A Retrospective Analysis of Background Lesions and Tissue Accountability for Male Accessory Sex Organs in Fischer-344 Rats

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ABSTRACT

Because the paired lobes (ventral, dorsal, lateral, and anterior) of the rat prostate have not been consistently sampled in many carcinogenicity and toxicity studies, comparison among different investigations has been compromised. The lack of specific site identification for prostatic lesions further lessens the value of incidences reported. We present here the lobe-specific incidences and degree of severity of background prostatic, seminal vesicular, and ampullary glandular lesions in 1768 control Fischer-344 rats from 35 recent National Toxicology Program 2-year carcinogenicity and toxicity studies conducted in 4 laboratories. The dorsal and lateral lobes were combined and considered the dorsolateral lobe where inflammation, epithelial degeneration, mucinous cysts, and edema were observed. Inflammation in the dorsolateral lobes was significantly associated with pituitary gland adenoma whose prolactin was suggested to play an important role in pathogenesis of prostatic inflammation. Epithelial degeneration, epithelial hyperplasia, inflammation, edema, and adenoma were conspicuous in the ventral lobes. Inflammation and edema occurred in the anterior lobes (coagulating glands). Inflammation, dilatation, epithelial hyperplasia, edema, and adenoma were observed in the seminal vesicles. Inflammation was also present in the ampullary glands. We suggest an optimal embedding and trimming method in rat prostate and seminal vesicle to ensure adequate, consistent sampling.

Keywords. Accessory male sex glands; background data; histopathology; rodents; pituitary gland adenoma; prolactin; spontaneous.

INTRODUCTION

Assessment of the potential toxicological and carcinogenic effects of a chemical compound in male accessory sex glands including prostate, seminal vesicle, and ampullary gland may be difficult if the incidence of spontaneous histopathological background lesions is unknown. Although the rat prostate has been used as an experimental model to analyze the function of androgenic hormones (26, 28), few background histopathological data exist for this gland and other male accessory sex glands in Fischer-344 (F-344) rats commonly used in 2-year carcinogenicity and toxicity studies (6, 11, 18, 41, 50). Because the paired lobes of the rat prostate have not always been sampled consistently in past carcinogenicity and toxicity studies, comparison among different studies has been compromised. Omission of the identification of specific sites for prostatic lesions in controls lessens the value of reported background incidences. Histopathological lesions in the seminal vesicles and ampullary glands have seldom been reported (4, 6–8, 18, 22, 30, 46, 50).

This retrospective histopathological examination was undertaken to document lobe-specific tissue accountability and incidences of background histopathological lesions in the prostate, seminal vesicles, and ampullary glands in control F-344 rats from 35 recent 2-year carcinogenicity and toxicity studies conducted in 4 laboratories for the National Toxicology Program (NTP). In addition, possible associations between various lesions were explored statistically; in particular, possible correlation between prostatic inflammation and the presence of pituitary gland adenomas was evaluated, because exposure to prolactin (PRL) has been implicated in the development of prostatitis (40, 42, 51, 52), and prolactinomas are common in F-344 rats.
The method of tissue trimming is vital for accurate histopathological examination and interpretation of each lobe of the rat prostate. One sectioning method, sagittal, has been previously proposed for rat prostate (5–7). The standard operating procedure (SOP) of the NTP study, however, has been to make a single transverse section including both dorsolateral and ventral lobes of the prostate just posterior to the urinary bladder. Our survey indicated, however, that the prostate was not uniformly trimmed among the 4 laboratories performing these NTP studies. We detail a trimming and embedding guideline to ensure optimal histological sampling of the rat prostate.

**Methods**

Prostate, seminal vesicle, and ampullary gland tissue sections from 1768 control male F-344 rats were obtained from selected feed (n = 20), gavage (n = 12), and drinking water (n = 3) 2-year carcinogenicity and toxicity studies conducted using the NTP’s SOP. Twenty recent bioassay studies were chosen from Laboratory A, and the remaining 15 studies were selected from 3 other laboratories—B, C, and D—operating under Good Laboratory Practice guidelines. Housing conditions for the rats had been standardized according to NTP specifications (10). For each animal, a complete necropsy had been performed and included gross and histopathological examination of major organs and all gross lesions. Tissues had been fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Selected sections of prostate were cut from NTP blocks and stained with Masson-trichrome and Alcian blue (pH 2.5)-periodic acid Schiff (PAS).

Because distinguishing between dorsal and lateral lobes on a routine basis in carcinogenicity and toxicity studies is difficult, we combined the dorsal and lateral lobes from the NTP slides and considered them the dorsolateral lobe (4, 6, 7). Histopathological lesions were thus recorded as dorsolateral lobe, ventral lobe, coagulating gland, seminal vesicle, and ampullary gland.

The amount of tissue from dorsolateral and ventral lobes, coagulating gland, seminal vesicle, and ampullary gland was estimated subjectively as a percentage of an optimal transverse section and assigned a category: none, 1–25%, 26–50%, 51–75%, and 76–100%.

The severity of nonproliferative lesions was scored subjectively using 4 grades based on the size and/or multiplicity of the lesions expressed as the percentage of acini, as follows: (1) minimal—lesion occurring in less than 10% of acini; (2) mild—10–39%; (3) moderate—40–79%; (4) marked—more than 80%.

After documentation of lobe-specific lesions in the prostate, the incidence data for some of the common findings were evaluated statistically for the following possible associations:

a. Presence of pituitary gland adenomas with the incidence and severity of prostatic inflammation in the dorsolateral and ventral lobes,

b. Prostatic inflammation with incidence of hyperplasia in the ventral lobe,

c. Prostatic inflammation with mucinous cysts of the dorsolateral lobe.

The data from 5 recent studies conducted at Laboratory A (33–37) were utilized for this purpose because prostatic tissue accountability has been found to be optimal from this facility since 1985. Multiple and logistic regression statistical analyses (14, 49) were used after adjusting for survival differences and study-to-study variability.

For immunohistochemistry, 15 paraffin sections of pituitary gland containing adenomas were cut from randomly selected blocks from 5 recent studies (33–37) without regard to presence or absence of prostatic lesions and examined by the avidin-biotin complex (ABC) method (21) using rabbit antisera at the following dilutions as primary antibody: anti-rat prolactin (PRL), 1:400; anti-rat growth hormone (GH), 1:200; anti-rat thyroid-stimulating hormone (TSH), 1:400; anti-rat luteinizing hormone (LH), 1:200; and anti-human adrenocorticotropic hormone (ACTH), undiluted. Rabbit anti-human ACTH was obtained from DAKO Corporation, Santa Barbara, California, and other primary antibodies were purchased from Biogenesis, Inc., Sandown, New Hampshire. The biotinylated secondary antibody and ABC complex were obtained from Vector Corporation, Burlingame, California. Then, 3, 3'-diaminobenzidine (DAB) was applied as a substrate for the peroxidase reaction, and slides were counterstained with hematoxylin.

**Results**

The relative amounts of tissue from prostatic lobes, seminal vesicles, and ampullary glands are summarized in Table 1. The data show that the quantity of dorsolateral and ventral lobe tissue was highly variable among laboratories so that a preponderance of either dorsolateral or ventral lobe occurred depending on sampling. The quantity of these tissues varied within each individual laboratory as well, indicating inconsistency of sampling among studies. Tissues from the ventral lobe were missed altogether in Laboratories C and D in a large percentage of cases. Because more than 51% of the maximum transverse area was usually present in coagulating gland and seminal vesicle sections from each laboratory, consistent sectioning did not seem to be a problem for these tissues. Ampullary glands were found not to be sectioned in Laboratories C and D in a large percentage of cases.

Detailed criteria for defining normal histological and histopathological characteristics in prostate, coagulating glands, seminal vesicles, and ampullary glands have been reported in the literature (3, 4, 23, 28) and are described briefly next for comparison with characteristics of lesions encountered in our survey. Our own observations of lesion occurrence are noted following the summarized descriptions.

**Normal Prostate**

The lateral regions of the dorsolateral lobes (see Figure 1) are bound to the urethra by smooth muscle, connective tissue stroma, and ducts. The acini of the lateral lobes are large and loosely arranged within stroma and contain strongly eosinophilic secretions. The epithelium is cuboidal to columnar, and the nuclei of the epithelial cells are centrally located; supranuclear areas of cytoplasmic pallor are prominent.

The dorsal regions of the dorsolateral lobes are separated from the lateral regions by thin smooth muscle and connective tissue stroma. Secretion in the dorsal lobes is moderately eosinophilic; the acini are large, but with minimal infolding,
and loosely distributed within stroma. The epithelium is generally cuboidal, and the nuclei of the epithelial cells are centrally located; supranuclear areas of cytoplasmic pallor are observed.

The ventral lobes are attached to the urethra by smooth muscle, connective tissue stroma, and ducts whose epithelium is mainly cuboidal. Secretions in the acini, which were rather tightly packed in the stroma, are pale and slightly eosinophilic. The acini of the ventral lobes exhibit infrequent and varying degrees of infolding. The epithelium is columnar to cuboidal and basophilic, and the nuclei of the epithelial cells are basally located; supranuclear areas of cytoplasmic pallor are conspicuous.

**Normal Coagulating Glands**

The coagulating gland acini are tightly packed, surrounded by thick smooth muscle and connective tissue stroma and attached to the seminal vesicles. The acini are typically infolded and contain moderately eosinophilic secretion. The epithelium is generally columnar. The nuclei of the epithelial cells are centrally located; basilar areas of cytoplasmic pallor are prominent.

**Normal Seminal Vesicles**

Thick smooth muscle cells and connective tissue stroma surround the acini that are infolded peripherally and exhibit a strongly eosinophilic secretion in a centrally distended lumen. The epithelium is columnar, and the nuclei of the epithelial cells are basally located with areas of cytoplasmic pallor in the apical cytoplasm.

**Normal Ampullary Glands**

These glands encircle the vas deferens. The acini are large, uniform in size and surrounded by smooth muscle and connective tissue stroma. A strongly eosinophilic secretion containing clear vacuoles is typically artifactually separated from the epithelium by clear space. The epithelium is flattened to cuboidal, and the nuclei of the epithelial cells are centrally located.

The incidence of histopathological lesions in prostatic lobes, coagulating glands, seminal vesicles and ampullary glands is summarized in Table 2. Descriptions for the non-proliferative and epithelial proliferative lesions follow.

**Prostatic, Coagulating Glandular, Seminal Vesicular, and Ampullary Glandular Inflammation**

This lesion is characterized by various degrees of inflammatory cell infiltration both in the interstitium and acinus (Figure 2). The lumina contain neutrophilic infiltrations and include various amounts of secretion, often accompanied by accumulations of tissue debris, sometimes forming abscesses. Denudation of the epithelium often coexists with neutrophilic exudation. Reactive hyperplasia, squamous metaplasia, and vacuolar degeneration of epithelium are observed in involved acini (Figure 3). Lymphocytes and plasma cells infiltrate thickened periacinar interstitial connective tissue in varying degrees. Fibrosis as a part of inflammatory reactions is frequently observed in the interstitium.

Our observations indicated that inflammation occurred from greater to lesser frequency in, respectively, the dorsolateral lobe, ventral lobe, ampullary gland, coagulating gland, and seminal vesicle.
Prostatic Mucinous Cysts

This noninvasive lesion (see Figures 4–6) is characterized by dilated acini filled with pale amphophilic mucinous material and a small amount of debris surrounded by dense fibrous connective tissue and inflammatory cells. The size and appearance of the cysts vary. The epithelial lining for individual cystic acini is metaplastic and varies from hypercellular columnar cells producing mucin to low columnar and atrophic squamoid cells. These different types of epithelial cells occur adjacent to each other. Localized areas of mucosal denudation may be present. We noted that mucinous cysts appeared only in the dorsolateral lobe and at a low incidence.

Prostatic Epithelial Degeneration

Distention of the cytoplasm of the prostatic epithelium and pale eosinophilic staining typify this lesion (Figure 7). The nuclei are shrunken and have lost polarity. Occasionally there is autophagy or phagocytosis of the epithelial cells by the macrophages between them. We observed that epithelial degeneration occurred more frequently in the ventral than the dorsolateral lobe.

Seminal Vesicular Dilatation

The lumen is filled with secretion and shows great distention associated with thinning of the muscular wall and flattening of the epithelium. We rarely observed this change, and the severity of this lesion was estimated by the degree of dilatation.

Prostatic, Coagulating Glandular, and Seminal Vesicular Edema

A distended interstitium contains faintly eosinophilic material. Few inflammatory cells are observed either in interstitia or acini (Figure 8). We observed a low frequency of edema in the different lobes of the prostate, coagulating gland and seminal vesicle; the severity of this lesion was estimated by the degree of expansion of the interstitium.

Prostatic Epithelial Hyperplasia

Hyperplasia, defined also as atypical hyperplasia by Bosland et al (5–7), consists of a proliferation of epithelial cells occurring in a single acinus or several adjacent acini without distention of the acini or compression of the surrounding tissues (Figures 9 and 10). Gradual transitions
<table>
<thead>
<tr>
<th>Laboratory</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td>Number of studies</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Number of animals</td>
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<td>253</td>
<td>260</td>
<td>252</td>
<td>3,768</td>
</tr>
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<td>Dosing day</td>
<td>53-857</td>
<td>25-732</td>
<td>151-734</td>
<td>113-736</td>
<td>25-857</td>
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<tr>
<td>Dorsolateral lobe</td>
<td>(969)</td>
<td>(246)</td>
<td>(26)</td>
<td>(142)</td>
<td>(1,383)</td>
</tr>
<tr>
<td>Edema</td>
<td>4 (0.4%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>4 (0.3%)</td>
</tr>
<tr>
<td>Epithelial degeneration</td>
<td>(3.5)</td>
<td>[ND]</td>
<td>[ND]</td>
<td>[ND]</td>
<td>[3.5]</td>
</tr>
<tr>
<td>Inflammation</td>
<td>96 (9.9%)</td>
<td>14 (5.7%)</td>
<td>6 (21.3%)</td>
<td>15 (10.6%)</td>
<td>131 (9.5%)</td>
</tr>
<tr>
<td>Mucinous cysts</td>
<td>655 (67.6%)</td>
<td>197 (80.1%)</td>
<td>21 (80.8%)</td>
<td>104 (71.1%)</td>
<td>974 (70.4%)</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>(1.6)</td>
<td>(1.5)</td>
<td>[1.5]</td>
<td>[1.8]</td>
<td>[1.9]</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>29 (3.0%)</td>
<td>11 (4.5%)</td>
<td>3 (11.5%)</td>
<td>1 (0.7%)</td>
<td>44 (3.2%)</td>
</tr>
<tr>
<td>Ventral lobe</td>
<td>(534)</td>
<td>(52)</td>
<td>(242)</td>
<td>(190)</td>
<td>(1,038)</td>
</tr>
<tr>
<td>Edema</td>
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<td>2 (0.8%)</td>
<td>0 (0.0%)</td>
<td>3 (0.3%)</td>
</tr>
<tr>
<td>Epithelial degeneration</td>
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<td>[2.0]</td>
<td>[2.0]</td>
<td>[2.0]</td>
<td>[2.0]</td>
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<tr>
<td>Inflammation</td>
<td>197 (37.6%)</td>
<td>5 (6.1%)</td>
<td>83 (34.3%)</td>
<td>40 (21.1%)</td>
<td>325 (31.3%)</td>
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<tr>
<td>Epithelial hyperplasia</td>
<td>52 (9.9%)</td>
<td>3 (3.7%)</td>
<td>30 (12.4%)</td>
<td>13 (6.8%)</td>
<td>98 (9.4%)</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Coagulating gland</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Edema</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>9 (1.1%)</td>
<td>1 (1.4%)</td>
<td>0 (0.0%)</td>
<td>2 (0.9%)</td>
<td>14 (0.9%)</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Dilatation</td>
<td>4 (0.4%)</td>
<td>1 (0.4%)</td>
<td>0 (0.0%)</td>
<td>2 (0.8%)</td>
<td>7 (0.4%)</td>
</tr>
<tr>
<td>Edema</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>8 (0.8%)</td>
<td>6 (2.4%)</td>
<td>0 (0.0%)</td>
<td>5 (2.0%)</td>
<td>19 (1.1%)</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>2 (0.2%)</td>
<td>2 (0.8%)</td>
<td>1 (0.4%)</td>
<td>0 (0.0%)</td>
<td>5 (0.3%)</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Ampullary gland</td>
<td>72 (7.3%)</td>
<td>35 (13.1%)</td>
<td>17 (6.6%)</td>
<td>12 (4.8%)</td>
<td>134 (7.7%)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>147 (23.3%)</td>
<td>37 (16.7%)</td>
<td>2 (25.0%)</td>
<td>8 (11.6%)</td>
<td>194 (20.8%)</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

(1): Number of animals with available tissue for evaluation.
(2): Percentage of total number of animals.
(3): Mean severity of nonproliferative histopathological lesions.
[ND]: Not detected.
Nonproliferative lesions were scored using 4 semiquantitative grades as follows: 1—minimal, 2—mild, 3—moderate, 4—marked; mean severities are based on affected animals only.

Occur from normal epithelium to hyperplastic areas with papillary and cribriform formation. No inflammatory reaction in or around this lesion is seen. No capsule formation is observed. Hyperplastic epithelium is generally columnar, and cytoplasm is tinctorially similar to that of normal epithelium. Cellular polarity tends to be lost, and mitotic figures are rarely observed. Reactive hyperplasia associated with inflammation is considered a component of the inflammatory lesion and not recorded as a separate finding as suggested by Bosland et al (5–7). Cases of functional hyperplasia described by Bosland et al (5–7) and characterized by increased infolding of the epithelium at the periphery of the lobes are not included. In our investigation, epithelial hyperplasia was observed at low incidence only in the ventral lobe.

**Seminal Vesicular Epithelial Hyperplasia**

Hyperplasia is defined as a proliferation of epithelial cells without distention of the acini or compression of the surrounding tissues (Figure 11). Gradual transitions occur from normal epithelium to hyperplastic areas with papillary formation. No inflammatory reactions in or around this lesion are seen. Hyperplastic epithelial cells are generally columnar and contain much more cytoplasm than normal epithelium. Cellular polarity tends to be lost, and nuclei are hyperchromatic. Mitotic figures are rarely observed. We rarely encountered epithelial hyperplasia in the seminal vesicle.

**Prostatic Adenoma**

Adenoma (Figures 12 and 13) is defined as an intraglandular epithelial proliferation filling the lumen of one or more adjacent acini. No inflammation is associated with adenomas, but in some lesions foamy macrophages are seen. Distortion or compression of surrounding tissue is observed. The epithelial cells are usually arranged in a cribriform or papillary pattern. The cells are cuboidal to columnar and slightly eosinophilic. The nuclei are round and slightly
FIGURE 2.—Marked (grade 4) inflammation in the dorsolateral lobe. Accumulation of neutrophils in the acinar lumen and increase in connective tissue with lymphocytic infiltration in the interstitium are prominent. H&E stain. Bar = 100 μm. 3.—Marked (grade 4) inflammation in the dorsolateral lobe. Note reactive hyperplasia of the epithelium. H&E stain. Bar = 100 μm. 4.—Moderate (grade 3) mucinous cysts in the dorsolateral lobe are surrounded by fibrous tissue and distended into various sizes by pale amorphophilic material. H&E stain. Bar = 500 μm. 5.—Moderate (grade 3) mucinous cysts in the dorsolateral lobe are surrounded by fibrous tissue and lined by columnar to squamous cells. Masson-trichrome stain. Bar = 100 μm. 6.—Moderate (grade 3) mucinous cysts in the dorsolateral lobe. Metaplastic columnar epithelial cells have PAS-positive (arrow) or Alcian blue-positive material. Alcian blue (pH 2.5)-PAS stain. Bar = 50 μm. 7.—Mild (grade 2) epithelial degeneration in the ventral lobe. Epithelial cells are distended by pale eosinophilic material. The nuclei are located peripherally. H&E stain. Bar = 100 μm. 8.—Mild (grade 2) edema in the ventral lobe. Faintly eosinophilic material is present in the interstitium. H&E stain. Bar = 500 μm. 9.—Focal intra-acinar epithelial hyperplasia in the ventral lobe. Note cribriform and papillary growth into the lumen. H&E stain. Bar = 100 μm. 10.—Epithelial hyperplasia in the ventral lobe. Note minimal cribriform and papillary growth. H&E stain. Bar = 100 μm. 11.—Epithelial hyperplasia in the seminal vesicle. Note papillary growth. H&E stain. Bar = 100 μm. 12.—Adenoma in the ventral lobe. The tumor is compressing the surrounding tissue. A cribriform growth pattern is prominent. H&E stain. Bar = 100 μm. 13.—Higher magnification of Figure 12. Epithelial cells have lost their polarity. Nuclear pleomorphism is shown. H&E stain. Bar = 50 μm. 14.—Adenoma in the seminal vesicle. The neoplastic epithelium has formed as a papillary bud (arrows) extending into the lumen. H&E stain. Bar = 500 μm.
hyperchromatic. Cells have lost their normal polarity, and mitotic figures are occasionally seen. The basement membrane of the adenomatous acini remains intact. We observed that adenoma in the prostate was mostly confined to the ventral lobe.

**Seminal Vesicular Adenoma**

Gradual transitions occur from normal epithelium to proliferative areas (Figure 14). No inflammatory reactions in or around this lesion are seen. The epithelial cells are predominantly arranged in a cribriform or papillary pattern. The epithelial cells are generally columnar and contain much more cytoplasm than normal epithelium. Cellular polarity tends to be lost, and nuclei are hyperchromatic. Mitotic figures are rarely observed. The one adenoma that we observed was characterized by a proliferation of epithelial cells with peduncular attachment.

**Statistical Analysis of the Relationship Between Prostatic Inflammation and Pituitary Gland Adenoma, Epithelial Hyperplasia, and Mucinous Cysts**

Inflammation of the ventral lobe occurred at a much lower incidence and severity than in the dorsolateral lobes (Table 3) and showed no correlation with the incidence of pituitary gland adenoma. In contrast, the incidence and severity of inflammation in the dorsolateral lobes of prostate showed a highly significant \( p < 0.001 \) association with the presence of pituitary adenomas (Table 4). No association was found between prostatic inflammation and the incidence of hyperplasia in the ventral or dorsolateral lobe (data not presented). Multiple and logistic regression analyses revealed a significant \( p < 0.05 \) association between inflammation and mucinous cysts in the dorsolateral lobes (Table 5).

**Immunohistochemical Expression of Pituitary Gland Adenoma**

Most pituitary gland adenomas demonstrated PRL positivity, and most PRL-positive pituitary gland adenomas were accompanied by inflammation of the dorsolateral prostatic lobe (Table 6). One PRL-positive pituitary gland adenoma had TSH and LH positivity. TSH and LH positivity, but PRL negativity, were observed in one pituitary gland adenoma. ACTH positivity was detected in one adenoma; no other hormonal reactivity was observed.

**Discussion**

Comparison of histopathological findings from the 4 laboratories revealed that the recorded incidence of proliferative lesions and epithelial degeneration in the ventral lobes was higher in laboratories that sampled primarily the ventral lobe, whereas recorded observation of inflammation and mucinous cysts in the dorsolateral lobe was higher from laboratories that sampled mainly dorsolateral lobes. These results indicate obvious sampling bias and suggest the importance of histopathological examination of both dorsolateral and ventral lobes of rat prostate to detect proliferative as well as nonproliferative lesions.

Although the coagulating glands and seminal vesicles obtained from the 4 laboratories were sectioned optimally, sectioning of both dorsolateral and ventral lobes was not consistent. Because of anatomical similarities of the human prostatic posterior, middle, and lateral lobes to the rat dorsal, anterior (coagulating gland) and lateral lobes, respectively (48), inclusion of both dorsolateral and ventral lobes in histopathological examinations to determine precise lobe localization of lesions in rat prostate might have relevance to humans.

**Table 4.** Summary of association between incidence of pituitary gland adenoma and prostatic inflammation in dorsolateral lobes in male F-344 control rats from 5 NTP studies.

<table>
<thead>
<tr>
<th>Severity of inflammation in dorsolateral lobes</th>
<th>Incidence of pituitary gland adenoma</th>
<th>Survival time (days) (Mean ± SD)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>15.3% (11/72)</td>
<td>693 ± 96</td>
</tr>
<tr>
<td>1</td>
<td>14.8% (9/61)</td>
<td>676 ± 128</td>
</tr>
<tr>
<td>2</td>
<td>41.2% (28/68)</td>
<td>685 ± 82</td>
</tr>
<tr>
<td>3</td>
<td>56.8% (25/44)</td>
<td>673 ± 83</td>
</tr>
<tr>
<td>4</td>
<td>88.9% (8/9)</td>
<td>666 ± 71</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) versus animals with no inflammation; overall trend: \( p < 0.001 \).

**Table 5.** Summary of association between incidence and severity of mucinous cysts and prostatic inflammation in dorsolateral lobes in male F-344 control rats from 5 NTP studies.

<table>
<thead>
<tr>
<th>Severity of inflammation</th>
<th>Incidence</th>
<th>Mucinous cysts</th>
<th>Severity * (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4% (1/72)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.6% (4/61)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8.8% (6/69)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.1% (4/44)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22.2% (2/9)</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

*Significance of overall trend \( p < 0.05 \), including studies having zero severity.
Table 6.—Summary of immunohistochemical expression of 15 (3/study, selected at random) pituitary gland adenomas and their association with prostatic inflammation*.

<table>
<thead>
<tr>
<th></th>
<th>PRL</th>
<th>GH</th>
<th>TSH</th>
<th>LH</th>
<th>ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of immuno-positive pituitary gland adenomas</td>
<td>13/15 (86.7%)</td>
<td>0/15 (0.0%)</td>
<td>2/15 (13.3%)</td>
<td>2/15 (13.3%)</td>
<td>1/15 (6.7%)</td>
</tr>
<tr>
<td>Number of pituitary gland adenomas with inflammation in dorsolateral lobe</td>
<td>12/13 (92.3%)</td>
<td>0/0 (---)</td>
<td>2/2 (100.0%)</td>
<td>2/2 (100.0%)</td>
<td>1/1 (100.0%)</td>
</tr>
<tr>
<td>Number of pituitary gland adenomas without inflammation in dorsolateral lobe</td>
<td>1/13 (7.7%)</td>
<td>0/0 (---)</td>
<td>0/2 (0.0%)</td>
<td>0/2 (0.0%)</td>
<td>0/1 (0.0%)</td>
</tr>
<tr>
<td>Number of pituitary gland adenomas with inflammation in ventral lobe</td>
<td>2/13 (15.4%)</td>
<td>0/0 (---)</td>
<td>0/2 (0.0%)</td>
<td>0/2 (0.0%)</td>
<td>0/1 (0.0%)</td>
</tr>
<tr>
<td>Number of pituitary gland adenomas without inflammation in ventral lobe</td>
<td>11/13 (84.6%)</td>
<td>0/0 (---)</td>
<td>2/2 (100.0%)</td>
<td>2/2(100.0%)</td>
<td>1/1 (100.0%)</td>
</tr>
</tbody>
</table>

(%) Percentage of number of pituitary gland adenomas expressing immunohistochemical positivity.
PRL: prolactin; GH: growth hormone; TSH: thyroid-stimulating hormone; LH: luteinizing hormone; ACTH: adrenocorticotropic hormone.
* 13/15 cases had inflammation in the dorsolateral lobe and 2/15 cases had inflammation in the ventral lobe.

The association between the quantity of tissue present in a section and the incidence of prostatic lesions is illustrated in Table 7 using epithelial degeneration of the ventral lobe as an example. Note that the incidence of this degeneration is clearly related to the amount of tissue present at Laboratories A, C, and D; Laboratory B had an insufficient quantity of tissue to show this association. For example, the 3 to 4-fold increase in the overall incidence of epithelial degeneration of ventral prostate at Laboratory D (21%) relative to Laboratory B (6%) is highly significant (p < 0.01 by a Fisher's exact test) (47) if the amount of tissue sampled is not taken into account. If tissue amount is considered, however, by comparing incidence rates for groups with equivalent tissue amounts using a Mantel–Haenszel test (49), then the difference in incidences between the two laboratories becomes insignificant (p > 0.20). The reason that the incidence of epithelial degeneration of the ventral lobe is much lower in Laboratory B (6%) than at the other 3 laboratories (21–38%) likely simply reflects the fact that 79% (65/82) of the ventral lobe samples at this laboratory involved only 1–25% of the lobe. In contrast, 80–98% of the samples from the other 3 laboratories involved 26–100% of the lobe, so a higher incidence of epithelial degeneration would be expected and was observed. The amount of tissue sampled by a laboratory clearly influences the reported incidence of epithelial degeneration of the ventral lobe. These observations illustrate that the quantity of tissue sampled is an important variable that must be standardized if historical control incidence data from different laboratories are to be compared.

The histopathological characteristics of spontaneous proliferative and nonproliferative lesions in prostate and seminal vesicles of F-344 rats compiled for this study were similar to those previously reported for this and other rat strains (4, 18, 30, 50). Quantification of the incidence and characterization of proliferative and nonproliferative lesions in each lobe of the prostate, seminal vesicle and ampullary gland is important because of differential lobe sensitivity to spontaneous and induced lesions (4–7, 22).

Although less tissue from the ventral than the dorsolateral lobe was present in slides from all four laboratories, our findings demonstrate that proliferative lesions of the rat prostate, including epithelial hyperplasia and adenoma, were limited exclusively to the ventral lobes. Spontaneous proliferative lesions are known to occur more frequently in ventral than dorsolateral lobes, while chemical carcinogens induce neoplastic changes predominately in the dorsolateral lobe (4–6, 22, 30, 41). As reported previously (30, 41), no spontaneously occurring adenocarcinoma was seen in ventral lobes of F-344 rats. Adolescent F-344 rats have been shown to be resistant to epithelial hyperplasia of the ventral lobe compared to Wistar and Sprague–Dawley rats exposed to tumor promoters during the ontogenetic and postcastration growth and differentiation periods (44). This interstrain susceptibility was suggested to be related to a possible decreased sensitivity of androgen receptors in F-344 rats (44). Epithelial hyperplasia and adenoma, as well as adenocarcinoma, are rarely reported in the seminal vesicle in the F-344 rat (22, 30, 46); similar findings were observed in the present investigation. Spontaneous proliferative lesions including hyperplasia and adenoma have not been reported in the ampullary gland in rats (7).

In the male accessory sex glands, inflammation is frequently seen in prostatic tissue of the rat (4, 6). Spontaneous prostaticis occurs in the lateral lobe of aged rats and has been used as a model of nonbacterial prostatitis in humans (2, 29, 31). In our study, histopathological features of inflammation in the dorsolateral lobes were similar to those described for various strains of rats used for toxicity and carcinogenicity

Table 7.—Incidence of epithelial degeneration in ventral lobe as a function of amount of tissue examined.

<table>
<thead>
<tr>
<th>Quantity of tissue</th>
<th>Laboratory A</th>
<th>Laboratory B</th>
<th>Laboratory C</th>
<th>Laboratory D</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–25%</td>
<td>9% (9/105)</td>
<td>6% (4/65)</td>
<td>17% (1/6)</td>
<td>0% (0/19)</td>
<td>7% (14/195)</td>
</tr>
<tr>
<td>26–50%</td>
<td>36% (37/104)</td>
<td>7% (1/14)</td>
<td>32% (25/79)</td>
<td>20% (14/71)</td>
<td>29% (77/268)</td>
</tr>
<tr>
<td>51–75%</td>
<td>48% (70/145)</td>
<td>0% (0/2)</td>
<td>28% (22/79)</td>
<td>24% (11/46)</td>
<td>38% (103/272)</td>
</tr>
<tr>
<td>76–100%</td>
<td>48% (81/170)</td>
<td>0% (0/1)</td>
<td>45% (35/78)</td>
<td>28% (15/54)</td>
<td>43% (131/303)</td>
</tr>
<tr>
<td>Total</td>
<td>38% (197/524)</td>
<td>6% (5/82)</td>
<td>34% (83/242)</td>
<td>21% (40/190)</td>
<td>31% (325/1038)</td>
</tr>
</tbody>
</table>
studies (2, 4, 6, 8, 12, 31, 39). Bacterial infection (6, 31, 39), endocrine influence (6, 24, 29, 39, 42, 51, 52), immunological dysfunction (13, 38, 39), and stress (1, 6, 17) may be factors in development of prostatic inflammation. Because chronic inflammation of the prostate was suggested to promote hyperplasia in this tissue (25), we examined the possible relationship between these two processes in our study and found no clear association.

Neonatal hyperprolactinemia induced by estradiol or PRL can later result in inflammation in the lateral prostate of the adult rat with histopathological features similar to those of spontaneous inflammation in the lateral lobe in rats (42, 53). Recently, hyperprolactinemia in the adult male rat has been implicated in the development of prostatic hyperplasia of the lateral lobes (40, 42, 51, 52). A dose-response relationship between exogenous prolactinemia and the severity of lateral prostatic inflammation was observed (53). Because hyperprolactinemia or manipulation of PRL can either enhance or suppress humoral and cellular immune responses, inflammation in the lateral lobe may be related to an altered immune function (32, 52). PRL is likely involved because the lateral lobes appear more sensitive than the other 3 lobes to the action of this hormone (6). The majority of spontaneous pituitary gland adenomas, relatively common among F-344 rats, are immunohistochemically reactive for PRL (43). Pituitary gland adenomas of aging rats show mammosotropic or mammotrophotropic functions, and rats with mammosotropic neoplasms have elevated serum PRL activity (9, 15). Although no statistical analysis was performed due to small sample size, our limited immunohistochemical investigation showed that PRL-positive adenomas were the most common finding (13/15 cases) in the pituitary gland. Twelve of these cases were associated with inflammation in the dorsolateral prostatic lobe. In contrast, in 2 of these animals showing 13 adenomas positive for PRL, inflammation of the prostate ventral lobe was noted. The significant relationship between the severity of inflammation in the dorsolateral lobes and the incidence of pituitary gland adenoma and immunohistochemical findings in this study suggests that excess PRL produced by a pituitary gland adenoma may be a predisposing factor for inflammation in the dorsolateral lobes.

Mucinous cysts, a lesion in the dorsolateral lobes in this study, have been referred to as mucoid metaplasia or cystic changes in rat prostate (6). Differentiating between mucinous metaplasia and prostatic carcinoma is important because some histological features of mucinous metaplasia resemble those of low-grade prostatic carcinoma (45). Mucinous metaplasia occurs mainly in perirenal areas of human prostate and is associated with an embryological remnant resembling the prostatic utricle (16). The association of mucinous cysts and inflammation in the dorsolateral lobe in our study suggests that chronic irritation such as inflammation could be a causative factor.

Epithelial degeneration, the most common lesion that we observed in the ventral lobes, had histopathologic features similar to those reported previously (3, 4, 6, 29). The decline of androgenic hormone by castration has been reported to induce prostatic epithelial degeneration, leading to both autophagy and phagocytosis of the epithelial cells by macrophages (4, 29). The frequency of this lesion in the dorsolateral and ventral lobes suggests sensitivity of these lobes to androgen withdrawal (3).

Other histopathological lesions including edema in the dorsolateral and ventral lobes, coagulating gland and seminal vesicle; dilatation in seminal vesicle; and secondary tumors metastasizing in the dorsolateral and ventral lobes, coagulating glands, seminal vesicles, and ampullary glands appear to have unrelated causes (4, 6).
The conclusion from our survey is that greater attention should be given to optimal technical sampling of prostate. Although ventral lobes have, in the past, been collected and embedded separately from the dorsolateral lobes for various reasons, e.g., weighing (7), we recommend that the collection and fixation of prostate, seminal vesicle, and urinary bladder be achieved together as a unit in order to maintain anatomic relationships for histopathological examination. Trimming should be performed to include both dorsolateral and ventral lobes as shown in Figure 15 (5, 7). We recommend that examiners make a midtransverse cut of 2–3 mm in thickness at the mid area of the ventral lobes. This anterior face of the posterior portion of the prostate should correspond to the prostatic region that obscures the base of the urinary bladder. This cut surface is then embedded downward for microscopic examination. We found that transverse sectioning was superior to frontal sectioning for yielding the optimal amount of prostate examined in a single section. Consistent collection of all appropriate regions by proper trimming and embedding is essential to ensure accurate histopathological diagnoses of prostatic lesions.

ACKNOWLEDGMENTS

The authors are grateful to JoAnne Johnson of the National Institute of Environmental Health Sciences; Peter B. Little of Pathology Associates International, Inc; and Sundeep Chandra of Experimental Pathology Laboratories, Inc, for their helpful comments. The authors thank William C. Hall of Pathology Associates International, Inc. for his assistance with immunohistochemical examination and Norris Flagler of the National Institute of Environmental Health Sciences for his assistance with the photography.

REFERENCES


