Plasminogen Activator Content and Secretion in Explants of Neoplastic and Benign Human Prostate Tissues


ABSTRACT

The plasminogen activators of surgically excised prostate cancers (43 specimens) and benign prostatic hyperplasias (33 specimens) were extracted with an acetate:arginine:detergent buffer, and the activities were quantitated with azocaseinolytic tests. Immunoinhibition with anti-urokinase antibody served to distinguish between activator types. The mean activator activities (total; urokinase type; and nonurokinase type) of the neoplastic group were about 2 times higher (p < 0.05) than that of the benign prostate hyperplasia tissues. Each group had more nonurokinase-type activator activity relative to urokinase type. Studies with autopsies tissues (13 specimens) revealed that different anatomical compartments of the prostate contain about the same mean activator activity, indicating that the site of origin of the disease did not influence the results. Immunoperoxidase staining for urokinase revealed its presence in the tumor cells and, to a lesser extent, in the epithelial elements of some benign ducts and glands but not in the connective tissue. The secretion and synthesis of activator activities were monitored in short-term (~120 hr) organ cultures (serum-free media) of 21 neoplastic neoplastic tumor explants cultured in serum-free medium, and these activators were consistently secreted and synthesized by prostatic tumors is significantly higher than that of BPH. Plasminogen activator activity has been introduced (4). This paper contains results with 10 benign prostatic hyperplasia specimens. Wide individual variations were present in both groups (1.5 to 322 times more activity than the initial values). Except for one case, urokinase-type activity was secreted continuously, while nonurokinase was secreted only initially in quantities similar to that present in preculture tissue extracts. After culture, tissue explants contained higher quantities of both activator activities than were present initially. Dexamethasone (10 or 100 μM) decreased secretion and/or synthesis of activators by about 70%. This human organ culture model appears to be a reproducible system for individual tissues and may prove to be a valuable tool for further studies.

INTRODUCTION

Plasminogen activators are essential enzymes in physiological hemostatic processes. Basic studies of in vivo and in vitro systems have shown that the levels of fibrinolytic plasminogen activator activities in normal cells or tissues may vary according to hormonally controlled cycles of tissue function and also that malignant transformation of cells can induce the synthesis and release of these enzymes (2, 18, 26, 27). We are currently involved in studies to quantitate and characterize the plasminogen activator activities present in surgically excised malignant tissues. There are 2 immunologically distinct types of human activators, the urokinase (EC 3.4.99.26)-type and the nonurokinase-type activator, sometimes called tissue or vascular activator. The latter has been shown to have augmented activator activity when assayed (or visualized) in one-stage fibrin tests (3). Because we have quantitated activator activities with a nonfibrinolytic assay system, it has been possible to avoid some of the complications which arise in fibrinolytic systems when different ratios of the 2 classes of activators are present in the tissues of interest. In 4 different types of tissues examined to date (lung, colon, breast, and prostate), the mean enzyme activities of the cancer specimens were found to be significantly higher than those of the benign tissues (3, 7, 10, 20).

The study of surgically excised benign and neoplastic prostatic tissues has been expanded to include in vitro organ culture experiments in order to quantitate the secretion and synthesis of plasminogen activator activities. Unlike cell cultures, organ cultures may permit biochemical studies while retaining properties such as tissue organization and heterogeneity present in the original tissue. These experiments may serve as models to assist in identifying mechanisms which control cell life or function, and they may be useful for surveillance or control of the disease. Prostatic tumors are usually intermingled with normal tissue. It is not feasible to separate the normal and tumor portions of the tissue from these patients as was possible with other tissues studied. Other types of studies using prostatic explant models have been reviewed (5, 15, 22, 29). Since the time of our original investigation (3), a more efficient extradant for plasminogen activator activity has been introduced (4). This paper contains those results.

The studies in this paper confirm our earlier results and show that the mean plasminogen activator activity of prostatic carcinomas is significantly higher than that of BPH. Plasminogen activators were consistently secreted and synthesized by prostatic tumor explants cultured in serum-free medium, and these processes were quantitated in both the absence and the presence of dexamethasone, an inhibitor of these functions.

MATERIALS AND METHODS

Prostate Tissues. Surgical specimens were obtained from this institute (total, 37) as well as from 3 local hospitals (20 specimens from Our Lady of Victory Hospital, courtesy of Dr. J. P. Grimaldi; 3 specimens from Millard Fillmore Hospital, courtesy of Dr. P. A. Greco; and 2 specimens from Roswell Park Memorial Institute; PAP, peroxidase; TPA, tissue activator; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CTA, Committee on Thrombolytic Activity.)

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4 The abbreviations used are: BPH, benign prostatic hyperplasia; RPMI, Roswell Park Memorial Institute; PAP, peroxidase; antiperoxidase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CTA, Committee on Thrombolytic Activity.
specimens from Buffalo General Hospital, courtesy of Dr. B. T. Mainl and from the National Prostatic Cancer Project Tissue Bank, Miami, Fla. (14 specimens, courtesy of Dr. T. I. Malinin, Jackson Memorial Hospital). Tissues to be placed in organ culture were collected aseptically and stored at 4–10°C for up to 5 hr; other tissues were stored at −85°C. The majority of tissues were extracted for enzyme studies within 3 weeks. No infiltrates of macrophages, lymphocytes, or plasmacytes were noted in the pathology reports.

Autopsy tissue which was used to quantitate enzymes in different zones of benign prostates (total, 13) was excised less than 12 hr after death. A piece of tissue was taken from the “central” periurethral zone close to the ejaculatory ducts at the level of the verumontanum or above; it probably included the “transition zone” as described by McNeal (21). A piece of tissue was also taken from the “periipheral” zone, from the outer parts of the lateral and/or posterior lobe at the mid-level of the prostate. Immediately adjacent, matched tissues were fixed for histological studies.

Organ Culture. Organ culture techniques were similar to those reported by others (12, 34, 37). Specimens were rinsed in RPMI Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.) and cut into ~1-cm fragments. Pieces from each section of the specimen were used for both cultures and extraction, so that, as far as it was possible, each contained the same heterogeneity as may have been present in the intact specimen. Usually, 8 to 10 pieces (total weight, 10 to 30 mg) were supported at the surface of 1 ml RPMI Tissue Culture Medium 1640 in a culture dish with a surface area of ~7 sq cm. The dish was a polyethylene cap of ~22 mm diameter and an inner raised ridge of ~19 mm diameter. The interface support was siliconized lens paper or a 20-mm-diameter disposable disc cut from 1000-Mm mesh polypropylene monofilament screen cloth (Small Parts, Inc., Miami, Fla.). RPMI Tissue Culture Medium 1640 always contained 100 units of penicillin and 100 μg of streptomycin (Grand Island Biological Solution 600-5140) per ml but no fetal calf serum. Cultures were maintained at 37°C in a 10-liter closed system flushed with a 5% CO2:95% air atmosphere from a line bubbled through water at about 2 liters/min. Media were cycled and replaced about every 24 hr for 4 to 6 days. When cultures were terminated, the tissue fragments were blotted on paper (Whatman No. 3MM) and weighed. Depending on the quantity of tissue available, from 2 to 4 culture dishes were set up for each control or variable to be examined. Tissue fragments from one of the cancer specimens were removed from culture after 2 days and 4 days. These were subsequently sectioned and examined after staining with hematoxylin:eosin. After the second day of culture, the nuclei of cancer cells were well stained with hematoxylin. The nuclear edge was sharp, and the nucleoli were obvious in most of these nuclei; however, mild perinuclear vacuolar changes and slight karyorrhexis were observed in some parts. After the fourth day, nuclear configuration and staining of the cancer cells were still retained in approximately one-half of the cancerous glands, and an increase in the nuclear debris in the basal area of these cancer cells was observed. From these morphological findings, it was concluded that, even though there was evidence of progressive deterioration, the cancer tissue was still viable after 4 days in culture. Dexamethasone, where used, was Decadron, marked 24 mg per ml dexamethasone phosphate equivalent (Merck Sharp and Dohme, West Point, Pa.). Cycloheximide was from Sigma Chemical Co.

Immunohistochemical Staining. The PAP method was carried out according to Sternberger (31) and Taylor (33) with some modifications. Surgically resected prostatic tissues were fixed with formalin:0.9% NaCl solution and embedded in paraffin. These tissues were cut into 4-μm thick sections and mounted in glass slides. After deparaffinization with xylol and alcohol, endogenous peroxidase activity was blocked with pure methanol containing 0.3% hydrogen peroxide for 30 min. Subsequently, these sections were rinsed in distilled water and places in 0.05 M Tris:0.1 M NaCl, pH 7.6. Specimens were incubated with the primary antiserum, anti-human urokinase goat IgG (10), diluted 1:80 with 0.05 M Tris:0.1 M NaCl containing 1% rabbit serum at 37°C for 60 min in a moist chamber. After sufficient washing with 3 changes, each 300 ml 0.05 M Tris:0.1 M NaCl, specimens were incubated with the secondary antiserum (antigoat rabbit IgG; Accurate Chemicals, Westbury, N. Y.; diluted 1:20 with normal 0.05 M Tris:0.1 M NaCl) at 37°C for 30 min, followed by 3 washings, as before, and then incubated with PAP (DAKO; Accurate Chemicals; diluted 1:100 with 0.05 M Tris:0.1 M NaCl containing 1% rabbit serum) at 37°C for 30 min and washed. Antibody localization was determined by detection of peroxidase activity by incubation with freshly prepared 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.) and 0.01% hydrogen peroxide in 0.05 M Tris:0.1 M NaCl for 10 min at room temperature. These specimens were rinsed in distilled water, counterstained lightly with hematoxylin, dehydrated, and mounted in Permount. Controls for the PAP methods were carried out using the nonimmune goat IgG in place of primary antiserum.

Enzyme Assays and Other Tests. Plasminogen activators were extracted from the tissues using the acetate:detergent buffer described recently composed of 0.075 M potassium acetate, 0.3 M NaCl, 0.1 M L-arginine, 0.01 M EDTA, and 0.25% Triton X-100, pH 4.2 (4). Buffer volumes from 1.8 to 5.0 ml were used with the Tekmar Tissumixer and Microprobe, and sample:buffer dilution varied from about 1:10 to 1:200 depending on the quantity of tissue available. Tissue extracts from 2 or more organ culture dishes were combined for the postculture extractions. The homogenates were centrifuged for 15 min at 3000 × g, and the supernatants were assayed. Plasminogen activator activities were determined by azocaseinolysis with human plasminogen added as reported in detail (20). The results for activator activity were corrected for the presence of plasminogen-independent proteases in the extracts by subtraction of absorbances obtained in an identical system to which no plasminogen was added. This correction was usually less than 5% of total activity. Abbokinase (kindly supplied by Dr. E. L. Platcow, Abbott Laboratories, North Chicago, Ill.) was used as the urokinase standard. The percentage of urokinase-like activator was a measure of the plasminogen activator activity that could be inhibited with purified goat anti-urokinase antibody (10). Because crude extracts of both human benign hyperplastic prostate tissues and metastatic lymph nodes have been shown to contain proteolytic inhibitory activity (8), we monitored this activity in the tissue extracts we have studied. Inhibitory activity against trypsin was measured as before using the azocaseinolytic assay (3). As found before, the trypsin-inhibitory activity did not inhibit urokinase activator activity or plasmin in our assays. Culture media were tested between 25 and 200 μl. Negligible protease and trypsin-inhibitory activity were found in the media. Protein was determined according to the method of Lowry et al. as reported earlier (3), except that SDS (0.5 ml of 10% SDS for each 1.15 ml of sample:alkaline copper mixture) was used to prevent the formation of precipitates in the presence of Triton X-100 as reported by Wang and Smith (36). A similar wide variation was found in the protein content of both the benign and cancer tissue extracts. Studies with a few of the tissues revealed that the buffer present during mincing of the tissue before homogenization in the presence of extraction buffer contained over 50% of the soluble protein recovered from the tissue and less than 10% of the activator activity. Since this was a variable which could not be controlled, all results of activator activities are reported in units per g of wet tissue (rather than mg of protein). SDS:PAGE and zymography were described previously (10) using 8.5% acrylamide and copolymerization with (a) casein (1 mg/ml) for identification of protease migration; (b) casein plus human plasminogen and goat anti-human urokinase antibodies (~50 μg/ml) to view plasminogen activator and protease activity; or (c) casein, plasminogen, and goat anti-human urokinase antibodies (~50 μg/ml) to locate the urokinase-type activator activity.

RESULTS

Activator Content of Benign and Neoplastic Prostatic Tissues. The results obtained for the surgical specimens of BPH (33 patients) and carcinoma tissue (43 patients) are shown (Table

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1; Chart 1). As in the previous series (3) when a less efficient solubilizing extraction buffer was used, these results show that the prostatic cancers have significantly higher mean plasminogen activator content than do the benign tissues (9.3 CTA units/g versus 4.8). There was a wider range of activator levels for the neoplastic group (0.8 to 61 CTA units/g) than for the benign (1.1 to 15.2 CTA units/g), and as before, there was no apparent correlation of activator content with the grade or type of staging reported for the patient carcinoma tissues. As shown in Chart 1, 26% (11 of 43) of the cancer tissues had activator contents greater than the mean of 9.3 CTA units/g, while only 6% (2 of 33) of the BPH tissues were above this value. One of these 2 BPH patients (age, 61 years; activator, 10.3 CTA units/g; 74% urokinase type; 119-hr secretion in culture, 186 CTA units/g) was diagnosed as having transitional-cell carcinoma of the bladder (Grade III, invasive) and expired 6 months after surgery (radical cystectomy) from which the BPH prostate specimen was obtained for assays. The second patient (age, 84 years; activator, 15.2 CTA units/g; 19% urokinase type; 140-hr secretion in culture, 33 CTA units/g) was diagnosed as having diverticulitis 16 months after transurethral resection of the prostate gland at which time the assay was made. About 60% of the benign group had activator content less than the mean of 4.8 CTA units/g, while only ~40% of the neoplastic tissues were below that value. The mean values of both urokinase type of activator activity and nonurokinase type were each significantly higher in the cancer than in the BPH groups (Table 1). Each group had more nonurokinase-type activator activity than urokinase type. The mean inhibitory activity as measured by inhibition of trypsin (not shown) was 24 ± 11% (n = 26) and 18 ± 11% (n = 36) for BPH and cancer specimens, respectively, with similar wide ranges of 0 to 52% for both series. These means are about 40% lower than those obtained in the original study, due possibly, as has already been noted (4), to inactivation of the inhibitory activity by the new acid extractant. Such inactivation was indeed found when comparisons with pH 7.8 buffer were made on the same individual tissue specimens. Unlike trypsin, plasmin and urokinase activities were not found to be inhibited in azocaseinolytic assays. Fig. 1 shows an example of zymography results with proteins separated by SDS-PAGE and visualized by proteolytic activities in casein substrate copolymerized with the acrylamide matrix. The lysed zones remain clear when the gel is stained for protein with Coomassie blue. The extract of a neoplastic prostate tissue is shown in Fig. 1, Lanes 3 to 5; Lane 3 shows total activator and protease activities, Lane 4 shows the same after inhibition of urokinase by inclusion of antiurokinase antibody, and Lane 5 shows only protease activity due to omission of plasminogen (Lane 5 was incubated for a longer period of time to emphasize any proteolytic bands). Lane 2 shows a mixture of M, 55,000 and 33,000 standard urokinase activators, and Lane 1 shows these urokinase standards mixed with the tissue extract. Lanes 6 to 8 show tissue extract of the same specimen after organ culture (see below), where the presence of a high-molecular-weight-activity (~100,000) urokinase-type activator can also be observed. Lane 9 contains 3 variants of M, ~90,000 plasmin, included to confirm the molecular weights of the Bio-Rad standards indicated in the left margin. These zymography data are not intended as rigorous studies of activator molecular weights but rather to show that more than one activator type is present as also indicated by the percentage of urokinase values obtained in azocaseinolyis in the presence of antibodies to urokinase. We note here that rigorous studies of molecular weights using gel electrophoresis require great care, since we have seen changes in molecular weight distribution depending on sample shelf life, freeze-thaw cycling, and solvent-exchange techniques. These changes were observed for the most part where no apparent changes in azocaseinolytic activator activities were obtained. We have not isolated or identified the proteolytic activity, although 2 different types of "direct proteolytic activities" have been reported for these tissues, including one with a molecular weight ≥800,000 (8). Fig. 2 shows the results of the immunocytochemical technique (PAP) used to visualize the urokinase-type activator. The reaction for urokinase was positive in many cancer cells; however, in some areas of fairly well-differentiated glandular pattern, the reaction was either weak or limited to the basement membrane. Positive reaction was also observed in normal glandular epithelium, especially in the dilated glands or dilated ducts. Fibromuscular stroma and vascular wall showed negative reaction. This study is continuing and will be expanded to include the nonurokinase reactions as sufficient antibody becomes available.

Activator Content of Central and Peripheral Zones of the Prostate. This study was made to determine if different com-

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**Table 1**

Comparison of the quantity and type of plasminogen activators in extracts of BPH and neoplastic human prostates

<table>
<thead>
<tr>
<th>CTA units/g tissue</th>
<th>Neoplastic activator</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH</td>
<td>Neoplastic</td>
</tr>
<tr>
<td>Activator</td>
<td>BPH</td>
</tr>
<tr>
<td>4.8 ± 3.2</td>
<td>9.3 ± 11.9 (43)</td>
</tr>
<tr>
<td>Urokinase-type activator</td>
<td>1.5 ± 1.4 (33)</td>
</tr>
<tr>
<td>Nonurokinase-type activator</td>
<td>3.4 ± 2.6 (33)</td>
</tr>
<tr>
<td>Activator</td>
<td>33 ± 20</td>
</tr>
</tbody>
</table>

*a* Student's t test for the significance of the means between 2 groups.

*b* Mean ± S.D.

*c* Numbers in parentheses, number of specimens.

*d* Eighty-two percent of the BPH specimens and 60% of the neoplastic specimens had a lower ratio of urokinase-type activator relative to nonurokinase type (percentage of urokinase < 50%).

*e* Percentage of urokinase type.

*f* NS, not significant.

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**Chart 1.** The distribution of prostate specimens according to plasminogen activator activity ranges. There were 33 benign and 43 cancer tissues in these groups, and the means are shown in Table 1.
departments or zones of benign prostate have different quantities of plasminogen activator activities. It is known that BPH and cancer originate in different zones of the prostate, and these regions, as described by McNeal (21), were the focus of this study. The results in Table 2, using benign autopsy tissue, show that no obvious differences in activator activity can be observed in the central (periurethral) zone where BPH originates and the peripheral zone where cancer originates. The average age of these patients was 50 ± 15 (n = 13; range, 24 to 75), somewhat lower than the BPH and cancer groups of Table 1, which gave means of 68 ± 10 (n = 30; range, 52 to 94) and 64 ± 6 (n = 41; range, 49 to 77), respectively. In a shorter series (4 specimen pairs), fibrin augmentation factors (3) were also calculated and found to be similar for the tissues of the central and peripheral zone (mean ± S.D. for central and peripheral, respectively: azocaseinolysis, 4.15 ± 2.0 and 6.0 ± 2.8 CTA units/g; percentage of urokinase, 29 and 30; fibrin plate lysis zone assay, 28.2 ± 10.0 and 40 ± 10 CTA units/g; fibrin augmentation factor, 7.4 ± 2.3 and 7.05 ± 1.6). In this study of autopsy tissue, 2 unsuspected prostate cancer tissues (not included in the data shown in Table 2) were received. One prostate had plasminogen activator activity of 10.5 and 13.3 CTA units/g in the central and peripheral regions, and the other had 10.0 and 8.7 CTA units/g, respectively, in these zones. Thus, both prostates had higher activities than the average shown in Table 2, and the higher activities were present in both compartments (periurethral and peripheral) tested.

Organ Culture Studies. Subpopulations of the BPH and cancer tissues shown in Table 1 were placed in organ culture, and plasminogen activator activities were measured in both the daily changes of culture media and in the tissue after termination of culture (4 to 6 days). The results are summarized in Table 3 which shows that, although the mean plasminogen activator activities of the preculture benign and neoplastic specimens are in agreement with those shown for the much larger group of Table 1, the difference between the means in this small group (n = 21 for cancer and n = 10 for benign) has not reached significance (p > 0.05). There was a significant difference, however, in the mean values of the urokinase-type activator activity of the 2 groups (p < 0.02). All tissues placed in culture secreted activator into the medium (see Chart 2) and produced activator in the explants, so that the total enzyme activity was always greater than that measured in the original tissue. The total amount of activator activity produced during culture relative to that present in the original tissue varied widely (range of ratios for all the tissues shown in Table 3, 1.5 to 322), and there are apparently no correlations between these ratios and activator activities of the preculture tissues or the type of staging of the tumors. The data will be reviewed over the next few years to see whether they could be related to useful clinical information.

In a separate study, it was found that the secretion of plasminogen activator by colon tumors in short-term organ culture tended to be higher if the tumors were of the infiltrative type (19).

With the exception of one cancer specimen (poorly differentiated adenocarcinoma; original tissue, 21.8 CTA units/g; 14% urokinase type), all tissues secreted essentially urokinase-type activator (>97% urokinase activity at 4 to 6 days in culture) into the medium after the first or second medium exchange. Studies of the earlier specimens placed in culture during the course of this study showed that essentially all the nonurokinase-type activator activity present in the original tissue could be recovered in the RPMI medium in less than 48 hr of culture, and even though additional activator activity of this type was produced in the tissue, no additional amount was secreted. In some cases, this was confirmed with SDS:PAGE and zymography to ensure that the results were not the consequence of a specific activator inhibitor (8, 9, 17, 24), the presence of which could have been revealed after dissociation in SDS. Neither zymography nor mixing experiments with other nonurokinase activators have indicated that inhibitors were present, so we remain confident that only urokinase activators were secreted continuously by these tissues (with one exception as noted above), even though additional quantities of both types of activators were made in the tissues (Table 3). There were no obvious differences in the behavior of the BPH and neoplastic prostate tissues in organ

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### Table 2

Comparison of the plasminogen activator content in extracts of the central (periurethral) and peripheral zones of benign autopsy prostates.

<table>
<thead>
<tr>
<th>Zones</th>
<th>Activator activity (CTA units/g tissue)</th>
<th>Peripheral/central activator activity ratio</th>
<th>% of urokinase-type activator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>5.5 ± 2.6 (1.4-9.1)</td>
<td>1.1 ± 0.6 (0.4-2.3)</td>
<td>28 ± 10</td>
</tr>
<tr>
<td>Peripheral</td>
<td>5.2 ± 2.8 (1.7-12.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± S.D.

b Numbers in parentheses, range of activity.

### Table 3

Comparison of plasminogen activator activities of benign and neoplastic prostate tumors before, during, and after short-term organ culture.

Surgical specimens were placed in short-term organ culture as described in "Materials and Methods." Culture media were exchanged every day and stored frozen until assayed. Original tissue specimens as well as those recovered at termination of organ culture were extracted. Azocaseinolytic assays were used as described in the legend to Table 1.

<table>
<thead>
<tr>
<th>Activity (CTA units/g)</th>
<th>BPH (10 specimens; 115 ± 31 hr in culture)</th>
<th>Neoplastic (21 specimens; 120 ± 23 hr in culture)</th>
<th>Neoplastic:BPH ratio of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original tissue</td>
<td>5.6 ± 2.6*</td>
<td>10.9 ± 13.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Culture media</td>
<td>60.6 ± 100.1</td>
<td>86.3 ± 96.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Postculture tissue</td>
<td>43.5 ± 55.0</td>
<td>47.1 ± 48.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Total in media and postculture tissue</td>
<td>104 ± 116*</td>
<td>135 ± 134*</td>
<td>1.3</td>
</tr>
<tr>
<td>Urokinase activator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original tissue</td>
<td>1.7 ± 0.9</td>
<td>3.2 ± 2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Culture media</td>
<td>56.8 ± 100.9</td>
<td>79.6 ± 90.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Postculture tissue</td>
<td>34.0 ± 45.1*</td>
<td>31.8 ± 33.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Total in media and postculture tissue</td>
<td>91 ± 113*</td>
<td>110 ± 112*</td>
<td>1.2</td>
</tr>
<tr>
<td>Nonurokinase activator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original tissue</td>
<td>3.9 ± 2.7</td>
<td>7.7 ± 12</td>
<td>2</td>
</tr>
<tr>
<td>Postculture tissue</td>
<td>9.5 ± 10.3</td>
<td>15.4 ± 19.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Total in media and postculture tissue</td>
<td>13 ± 10.6</td>
<td>23 ± 29.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

b These means are significantly different (p < 0.05) from the means for the original tissue in the given group.

c Total amount of activator secreted during culture per g tissue placed into culture.

d Experience with the early cultures in the series showed that the nonurokinase-type activator activity of the preculture tissue was secreted in culture in <48 hr. With the exception of one neoplastic tissue (see text), no additional measurable quantities of this activator were secreted.
culture, but a larger number of specimens (particularly BPH) may be needed to confirm this. Some patterns of activator secretion are shown in Chart 2 for a BPH specimen (a) and cancer tissues (b to d). In only ~25% of the secretion experiments with either type of tissue were maximum slopes (i.e., rates of secretion of activator activity expressed as CTA units per g tissue per hr) confined to the first day in culture.

In order to verify that the total increase in plasminogen activator activity obtained during organ culture was due to de novo synthesis of activator, 3 specimens (2 neoplastic, one BPH) were cultured in the absence and presence of cycloheximide, a protein synthesis inhibitor. The compound, added to the culture medium at a concentration of 10 μg/ml, caused a 73 to 90% decrease in the total activator activities relative to those obtained in the unmodified cultures. With one of the specimens, the cycloheximide treatment was stopped after 2 days of culture; in this case, the rate of activator secretion, which had dropped to 23% of its first-day level, remained the same after the third day but returned to its original higher value during the fourth and fifth days in culture. The overall pattern suggested a reversal of the protein inhibition with the removal of the inhibitor.

When RPMI culture medium was supplemented with the synthetic glucocorticoid dexamethasone (10 or 100 μM), the total activator activity in organ culture was consistently decreased in the tissue populations examined. Subpopulations (15 neoplastic and 6 benign tissues) of the groups shown in Table 3 were studied with this modification, and the results can be seen in Table 4 and Chart 2. From the means of the individual ratios (Table 4), an approximate 70% decrease in the total plasminogen activator secreted in medium and produced in tissue by either the cancer or benign specimens was achieved (range of the ratios for cancer tissues, 0.09 to 0.61; that for BPH, 0.12 to 0.49). Attempts as above to determine if the presence of inhibitors could account for the decreased quantities of plasminogen activator secreted and produced in the dexamethasone environment failed to show any evidence for this. Such an inhibition has been reported for some cell culture systems (6, 11). It should be noted that, for each of the 21 specimens of Table 4, the decrease in plasminogen activator with dexamethasone was observed by measuring either the amount secreted or the total activator in tissue after organ culture (4 to 6 days). Furthermore, dexamethasone inhibited both urokinase and nonurokinase activator activity produced in tissue during culture (Table 4). Since only small quantities of tissue were available, it was not possible to do additional studies, but a limited study did show that decreasing the dexamethasone to 1 μM was not sufficient to produce the effects reported here. The first 12 specimens in this study were examined with 100 μM dexamethasone, while the more recent 9 specimens received 10 μM dexamethasone in culture. There was tendency for the former group to have a greater decrease in plasminogen activator activity, supporting dose dependence of this effect.

**DISCUSSION**

The 43 carcinoma tissues had, on the average, 1.9 times more plasminogen activator activity than did the 33 benign tissues which were collected and assayed over a 20-month period for this study. The results confirm our previous findings (3), where use of a less efficient extractant yielded lower activity values, especially for the nonurokinase type of activator of either group. Although there were wide ranges of activator activities in both neoplastic and benign tissues (Chart 1), about 26% (11 of 43) of the cancer specimens had levels above the group mean (±9.3 CTA units/g), while only 6% (2 of 33) of the BPH specimens were at that level. If the earlier study (3) is analyzed in this way, a similar result is obtained (10 of 25 or 40% of the cancer tissues were above the mean of that group of 3.33 CTA units/g, while only 2 of 29 or 7% of the BPH tissues were at that level). One
of the 2 BPH specimens in the "high" group of this report came from a local patient who had been diagnosed as having urinary bladder cancer (a total of 6 BPH specimens were excised from patients with this same disease). Both BPH specimens with high levels of activator from the first study were also urinary bladder carcinoma patients (one additional urinary carcinoma patient was also included in that BPH group). A study of different anatomical compartments (Table 2) indicated that there are no apparent differences which can be detected in different zones reported to be sites of BPH and cancer (21). Altogether, the information obtained from the results to date indicates that, using procedures readily adaptable to clinical laboratories (biopsy specimens, extraction of tissue with a defined buffer, and azocaseinolytic assays with quantitation at visible light spectrophotometry), it may be possible to identify and/or confirm the diagnosis of about one-fourth of patients with prostatic carcinoma. Furthermore, early evidence seems to indicate that prostatic tissues with "high" plasminogen activator levels may be indicative of either malignant prostate tumors or another type of pathological condition, such as the urinary bladder carcinoma of some of the patients included in this study. Wajsmann et al. (35) reported a correlation for urinary fibrinogen degradation products and/or urinary cytology with the activity of bladder cancer which may relate to the results we found.

Since the early reports of the identification of plasminogen activator in prostate tissues and its implications in fibrinolytic events and metastasis (1, 32), there have been a number of studies made to identify its source with apparently conflicting conclusions (8, 13, 14, 16, 28). While some reports indicated that fibrinolytic activity was confined to small blood vessels, others have reported activator activity in epithelial cells. We found urokinase-type activator present in epithelial elements, especially in cancer cells (see Fig. 2). The same immunocytochemical technique is used in work in progress to identify reactions with the nonurokinase-type activator.

The preliminary results of the urinary bladder cancer patients discussed above suggest a number of possibilities. It may indicate that benign tissue can respond to a pathophysiological stimulus which does not originate in its immediate environment, or it may indicate that the tissues may have absorbed activator secreted at another source. If this should correlate with metastatic potential of the bladder cancer or with exfoliative cytology (35), then it is possible that cells shed at the tumor site may leave a trail of activator enzyme which is absorbed by the venous and lymphatic systems they traverse. We intend to study plasminogen activator content of bladder cancers as part of our ongoing studies.

All human prostates placed in organ culture (Table 3, 31 total) were found to secrete and synthesize plasminogen activator. That this was a consequence of tissue viability rather than the result of the leakage of enzyme from disintegrating tissue is evident not only because the tissues yielded more than their original activator activities during culture, but also because the secretion and synthesis of activity could be inhibited by cycloheximide, an inhibitor of protein synthesis. Our brief morphological study which identified tissue viability in one specimen confirms a study of human prostatic tissue cultured under similar conditions which reported a retention of original structure (30).

Although some of the tissues which had high levels of extractable plasminogen activator activities yielded more than average activities in the culture systems, there was no consistent correlation between these 2 parameters for either the neoplastic or benign tumors. Follow-up of the clinical progress of the patient over the next few years should show whether the organ culture results obtained in this study correlate with any particular pattern of disease progression, regression, or drug response.

It should be noted that plasminogen activator secretion and synthesis are not specific properties of tumor explants but have also been reported for different types of normal human tissues (19) as well as normal rodent tissues (25, 37) placed in organ culture. To date, the mechanisms involved in the production of activator during culture are not known nor is it known if the same mechanisms operate in normal tissues and tumors. Possible factors which can contribute to the increase in activator activity reported in the model used in this study include: (a) an increase in cellular products as a consequence of increase in tissue proliferation (23); (b) absence of activator substrate (plasminogen) and/or protease inhibitors which may normally perfuse tissues in vivo; (c) derepression induced by the use of hormone-free medium during culture (thus, the inhibition with dexamethasone might indicate a restoration to an environment more similar to that found in vivo); and (d) acceleration of cell-aging processes. The in vitro organ culture model described here is apparently the first to quantitate the secretion and synthesis of the 2 types of activator activities as well as their inhibition by dexamethasone.

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Fig. 2. In A, cancer cells with irregular glandular structures show accumulation of dark reaction product. No positive reaction is observed in the stromal tissue. In B, control stain using the nonimmune goat IgG in place of antitrypsinase goat IgG shows negative reaction with only a minimal staining in some of the cancer cells. In C, cancer tissue (upper-right quadrant), normal (single arrow), and dilated prostatic glands (double arrow) show positive reaction. The heaviest reaction is observed in the epithelial cells of the dilated glands. In D, control stain in the serial section shows no remarkable staining. Immunoperoxidase staining (PAP) for urokinase in human prostate cancer counterstained with hematoxylin, x 480.
Plasminogen Activator Content and Secretion in Explants of Neoplastic and Benign Human Prostate Tissues


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