Models for Development of Nonreceptor Methods for Distinguishing Androgen-sensitive and -insensitive Prostatic Tumors

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ABSTRACT

From the original Dunning R-3327 rat prostatic adenocarcinoma, several distinct sublines have been obtained. These sublines include a well-differentiated, slow-growing, androgen-sensitive tumor (R-3327-H); a well-differentiated, slow-growing, androgen-insensitive tumor (R-3327-HI); and a fast-growing, androgen-insensitive, anaplastic tumor (R-3327-AT). These three sublines were compared in order to develop new model systems for the prediction of the androgen sensitivity and the degree of differentiation of prostatic adenocarcinomas. The R-3327-AT was very distinct in all parameters examined except the tissue protein electrophoretic patterns which contained a uniform pattern in all tumors. The significant differences between R-3327-H and -HI sublines were (a) the inability of testosterone to stimulate DNA synthesis in the R-3327-HI tumor and (b) the difference in the enzymatic profiles of these sublines. The specific activity of three enzymes (3a-hydroxysteroid dehydrogenase, leucine aminopeptidase, lactic dehydrogenase) increased while the activity of another three enzymes (6a,7a-hydroxylase, 5a-reductase, alkaline phosphatase) decreased in the sublines which are androgen insensitive and less differentiated. An arbitrary index was constructed, based upon these enzyme differences, which clearly discriminates the degree of androgen sensitivity and differentiation of these R-3327 rat prostatic adenocarcinomas.

INTRODUCTION

These experiments were undertaken in model systems to evaluate new approaches that might be developed as prognostic indicators in predicting which human prostatic tumors might respond to hormonal therapy. Hormone receptors have been utilized as prognostic indicators in breast cancer and have proven useful, although they have limitations (8). Receptor assay techniques are being developed for androgen receptors in prostatic cancer; however, at present, they have not reached the applicability that has been realized in breast cancer. The Dunning rat prostatic adenocarcinomas offer an opportunity to study these issues because of the many similar properties to that of human prostatic cancer (5, 11).

In 1963, Dunning (2) discovered a spontaneous adenocarcinoma originating in the dorsal lobe of the prostate of an aged syngeneic Copenhagen rat. This tumor has been passaged as a s.c. flank tumor since its original discovery. There are now several distinct sublines from the original tumor. The R-3327-H tumor (hereafter called the H subline), which has been extensively characterized in this laboratory, is a slow-growing, well-differentiated transplantable prostatic adenocarcinoma (5, 11, 14). This subline is composed of both androgen-sensitive and androgen-insensitive cells (5, 11) and possesses the cytoplasmic receptors for DHT (4, 7, 14) and 5aR activity (5, 13). The H subline is therefore routinely passaged in intact adult male rats in order to preserve the androgen-sensitive cells. Passage of the H subline in castrated male rats results in the selective loss of these androgen-sensitive cells with no effect on the proliferative growth of the androgen-insensitive cells. Therefore, by long-term passage of the H subline in castrated male rats, a new, slow-growing, well-differentiated, androgen-insensitive subline has been established and designated the R-3327-HI (hereafter called HI) subline (5). This HI subline contains both cytoplasmic receptor for DHT (4) and 5aR activity (5) but does not respond to testosterone in terms of a stimulation of growth. The HI tumor is therefore routinely passaged in castrated male rats.

A third tumor subline has spontaneously arisen from the R-3327-H tumor (5, 11). This is a rapidly growing, androgen-insensitive, anaplastic tumor termed the R-3327-AT (hereafter called AT) subline. This anaplastic tumor does not contain receptor for DHT (4, 7), nor does it possess 5aR activity (5).

Using these 3 characterized R-3327 tumor sublines, a series of cellular parameters were examined to determine which of these indices could be utilized to predict the androgen sensitivity and the degree of differentiation of the prostatic adenocarcinomas.

MATERIALS AND METHODS

Tumors and Animals. The Dunning H androgen-sensitive prostatic adenocarcinoma was originally obtained from the Mason Research Institute and is carried in Fischer X Copenhagen F1 rats. The development of sublines HI and AT and the methods of passage of these tumors have been described previously (5). Castration, when performed, was via the scrotal route, using ether anesthesia.

Enzyme Assays. Homogenates were made from various tissues with an all-glass conical homogenizer, using 50 mM Tris, pH 7.4, as buffer. Dorso lateral prostate, H, HI, and AT homogenates were assayed for the following enzymatic activities: (a) 5aR; (b) 3aHSD; (c) LAP; (d) LDH; (e) 7a,6a HYD; and (f) ALKP. All assays were performed as outlined previously (5).

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2 To whom requests for reprints should be addressed.

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Testosterone Metabolism. For the determination of the ability of the H, HI, and AT tumors to metabolize testosterone, the following standard format was utilized, based on the method of Glioyna and Wilson (3). Tissues from the dorsolateral prostate, H, HI, and AT tumors were rapidly removed from the animals, immediately placed on ice, and minced with a pair of fine scissors. Aliquots (100 mg) of these various tissue minces were then placed separately in a 25-ml Erlenmeyer flask containing 2.5 ml of Medium 199, containing $[^{3}H]$testosterone (0.48 μM final concentration with 10$^5$ cpm). The flasks were then incubated in a shaking water bath at 37°C in an atmosphere of 95% O$_2$-5% CO$_2$. In addition, zero time control flask was run for each set of specific tissue minces. This was performed by adding 10 ml of chloroform:methanol (2:1) directly to the medium before the addition of the tissue mince. The remaining flasks were then terminated at 15-min intervals by the addition of 10 ml chloroform:methanol (2:1). The entire 12.5-ml mixture from each flask was then separately homogenized with an allglass homogenizer, and the homogenates were centrifuged at 100 × g for 5 min. The chloroform:methanol layers were taken to dryness and redissolved with 50 μl of unlabeled reference steroid solution. This reference solution contained testosterone, DHT, 5α-diol, 4-androstene-3,17-dione, and androstenedione, each at 0.25 mg/ml. The redissolved mixture from each separate flask was then applied to a silica gel plate (Quanta Gram plate, Type LKG6) and developed in a chloroform:methanol (98:2.5) system with 2 ascents. Reference steroids were localized by spraying with methanol:sulfuric acid (70:30) followed by heating at 110°C for 5 min. The reference steroids were marked, the corresponding areas were scraped into scintillation vials, and radioactivity was determined. All areas of the chromatogram were counted. The pmol of various steroids were calculated from the percentage of the total cpm isopolar with a particular reference steroid × the total amount of starting testosterone substrate. The results are expressed as pmol steroid per 100 mg tissue.

Histology. Aliquots of tissue were fixed in buffered formalin and processed for routine light histology. This included staining with hematoxylin and eosin, periodic acid-Schiff reagent, and Masson trichrome stain. Electron microscopy was performed on tissue fixed with 4% glutaraldehyde buffered at pH 7.4 with 0.1 M cacodylic acid, postfixed in 2% osmium tetnoxide buffered at pH 7.4 with 0.1 M cacodylic acid, dehydrated in graded concentrations of ethanol which included 1% uranyl acetate in 25% ethanol for prestaining, and stained with lead citrate.

In Vitro Rate of DNA Synthesis. The measurement of the in vitro rate of DNA synthesis was determined according to the technique of Sufnin and Coffey (12). The in vitro rate of DNA synthesis is expressed as the absolute cpm of $[^{3}H]$thymidine incorporated into DNA per hr per 100 μg DNA following a 1-hr incubation of 100 mg of minced tissue in Medium 199.

Tissue Electrophoresis. Standard SDS-10% polyacrylamide gel electrophoresis was performed on whole homogenates of various tissues by the method of Laemmli (6).

Electrophoretic Separation of the Lysosomal and Secretory Forms of Acid Phosphatase. The gel electrophoretic technique of Rennie et al. (10) was utilized to separate the secretory and lysosomal forms of acid phosphatase of the soluble fraction following 700 × g centrifugation of a tissue homogenate. After electrophoresis, the gels are reacted with a $\alpha$-naphthylphosphate and stained with Fast Garnet GBC. The relative percentages of the secretory and lysosomal acid phosphatase activities were determined by densitometric traces of the gels at 600 nm. The actual specific activities of the lysosomal and secretory forms of acid phosphatase were calculated by multiplying the relative percentage of the activity in each form on the gel by the total specific activity determined on a separate aliquot of whole homogenate using $\rho$-nitrophenylphosphate as substrate (10). The specific activities are expressed as μmol/hr/mg DNA processed.

Material. All nonradioactive substrates for enzyme assays were purchased from Sigma Chemicals St. Louis, Mo. [1,2-3H]-Testosterone (51 Ci/mmole) and [1,2-3H]5α-dihydrotestosterone (51 Ci/mmole) were purchased from Amersham/Searle (Arlington Heights, Ill.) [methyl-3H]-Thymidine (59 Ci/mmole) was purchased from Schwarz/Mann (Orangeburg, N. Y.). Testosterone propionate was obtained from Steraloids (Wilton, N. H.). Medium 199 was purchased from Grand Island Biological Co. (Grand Island, N. Y.). Quanta Gram silica gel plates (LKG6) were purchased from Whatman, Inc. (Clifton, N. J.).

RESULTS

Comparative Morphology of the R-3327 Sublines. The morphology of the H and HI tumors is very similar at the light microscopic level, both sublines being composed of glandular acini filled with PAS-positive secretions (Fig. 1, left column). The anaplastic AT tumor, in contrast, is composed of sheets of cells with no indication of secretory activity. The nuclei or the H and HI tumors are also very similar and are markedly different from the nuclei of the AT tumor (Fig. 1, center column). The AT tumor nuclei are much larger and more pleomorphic than either the H or HI nuclei. The AT tumor nuclei also appear to stain consistently lighter than do the nuclei of the other sublines when processed routinely for hematoxylin and eosin histology. At the electron microscopic level, the H acinar cells have the characteristic appearance of epithelial cells (Fig. 1, right column). These cells possess both microvilli and desmosomes. The electron microscopic appearance of the HI tumor is slightly different. The HI glandular cells have less prominent microvilli. Both the H and HI tumors contain cells which possess large filled vacuoles which approach the size of the nuclei. Frozen sections at the light microscopic level demonstrate that these vacuoles are filled with lipid. These large lipid-filled vacuoles appear to be more numerous in the HI tumor but are also normally found in the H tumor. Electron microscopy of the AT tumor reveals that there are few if any microvilli. Stains for collagen or reticular fibers fail to demonstrate any production of these components by the AT tumor. The AT tumor also does not contain cells which possess the large vacuoles.

Testosterone Metabolism of the R-3327 Sublines. The ability of these three tumor sublines to metabolize testosterone was compared to that of the dorsolateral prostate. This is appropriate since it has been shown that the original R-3327 tumor not only arose in the dorsolateral lobe (2) but also retains a close enzymatic similarity to this tissue of origin (5). The dorsolateral prostate metabolizes testosterone chiefly via the 5αR pathway (Chart 1). The total 5α-reduced products (DHT plus 5α-diols plus androstanediol) are a measure of the 5αR activity which is characteristic of most androgen-sensitive tissues. The main metabolite of testosterone in the dorsolateral....
Tissue Electrophoretic Patterns. SDS-polyacrylamide gel electrophoresis was performed on the proteins of the whole homogenate of a variety of normal tissue as well as the R-3327 tumor sublines (Chart 2). Surprisingly, while the seminal vesicle and ventral and dorsolateral prostate gave unique electrophoretic patterns, all the patterns for R-3327 tumor sublines appeared to be practically identical. To rule out the possibility that the electrophoretic pattern of the R-3327 subline is a general property of all tumors, a spontaneous mammary tumor arising in an F1 female of our breeding colony was also analyzed. The electrophoretic pattern of this mammary tumor was entirely different from the characteristic similar patterns seen with all 3 of the R-3327 tumor sublines.

Content of the Lysosomal and Secretory Forms of Acid Phosphatase. The content of lysosomal and secretory acid phosphatase was determined for the seminal vesicle, liver, the dorsolateral and ventral prostate, and the R-3327 tumor sublines. The separation of these 2 forms of acid phosphatase was achieved by means of polyacrylamide gel electrophoresis. Secretory acid phosphatase is believed to be found specifically only in the prostate gland. The lysosomal form of this enzyme is universally found in all tissues. Indeed, the seminal vesicle and liver possess no secretory acid phosphatase (Table 1). The ventral prostate has the highest level of this secretory form of the enzyme; the dorsolateral prostate also possesses relatively high levels. The H and HI tumors both possess the secretory form of the enzyme, and its activity is approximately equal to that of the dorsolateral prostate of a noncastrated rat. In contrast, the AT tumor does not possess any of the secretory forms of this enzyme.

Effects of Testosterone on the Induction of DNA Synthesis. The effects of daily injections of testosterone propionate on the rate of DNA synthesis of the dorsolateral prostate and the tumor sublines were examined. The rate of prostatic DNA synthesis has been demonstrated to be related to prostatic growth rates (12) and to reflect androgen stimulation (12).
When an intact adult rat is castrated and allowed 7 days without androgenic steroid, the rate of DNA synthesis in the dorsolateral prostate decreases slightly (Chart 3). If, at Day 0 (7 days postcastration), daily injections of testosterone propionate (10 mg/day) are begun and continued, a sharp rise in the rate of DNA synthesis is observed with time, as determined by an in vitro assay (12). The rate of DNA synthesis is maximal at Day 3 and is followed by a return to original base-line levels within 1 week of continued androgen treatment (Chart 3). When an animal bearing an H tumor is castrated and not given an injection of androgenic steroid for 7 days, the rate of DNA synthesis of the H tumor decreases significantly (Chart 3). If, after this 7-day period (Day 0), testosterone propionate (10 mg/day) is injected daily, the rate of DNA synthesis of the H tumor returns within 3 to 4 days to the high level seen in the H tumor borne in an intact animal (Chart 3). There is no overshoot in the rate of DNA synthesis for the H tumor as is seen with the dorsolateral prostate under identical androgen stimulation conditions. Also, the high rate of DNA synthesis in the H tumor remains constant if the steroid injections are continued (Chart 3). This is in direct contrast to the androgen stimulation of DNA synthesis seen in dorsolateral prostate which wears off with time even with continued androgen treatment. In comparison, neither the HI nor the AT tumors respond to exogenous androgen (Chart 3). This is highly significant since, in an earlier study, the HI tumor was found to possess cytoplasmic androgen receptor equal in amount to that of the dorsolateral prostate (4). Therefore, the inability of testosterone to induce a response in the rate of DNA synthesis of the HI tumor is most interesting. The growth rate of the HI tumor, as monitored by its doubling time, is also unaffected by androgen stimulation (data not shown). This may be related to the fact that the rate of DNA synthesis of the HI tumor carried in a castrate animal is essentially identical to that of the H tumor carried in either an intact or testosterone-injected castrated animal; both of these tumors have identical growth rates (21-day doubling time). The AT tumor, however, grows more rapidly (2-day doubling time) (5).

REI. In an earlier study, it was found that 3 enzymes (3aHSD, LAP, and LDH) increased while another 3 enzymes (5aR, 7a,6aHYD, and ALKP) decreased in the R-3327 sublines which were androgen insensitive (5). These enzymatic changes are of value as discriminatory factors, particularly when combined into an arbitrary enzymatic index value. Therefore, normal dorsolateral prostates, H, HI, and AT tumor tissues were obtained from 10 different animals of each group, and these 30 tumor samples were assayed for the specific activities of the enzymes discussed above. Table 2 presents the mean specific activities ± S.E. for each of these individual enzymes. The enzymatic activities in this table are expressed per unit DNA. These specific activities can then be presented as relative values by normalizing each enzymatic activity separately based on the mean activity for that enzyme in the dorsolateral prostate (mean activity of each enzyme in the dorsolateral prostate = 1). The average relative enzymatic activity for each enzyme in the respective tissue groups is given in parentheses in Table 2. These average relative values can be used to construct an arbitrary index termed the REI, according to the formula:

\[
\text{REI} = \frac{\text{Product of relative activities of the 3 enzymes which increase}}{\text{Product of relative activities of the 3 enzymes which decrease}}
\]

\[
= \frac{(3a\text{HSD}) \times (\text{LAP}) \times (\text{LDH})}{(5aR) \times (7a,6a\text{HYD}) \times (\text{ALKP})}
\]

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Secretoy</th>
<th>Lysosomal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;1</td>
<td>182 ± 14 *</td>
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<tr>
<td>Seminal vesicles</td>
<td>&lt;1</td>
<td>65.0 ± 7.3</td>
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<tr>
<td>Ventral prostate</td>
<td>15.5 ± 2.5</td>
<td>70.5 ± 8.9</td>
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<tr>
<td>Dorsolateral prostate</td>
<td>8.24 ± 3.2</td>
<td>74.2 ± 11.4</td>
</tr>
<tr>
<td>Dunning tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>7.2 ± 2.5</td>
<td>28.8 ± 4.2</td>
</tr>
<tr>
<td>HI</td>
<td>8.25 ± 1.7</td>
<td>24.8 ± 3.2</td>
</tr>
<tr>
<td>AT</td>
<td>&lt;1</td>
<td>40 ± 5.6</td>
</tr>
</tbody>
</table>

*Mean ± S.E.
Table 2
Comparative enzymatic activities in tissues

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Dorsolateral prostate</th>
<th>R-3327-H androgen sensitive</th>
<th>R-3327-HT androgen insensitive</th>
<th>R-3327-AT anaplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3aHSD (pmol/hr/100</td>
<td>1228 ± 171 (1.00)</td>
<td>618 ± 129 (0.50)</td>
<td>1267 ± 129 (1.03)</td>
<td>1876 ± 246 (1.53)</td>
</tr>
<tr>
<td>µg DNA)</td>
<td>7.14 ± 0.86 (1.00)</td>
<td>4.29 ± 0.16 (0.60)</td>
<td>8.71 ± 3.39 (1.22)</td>
<td>8.19 ± 0.99 (1.15)</td>
</tr>
<tr>
<td>LAP (nmol/min/100</td>
<td>2071 ± 143 (1.00)</td>
<td>1798 ± 189 (0.86)</td>
<td>2954 ± 265 (1.43)</td>
<td>4485 ± 370 (2.16)</td>
</tr>
<tr>
<td>µg DNA)</td>
<td>17.0 ± 5.4 (0.53)</td>
<td>12.7 ± 3.16 (0.39)</td>
<td>1.3 ± 0.5 (0.04)</td>
<td>1.3 ± 0.5 (0.04)</td>
</tr>
<tr>
<td>LDH (nmol/min/100</td>
<td>32.2 ± 10.9 (1.00)</td>
<td>180 ± 60 (0.72)</td>
<td>100 ± 35 (0.40)</td>
<td>90 ± 15 (0.36)</td>
</tr>
<tr>
<td>µg DNA)</td>
<td>197 ± 3.15 (0.76)</td>
<td>74 ± 12.5 (0.28)</td>
<td>250 ± 100 (1.00)</td>
<td>20 ± 8.5 (0.08)</td>
</tr>
<tr>
<td>5aR (pmol/hr/100</td>
<td>250 ± 100 (1.00)</td>
<td>197 ± 3.15 (0.76)</td>
<td>258 ± 13.6 (1.00)</td>
<td></td>
</tr>
<tr>
<td>µg DNA)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
<td></td>
</tr>
<tr>
<td>7a,6a-Hyd (pmol/min/</td>
<td>250 ± 100 (1.00)</td>
<td>197 ± 3.15 (0.76)</td>
<td>258 ± 13.6 (1.00)</td>
<td></td>
</tr>
<tr>
<td>100 µg DNA)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
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</tr>
<tr>
<td>ALKP (nmol/min/</td>
<td>250 ± 100 (1.00)</td>
<td>197 ± 3.15 (0.76)</td>
<td>258 ± 13.6 (1.00)</td>
<td></td>
</tr>
<tr>
<td>100 µg DNA)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
<td>258 ± 13.6 (1.00)</td>
<td></td>
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*a Mean ± S.E.
*b Numbers in parentheses, relative values.

Table 3
Determination of the REI

| Tissue analyzed | Growth rate | Androgen response | Histological differentiation | Equation | Av. REI
|-----------------|-------------|-------------------|-----------------------------|----------|--------|
| Normal dorsolateral prostate | Very slow | Sensitive | Well | (1.0) (1.0) (1.0) | 1.00 (0.8-3)
| R-3327-H tumor | Slow | Sensitive | Well | (1.53) (1.15) (1.15) | 41.4 (26-110)
| R-3327-HT tumor | Slow | Insensitive | Poor anaplastic | (0.04) (0.36) (0.08) | 2533.3 (2337-9260)
| R-3327-AT tumor | Fast | Insensitive | Poor anaplastic | (0.04) (0.36) (0.08) | 2533.3 (2337-9260)

a Product of REI:

\[ \text{REI} = \frac{(3aHSD) (LAP) (LDH)}{(5aR) (7a,6a-Hyd) (ALKP)} \]

b Average equation values from 10 determinations.

c Numbers in parentheses, range of individual values.

The average REI for each tissue as a group was determined, based on the average relative values of each of the respective enzymes for that particular group (Table 3). The individual REI values for each individual sample of a tissue group (N = 10) was determined based on the actual relative enzymatic values that each separate sample possessed. These individual REI values are presented as a scattergram in Chart 4. Using the REI as a discriminatory factor, one can clearly separate the androgen-sensitive (H) and -insensitive (HI) tumors. In making the proper classification of the 30 tumors from the REI values, we encountered no overlap of values that would mistakenly produce misclassification of the tumors.

**DISCUSSION**

In an attempt to develop new indices for predicting which prostatic cancers will respond to hormonal therapy, we have compared several possible approaches. These approaches have been tested for their abilities to discriminate between tumors of known androgen sensitivity, utilizing the well-characterized Dunning R-3327 rat prostatic adenocarcinoma sublines as models. The results obtained are shown in Table 4. It is clear from these results that histological examination can only distinguish between the well-differentiated (H and HI) and anaplastic (AT). This type of examination is totally unable to distinguish between the androgen-sensitive H and -insensitive HI tumors. It is possible that, with the more sophisticated morphometric techniques (1) now available, the content of large lipid vacuoles and relative amounts of microvilli might...
be of some predictive value in discriminating between the androgen-sensitive and -insensitive well-differentiated tumors. It remains to be documented whether the relative loss of microvilli or the increase in large lipid vacuoles seen in the HI tumor as compared to the H is a general process.

The ability of the various tumor sublines to metabolize testosterone was not an effective discriminator. The well-differentiated androgen-insensitive HI tumor was able to metabolize testosterone equally as well as the H tumor. It would therefore appear that the 5aR activity may not be a good index of androgen sensitivity in this model. The presence of secretory acid phosphatase also was not predictive of androgen sensitivity. The presence of the secretory acid phosphatase activity, however, does demonstrate again the prostatic origin of the Dunning tumors. The fact that the anaplastic tumor does not contain the secretory form of the enzyme indicates a degree of dedifferentiation resulting in the loss of this marker. Other methods are needed to establish the prostatic origin of this AT tumor. This is accomplished through the study of the tissue protein electrophoretic profiles, which clearly demonstrate that the AT tumor is uniquely related to the H and HI tumors.

The type of SDS-gel electrophoresis performed tends to reveal mainly structural components of tissue, since only these components are of sufficient quantity to be detected. Most enzymes are at too low a total protein concentration to be stained individually on gels. The fact that each tissue examined possessed a unique protein profile is thus not surprising when this is taken into consideration. Thus, even though the well-differentiated H and HI tumors have such a different histology from the anaplastic AT tumor, their protein profiles are all virtually identical. Also, since the H and HI tumor are clearly of prostatic origin, the assumption that the AT tumor is also a prostatic tumor appears reasonable, especially since it arose spontaneously from the H subline.

The ability of testosterone to selectively stimulate the DNA synthesis of the H tumor demonstrates once again the androgen sensitivity of this subline. This may represent the primary requirement for androgen in the growth cycle of the H tumor. The DNA synthesis of neither the HI tumor nor AT tumor was stimulated by exogenous testosterone. The lack of androgen receptor in the AT tumor could explain why this tumor does not respond to androgens (7). This rationale is, however, unsatisfactory for the explanation of the androgen insensitivity of HI tumor. This tumor possesses androgen receptor equal in amount and $K_d$ with that of the normal androgen-sensitive dorsolateral prostate (4). Since the rate of DNA synthesis has been shown to be related to prostatic growth rates (12), androgen stimulation of DNA synthesis may be a more sensitive predictor of androgen sensitivity than receptors per se. The fact that the HI tumor would be falsely classified as androgen sensitive on the basis of its androgen receptor content points to the requirement that receptor content must be correlated with some type of growth response in order to be predictive. Testosterone stimulation of DNA synthesis would thus appear to be an excellent alternative method to androgen receptors for discriminating androgen-sensitive and -insensitive tumors. However, the limitations of this method as applied to human tumors are obvious. It is conceivable that a biopsy specimen from a human prostatic tumor might be either maintained in organ culture or grown in a nude mouse and that the effects of testosterone on DNA synthesis determined; however, this has not yet been accomplished.

The enzymatic profile appears to offer an alternative as a prognostic indicator. The REI has been tested with this model system and appears highly reliable in distinguishing the androgen sensitivity of these tumors. It is important to note that the 6 enzymes were chosen on a pragmatic basis related to the normal tissue of origin, in this case the normal adult rat dorsolateral prostate. By definition, the low values approaching one would indicate relatedness in enzymatic profile to the androgen-stimulated (intact adult) rat dorsolateral prostate. It appears that the more closely related a tumor is to the adult dorsolateral prostate, the higher is the probability that the tumor is androgen sensitive. The REI also appears to discriminate the degree of differentiation of a tumor. The more closely related to the differentiated state of the tissue of origin, the more a tumor is differentiated on a biochemical basis. Using the REI as a discriminator then, the H tumor is well differentiated, the HI tumor is less differentiated, and the AT tumor is anaplastic. It is important to note that differentiation is determined here by function as related through enzymatic profiles and not histology. In this case, however, the histology agrees completely with these interpretations.

One of the advantages of the REI is that this assay method could be scaled down and applied to needle biopsy samples and multiple assays could be performed rapidly and with a high degree of reliability.

The present study did not consider the use of steroid recep-
tor assays which might also prove useful in discriminating androgen-sensitive prostatic cancer; these studies are under way in several other laboratories (9). It is conceivable that the REI and steroid receptor assays might be used in concert to increase the discriminatory value of any prognostic indicators.

REFERENCES

Fig. 1. Histology of the R-3327 tumor sublines. Top, H; middle, Ht; bottom, AT. Left, × 60; center, × 410; right, × 6300.
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