhere (28). Tumors of the ear duct and/or Zymbal's gland were the most common tumors in Experiment A (MNU), while both ear duct/Zymbal's gland tumors and s.c. sarcomas occurred very frequently in Experiment B (DMBA). These tumors did not result in early mortality in comparison with the mean survival of each experimental group (data not shown). Generalized lymphomas, on the other hand, which occurred in an incidence between 0 and 20% in all three experiments, often caused early mortality (data not shown).

DISCUSSION

The major observation in this study is the 30% incidence of carcinomas of the dorsolateral prostate in a group of rats that received a single injection with the direct-acting nitrosamide MNU. Adenocarcinomas were found in 25% of these rats, while 5% had adenocarcinomas in situ. The carcinomas were found some 63 weeks after MNU injection, i.e., when the rats were about 75 weeks old. This is a highly significant finding, because the strain of rats used in this study has a known very low incidence of spontaneous prostate cancer, i.e., 0.03% in rats of over 2 years of age (39). Full development of the prostate gland prior to carcinogen exposure was not an absolute prerequisite for the CA plus TP plus MNU protocol to be carcinogenic to the dorsolateral prostate, inasmuch as carcinomas were induced in both rats with a juvenile prostate and in animals that were young adults at the start of the experiment.

The results of Experiment B and particularly those of Experiment A point to a decisive role of cell proliferation during the initiation phase of prostatic carcinogenesis in the rat. Invasive adenocarcinomas, carcinomas in situ, and atypical hyperplasias of the dorsolateral prostate were found only in groups that received TP or recovery following CA, and not in groups treated with only CA, without TP treatment or recovery. Cell proliferation in the prostate can be enhanced effectively only by androgen treatment in castrated animals and not in intact ones (24, 25). Thus, the chemical castration caused by treatment with CA preceding the TP injections is an essential step in enhancing cell proliferation in the prostate in the approach that was applied in this study. A 7-day recovery after CA treatment is probably also accompanied by enhanced cell proliferation in the prostate. There may well be differences between the various accessory sex gland structures in the timing of the cell proliferation response to sequential treatment with CA and TP. This might explain why (pre)neoplastic lesions were only found in the dorsolateral prostate in the experiments with MNU and DMBA, and not in the ventral prostate, seminal vesicle, or coagulating gland.

The carcinogen DMABP (Experiment C) yielded different results. Prostatic carcinomas and atypical hyperplasias were not found in Groups 1 and 2, as was the case in Experiments A and B, but only in Groups 3 and 4 and Groups 5 and 6. In the latter two groups, cell proliferation was strictly inhibited by CA at the time of carcinogen administration. Furthermore, neoplastic epithelial lesions were found in the seminal vesicles and coagulating glands, unlike results in Experiments A and B. DMABP is slowly metabolized to its putative active form (31). Therefore, activated DMABP may have "missed" the transient phase of maximally stimulated cell proliferation in the dorsolateral pros-

Fig. 4. Reactive hyperplasia of the ampullary gland. Note the normal epithelium in the upper left-hand corner and the presence of sperm in the lumen of the hyperplastic gland. H & E, x 90.
tate in Groups 1 and 2 and “hit” maximal cell proliferation in other accessory sex glands instead. In Groups 5 and 6 enhancement of cell turnover in the dorsolateral prostate perhaps occurred later than in Groups 1–4, coinciding with the formation of activated DMABP.

To our knowledge, this is the first report of a substantial incidence of invasively growing adenocarcinomas of the prostate induced in experimental animals by a single carcinogen administration without further treatment. Recently, Pollard and Luckert (40) reported that a single MNU injection without concomitant stimulation of prostatic cell turnover greatly enhanced testosterone-induced carcinoma incidence in the dorsolateral prostate of the Lobund-Wistar rat from 10–15% to 65–70%. The mechanism of this reported effect of MNU is puzzling. Pour and Stepän (41) similarly produced prostatic carcinomas by N-nitrosobis(2-oxopropyl)amine administered during androgen-enhanced cell proliferation in the prostate, but only when carcinogen injection was followed by long-term testosterone treatment. The incidence was 15–21%, and most tumors were adenocarcinomas occurring in the dorsolateral prostate; some were found in the ventral lobes, where squamous cell carcinomas also were found in a 19–29% incidence.

In Experiments A and B, atypical hyperplasias and carcinomas in situ were observed only in the dorsolateral prostate, and they occurred only in those groups in which invasive carcinomas were found. For reactive hyperplasia and squamous metaplasia such associations were not present. This suggests that (a) the dorsolateral prostate is the most likely site of origin of the tumors found in the dorsolateral prostate region and (b) the atypical hyperplasias and carcinomas in situ are precursor lesions of the adenocarcinomas. These putative precursor lesions have never been observed in 12-month-old control rats of the strains (11, 12); in the Wistar strain that was used in the present study, reactive hyperplasia and squamous metaplasia were found. For reactive hyperplasia and squamous metaplasia such associations were not present. This suggests that (a) the dorsolateral prostate is the most likely site of origin of the tumors found in the dorsolateral prostate region and (b) the atypical hyperplasias and carcinomas in situ are precursor lesions of the adenocarcinomas. These putative precursor lesions have never been observed in 12-month-old control rats of the same strain (N = 111).4 It is unlikely that any tumors have arisen from the seminal vesicles, coagulating glands, or ampullary glands in Experiments A and B, because there were no obvious preneoplastic lesions in these glands occurring in association with the treatments. In addition, none of the carcinomas was exclusively located in these structures in Experiments A and B. The ventral prostate was clearly not the site of origin of the carcinomas, because it was neither macroscopically nor microscopically involved in any of the tumor processes. Furthermore, no putative precursor lesions (atypical hyperplasia) occurred in association with the treatments. These lesions are commonly found in rats over 1 year of age in a range of strains (11, 12); in the Wistar strain that was used in the present study the incidence was 4.5% in a group of 44 rats (32). The papillary cystadenoma of the ventral prostate that was found in Group A-1 may have resulted from the treatment. This lesion has, to our knowledge, not been described before. The reactive hyperplasias in the various accessory sex glands and the focal hypertrophic-hyperplastic lesions in the seminal vesicles are most likely not related to the development of the prostatic tumors, since the incidence of these changes was comparable in all experimental groups.

An important conclusion of this study is that a variety of chemical carcinogens can, in combination with appropriate stimulation of cell proliferation, produce prostatic cancer in rats. The direct-acting carcinogen MNU was most effective, while the carcinogens DMBA and DMABP, both requiring metabolic activation, induced a lower tumor incidence. Interestingly, the occurrence of sarcomas in the dorsolateral prostate region was apparently not related to the presence of a high level of cell proliferation in the prostate. Rather, it was associated with the type of carcinogen. DMBA, which induced many sarcomas at other sites (see Ref. 28), was also the most effective carcinogen in causing dorsolateral prostate sarcomas.

The approach presented in this paper offers a promising animal model for human prostatic carcinogenesis. However, this model must comprise a number of essential characteristics, which are detailed in a companion paper (33). In this regard, it is important that the presence of most other tumors did not interfere with the risk to the animals of developing prostate tumors, since they did not cause early death, as indicated by a comparable survival of rats with and without prostatic carcinomas. Only lymphomas caused a reduction of survival.

Four important features make the model stand out favorably in comparison with other induction models: (a) the tumors are adenocarcinomas, and not squamous cell carcinomas as in the model described by Pour (20); (b) the tumors are invasively growing and are capable of metastasizing (see Ref. 33), unlike the lesions reported by Katayama et al. (19) and Shirai et al. (21); (c) no long-term hormonal treatment is required. After initial endocrine manipulation to stimulate cell proliferation, the animals remain untreated, unlike prostate cancer models that involve long-term, high dose androgen treatment (5, 17, 40, 41); and (d) the tumors do not originate from the ventral prostate as they do in some of the other chemical induction models mentioned (19–21), but from the dorsolateral lobes which are embryologically homologous to the parts of the prostate in which carcinomas develop in humans (8, 15, 16). In addition, the fact that only a single carcinogen injection is needed in the present model and not repeated carcinogen administration (19–21) or long-term hormonal treatment (5, 17, 40, 41) provides excellent opportunities for studying the promotion stage of prostatic carcinogenesis and its modification by environmental factors.

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REFERENCES


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CHEMICAL INDUCTION OF RAT PROSTATE ADENOCARCINOMAS


Induction of Dorsolateral Prostate Adenocarcinomas and Other Accessory Sex Gland Lesions in Male Wistar Rats by a Single Administration of \(N\)-Methyl-\(N\)-nitrosourea, 7,12-Dimethylbenz(a)anthracene, and 3,2\(^{\prime}\)-Dimethyl-4-aminobiphenyl after Sequential Treatment with Cyproterone Acetate and Testosterone Propionate

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