Decrease in Creatine Kinase in Human Prostatic Carcinoma Compared to Benign Prostatic Hyperplasia


ABSTRACT

Many previous studies have shown that a proportion of patients with carcinoma of the prostate have increased activity of the creatine kinase (EC 2.7.3.2) isoenzyme designated BB in sera from their peripheral blood. We have analyzed tissues from prostatic hyperplasia of 22 patients and from prostatic carcinoma of 23 additional patients. Prostatic carcinomas contain less (p < 0.001) creatine kinase activity (units/g) than do prostates with benign prostatic hyperplasia. The facts that (a) histochemical studies that we performed confirmed the observation reported previously by others that creatine kinase activity is found primarily in the epithelial elements of hyperplastic prostates and prostatic carcinomas, (b) the carcinomas that we examined had, on the average, a somewhat larger epithelial component than the hyperplastic prostates that we examined, and (c) prostate cancer was found to contain less creatine kinase activity than hyperplastic prostates suggest that the epithelial cells in prostate cancers contain less creatine kinase activity per cell than do those from hyperplastic prostates. The BB form of creatine kinase accounts for 98% of the activity in prostatic carcinoma and in prostates without cancer. Creatine kinase has been discussed as a possible marker for prostatic carcinoma, and we had hoped that it might be useful for the assay of tumor burden. Our data suggest that, if creatine kinase is to be useful in the monitoring of tumor burden, it will be useful only in the contexts of particular patients studied longitudinally since the creatine kinase activity varies enormously among different prostatic carcinomas.

INTRODUCTION

In 1979, Shatton et al. (39) studied the isoenzymes of CPK in a variety of rat and mouse tumors and normal tissues. They reviewed the limited previous work in this area of investigation and observed that “Data on creatine kinase in tumors are very sparse.” We were stimulated both by this sparsity of data and by the observation of Sjovall et al. (43) that there are large differences among human prostatic carcinomas with respect to their histochemically demonstrable, epithelial CPK. These authors (43) stated that “The activity in carcinomatous prostatic epithelial cells varied considerably between specimen (sic) from different patients but was uniform within a given specimen in contrast to the variation of epithelial CPK activity within non-carcinomatous tissue.” In light of the observations of Sjovall et al. (43), we hoped to find differences in CPK activities or isoenzymes from different tumors that might be valuable in assessing the prognoses of individual patients.

Our interest in CPK activity in prostatic carcinomas has been further stimulated by numerous recent reports that many patients with prostatic carcinoma have been observed to have elevated CPK-BB isoenzyme activities in sera from peripheral blood (8, 9, 18, 19, 40, 41). Feld and Witte (9) observed this abnormality in the sera of 9 of 19 patients with metastatic carcinoma of the prostate. Silverman et al. (40) noted a similar abnormality in the sera of 3 of 3 patients with prostatic carcinoma as did Hoag et al. (19) in 5 of 16 such patients. We now report our analysis of CPK and CPK isoenzymes in human prostatic carcinoma and benign hyperplasia of the prostate removed at surgery.

MATERIALS AND METHODS

Tissue. The majority of tissue was obtained from surgically resected prostates (Tables 1 and 2); some tissue was obtained from autopsies (Table 3). Tissues were made available by the Tissue Procurement Service of the Comprehensive Cancer Center of the University of Alabama in Birmingham. Tissues were placed in cold 0.9% NaCl solution in an ice bath in the operating room and brought to the Tissue Procurement Laboratory. In order to assure an adequate diagnostic evaluation of the tissue, chips were taken from transurethrally resected hyperplastic prostates only if the entire specimen weighed more than 25 g. Our assays were carried out with one chip/assay except for experiments that were carried out to test the variability among chips. Sagittal sections were obtained from each chip immediately after its arrival in the Tissue Procurement Laboratory. The 2 lateral halves of each chip were then frozen over liquid nitrogen until a frozen section of the sagittal section stained with hematoxylin and eosin could be examined. Chips from prostatic carcinomas were studied biochemically only when no benign epithelial elements were present in the sagittal sections from them. Morphometric studies for the estimation of glandular tissue, as opposed to fibromuscular stroma, were conducted with sagittal sections of transurethrally resected chips or sagittal sections of fragments of tissue of similar size obtained from total prostatectomies. We were assisted in these studies by Dr. S. Bishop and used a Zeiss projecting microscope and a Hewlett-Packard 9825A calculator interfaced with a Science Accessories Graf Pen as used by him previously (4).

In the case of total prostatectomies, carcinomatous tissue was obtained only when a previous histological diagnosis had been made either from a previous transurethral resection or from a needle biopsy. Frozen sections were carried out on the freshly resected tissue to confirm the previous diagnosis, and the tissue to be studied was stored over liquid nitrogen until the surgical pathological description of the
paraffin sections was formally reported. For carcinoma obtained by transurethral resection, frozen sections were performed on 10 chips. If frozen sections of sagittal slices of at least 5 of the 10 chips showed carcinoma, the lateral halves of these 5 chips were placed in formalin and processed for paraffin sections. Additional chips were then (a) processed for routine paraffin sections and (b) stored over liquid nitrogen as described above for biochemical investigation. Bilateral orchietomy was performed on one patient (10-1) 8 years prior to resection of the carcinoma used in this study; no other patients had received chemotherapy, irradiation, or hormonal therapy prior to this study.

Homogenization of Tissue. Assays were performed with the 2 lateral halves of prostate chips after examination of a sagittal section of the chips by frozen section stained with hematoxylin and eosin. The 2 lateral halves of single chips (weight range, 0.055 to 0.385 g) were minced and placed in a prechilled No. 8575 homogenizer microcontainer (Eberbach Corp., Ann Arbor, Mich.) at 4.0°C in 10 ml of the buffer for homogenization as described by Shatton et al. (39). The contents of the Eberbach microcontainer were homogenized for 3 min with a Waring high-speed blender. The homogenate was centrifuged for 30 min at 4.0°C at 31,300 x g. The supernatant was decanted, kept shielded from light, and stored in an ice bath for subsequent assays.

Assay. CPK was assayed by a modification of the method of Oliver (36) as described by Shatton et al. (39). Hexokinase (type III) and glucose-6-phosphate dehydrogenase (type XVI) were both obtained as salt-free lyophilized powders from the Sigma Chemical Company (St. Louis, Mo.); other chemicals were obtained from Sigma. Assays of adenylate kinase activity were carried out by making "blanks" with the same reaction mixture except that the creatine phosphate was omitted. The CPK activity was calculated by subtracting the reaction in the adenylate kinase "blank" from the reaction in the complete reaction mixture for CPK. Adenylate kinase activity was detectable in the soluble supernatants of centrifuged tissue homogenates; however, it was not detectable in the BB fraction after ion-exchange chromatography. A CPK standard (Sigma) was frozen in 40 aliquots over liquid nitrogen. Standards from the nitrogen freezer were assayed with each investigated tissue in order to monitor the consistency of the conditions for assay. Protein was measured by the Hartree (18) modification of the method of Lowry et al.

Isolation of BB Isoenzyme. The ion-exchange chromatographic technique of Mercer (28) was used to separate the BB isoenzyme from the supernatants of whole-tissue homogenates. Columns were prepared in 14.5-cm disposable Pasteur pipets with DEAE-Sephadex, particle size 40 to 120 µm. One ml of supernatant was layered over the column and collected in Tube 1. In preliminary experiments with prostates with and without cancer, according to the procedure of Mercer (28), 1-ml aliquots of eluents were used, and 10 fractions were collected. After many experiments, we observed that (a) we encountered only small amounts of MM or MB isoenzymes in prostates with or without cancer, (b) all BB activity was contained in Fractions 8 and 9, and (c) no CPK activity was detectable in Fraction 10. We therefore modified the procedure to collect only 4 fractions (1 for MM, 1 for MB, and 2 for BB). The first of these fractions was obtained by passing 1 ml of supernatant from the centrifuged homogenate and 3 ml of the first eluent (50 mm Tris and 100 µm NaCl, pH 8.0) through the column. This elutes the MM fraction from this column. When skeletal muscle was used for comparison instead of prostate, MM was eluted from these columns in the first fraction. The second fraction resulted from washing the column with 3 ml of 50 mm Tris in 200 µm NaCl (pH 8.0); this elutes MB CPK from the column. The third (BB) fraction consisted of an elution with 6 ml of 50 mm Tris with 300 µm NaCl (pH 7.0). This was followed by a fourth fraction eluted with an additional 3 ml of the same buffer to assure complete removal of the BB isoenzyme. All tubes were kept shielded from light in an ice bath and assayed for CPK activity immediately.

Electrophoresis of Isoenzymes. Two cellulose-acetate Titan III ISOFlo plates (Helena Laboratories Corp., Beaumont, Texas) were soaked for 20 min in Tris-barbital buffer (HR buffer; Helena Laboratories Corp.). The tissue was homogenized and centrifuged as described above, and 10 µl of the soluble fraction of the homogenate and control CPK isoenzyme (Helena Laboratories Corp.) were loaded into the multisample applicator (Helena Laboratories Corp.). The samples were applied simultaneously to the first cellulose acetate electrophoresis plate. This plate was placed (acetate side down) into the electrophoresis chamber with the sample placed near the cathode. Electrophoresis was carried out at constant voltage with 300 V for 10 min. The electrophoresis buffer in each compartment was the same Tris-barbital (HR) buffer used to soak the plates.

The second, unused cellulose acetate plate was layered with 1.5 ml of CPK Substrate (Helena Laboratories Corp.). After electrophoresis, the first plate was apposed to the CPK Substrate-soaked plate (acetate side to acetate side) and incubated for 25 min at 37°C. According to the manufacturer (Helena Laboratories Corp.), the materials they provide for the staining of the electrophoresis plate differ from the reaction described for our assay above in that NAD has been substituted for NADP in the last step of the reaction. The isoenzymes were quantitated by scanning the plates for fluorescence of NADH with a Quick-Scan Fluor-Vis densitometer (Helena Laboratories Corp.).

Histochemistry. The method of Sjovall (42) was used for the histochemical demonstration of CPK. Following the technique described by Sjovall et al. (43), a modification of the method of Lowry et al. (36) as described by Shatton et al. (39). Hexokinase (type III) and glucose-6-phosphate dehydrogenase (type XVI) were both obtained as salt-free lyophilized powders from the Sigma Chemical Company (St. Louis, Mo.); other chemicals were obtained from Sigma. Assays of adenylate kinase activity were carried out by making "blanks" with the same reaction mixture except that the creatine phosphate was omitted. The CPK activity was calculated by subtracting the reaction in the adenylate kinase "blank" from the reaction in the complete reaction mixture for CPK. Adenylate kinase activity was detectable in the soluble supernatants of centrifuged tissue homogenates; however, it was not detectable in the BB fraction after ion-exchange chromatography. A CPK standard (Sigma) was frozen in 40 aliquots over liquid nitrogen. Standards from the nitrogen freezer were assayed with each investigated tissue in order to monitor the consistency of the conditions for assay. Protein was measured by the Hartree (18) modification of the method of Lowry et al.

RESULTS

Prostatic tissue with benign hyperplasia and primary prostatic carcinoma were different (p < 0.001) with respect to CPK-BB activity per wet weight of tissue (Tables 1 and 2). Benign hyperplasia of the prostate (Table 1) contained less than 38 units/g of tissue in only 2 (Tissues 7-62 and 10-65) of 22 cases examined. Carcinomas of the prostate (Table 2) contained more than 38 units/g in only 3 (Tissues 3-38, 3-34, and 8-45) of the 23 carcinomas examined. To determine what proportion of the recoverable CPK was recovered in the supernatant from the homogenization, the pellet was again homogenized and centrifuged as before. In the study of prostatic tissue from 14 patients, 94.7 ± 3.2% (S.D.) of the recovered activity was obtained in the supernatant from the first homogenization; the supernatants from the second and third homogenizations contained 4.5 ± 2.9 and 0.8 ± 0.8%, respectively, of the recovered activity.

To test whether or not there might be a correlation between histological grade and the CPK activity in prostatic carcinoma, all cases were classified with the system for the grading of prostatic carcinomas proposed by Gleason et al. (11) and Mellinger et al. (27). With this system, the histological appearances of prostatic carcinomas are assigned numerical grades between 2 and 10 with the lower numbers representing the histologically better-differentiated tumors and the higher num-
Table 1

CPK activity in human prostatic hyperplasia

Values are given in units (μmol of ATP formed per min) per g tissue or per mg protein. The isoenzyme was separated from the supernatant of the centrifuged homogenate on DEAE-Sephadex columns prior to assay. BB isoenzyme was 98 ± 1% of CPK activity.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Amount of tissue (g)</th>
<th>Age of patient (yr)</th>
<th>BB isoenzyme (units/g)</th>
<th>BB isoenzyme (units/mg protein)</th>
<th>Proportion of tissue occupied by epithelial cells and/or glands (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-62</td>
<td>0.163</td>
<td>65</td>
<td>18.8</td>
<td>0.165</td>
<td>25.9</td>
</tr>
<tr>
<td>10-65</td>
<td>0.285</td>
<td>85</td>
<td>24.4</td>
<td>0.770</td>
<td>37.9</td>
</tr>
<tr>
<td>21-11</td>
<td>0.142</td>
<td>67</td>
<td>38.4</td>
<td>0.237</td>
<td>23.5</td>
</tr>
<tr>
<td>14-62</td>
<td>0.146</td>
<td>79</td>
<td>39.6</td>
<td>0.241</td>
<td>39.5</td>
</tr>
<tr>
<td>10-38</td>
<td>0.181</td>
<td>60</td>
<td>43.1</td>
<td>0.368</td>
<td>33.1</td>
</tr>
<tr>
<td>11-52</td>
<td>0.156</td>
<td>64</td>
<td>44.4</td>
<td>0.498</td>
<td>39.1</td>
</tr>
<tr>
<td>22-17</td>
<td>0.135</td>
<td>83</td>
<td>44.5</td>
<td>0.328</td>
<td>40.3</td>
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<tr>
<td>15-15</td>
<td>0.357</td>
<td>63</td>
<td>49.9</td>
<td>0.344</td>
<td>31.0</td>
</tr>
<tr>
<td>9-6</td>
<td>0.164</td>
<td>77</td>
<td>54.0</td>
<td>0.738</td>
<td>34.5</td>
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<td>22-2</td>
<td>0.227</td>
<td>75</td>
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<td>0.595</td>
<td>26.8</td>
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<td>9-32</td>
<td>0.176</td>
<td>81</td>
<td>55.6</td>
<td>0.461</td>
<td>23.1</td>
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<tr>
<td>10-51</td>
<td>0.100</td>
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<td>58.5</td>
<td>0.611</td>
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<tr>
<td>13-01</td>
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<td>0.483</td>
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<td>12-15</td>
<td>0.167</td>
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<td>65.7</td>
<td>0.946</td>
<td>28.2</td>
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<td>0.579</td>
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<td>12-11</td>
<td>0.241</td>
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<td>29.6</td>
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<td>68.0</td>
<td>0.509</td>
<td>28.5</td>
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<td>20-18</td>
<td>0.131</td>
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<td>69.4</td>
<td>1.486</td>
<td>51.1</td>
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<td>22-15</td>
<td>0.252</td>
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<td>72.1</td>
<td>0.858</td>
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<td>4-12</td>
<td>0.217</td>
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<td>94.7</td>
<td>1.719</td>
<td>29.3</td>
</tr>
<tr>
<td>9-58</td>
<td>0.127</td>
<td>62</td>
<td>98.3</td>
<td>0.944</td>
<td>24.0</td>
</tr>
<tr>
<td>16-10</td>
<td>0.090</td>
<td>76</td>
<td>129.0</td>
<td>57.5</td>
<td>93.5</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td>70.9 ± 8.5</td>
<td>59.9 ± 24.6</td>
<td>0.630 ± 0.369</td>
</tr>
</tbody>
</table>

* The values shown for these tissues are average values for the 2 or 3 chips presented in Table 4.

Table 2

CPK activity in human prostatic carcinoma

Values are given in units (μmol of ATP formed per min) per g tissue or per mg protein. The isoenzyme was separated from the supernatant of the centrifuged homogenate on DEAE-Sephadex columns prior to assay. BB isoenzyme was 99 ± 2% of CPK activity.

<table>
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<tr>
<th>Tissue</th>
<th>Amount of tissue (g)</th>
<th>Age of patient (yr)</th>
<th>BB isoenzyme (units/g)</th>
<th>BB isoenzyme (units/mg protein)</th>
<th>Proportion of tissue occupied by epithelial cells and/or glands (%)</th>
<th>Stage of disease</th>
<th>Degree of differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-1</td>
<td>0.181</td>
<td>66</td>
<td>2.23</td>
<td>0.022</td>
<td>53.4</td>
<td>D</td>
<td>9</td>
</tr>
<tr>
<td>21-6</td>
<td>0.132</td>
<td>69</td>
<td>2.62</td>
<td>0.022</td>
<td>33.7</td>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td>16-22</td>
<td>0.138</td>
<td>65</td>
<td>9.07</td>
<td>0.067</td>
<td>49.6</td>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td>22-35</td>
<td>0.089</td>
<td>68</td>
<td>10.4</td>
<td>0.080</td>
<td>64.1</td>
<td>C</td>
<td>8</td>
</tr>
<tr>
<td>24-1</td>
<td>0.071</td>
<td>62</td>
<td>10.4</td>
<td>0.045</td>
<td>71.8</td>
<td>C</td>
<td>8</td>
</tr>
<tr>
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<td>0.123</td>
<td>67</td>
<td>13.3</td>
<td>0.147</td>
<td>60.4</td>
<td>C</td>
<td>8</td>
</tr>
<tr>
<td>8-40</td>
<td>0.086</td>
<td>65</td>
<td>13.5</td>
<td>0.160</td>
<td>60.0</td>
<td>D</td>
<td>6</td>
</tr>
<tr>
<td>10-1c</td>
<td>0.142</td>
<td>77</td>
<td>14.3</td>
<td>0.117</td>
<td>50.7</td>
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<td>0.101</td>
<td>61</td>
<td>15.2</td>
<td>0.235</td>
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<td>D</td>
<td>10</td>
</tr>
<tr>
<td>7-21</td>
<td>0.065</td>
<td>69</td>
<td>16.0</td>
<td>0.099</td>
<td>21.8</td>
<td>D</td>
<td>6</td>
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<td>16-45</td>
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<td>60.3</td>
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<td>27.7</td>
<td>0.166</td>
<td>63.1</td>
<td>D</td>
<td>7</td>
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<td>52</td>
<td>31.4</td>
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<td>10</td>
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<td>34.5</td>
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<td>70.3</td>
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<td>0.193</td>
<td>65</td>
<td>37.5</td>
<td>0.314</td>
<td>39.4</td>
<td>D</td>
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<td>17-2</td>
<td>0.096</td>
<td>91</td>
<td>37.6</td>
<td>0.234</td>
<td>44.7</td>
<td>D</td>
<td>8</td>
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<tr>
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<td>0.105</td>
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<td>52.6</td>
<td>0.454</td>
<td>54.9</td>
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<td>7</td>
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<tr>
<td>3-38</td>
<td>0.350</td>
<td>54</td>
<td>75.5</td>
<td>1.96</td>
<td>72.0</td>
<td>B</td>
<td>7</td>
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<td>8-48</td>
<td>0.121</td>
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<td>0.816</td>
<td>70.4</td>
<td>C</td>
<td>8</td>
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<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td>69.3 ± 9.7</td>
<td>28.3 ± 22.3</td>
<td>0.298 ± 0.400</td>
<td>54.0 ± 16.3</td>
<td></td>
</tr>
</tbody>
</table>

* The values shown for these tissues are average values for the 2 or 3 chips presented in Table 4.

b Recurrent prostatic carcinoma that had been previously resected transurethrally.

d Bilateral orchiectomy 8 years prior to this surgery.

* Only specimens obtained from open prostatectomy.

Classified with the system proposed by Gleason et al. (11) and Mellinger et al. (27).
bers representing tumors with lesser degrees of differentiation.
It is interesting that all carcinomas with activities below 12
units/g received Gleason scores between 8 and 10.

Because of the precautions that we took in obtaining tissue,
our study was limited to patients with A2, B2, C, and D disease.
The proportion of patients with metastases at the time of
diagnosis is high in this series (Table 2); the general experience
with prostatic carcinoma has been reviewed recently (23). It
is interesting to note that our patients (Tissues 8-37, 3-34,
and 3-38) with the most limited disease had carcinomas that
contained relatively high CPK activities. The patient from whom
we obtained tissue 3-38 had a staging pelvic lymphadenectomy
which showed the examined lymph nodes to be free of tumor,
and he is currently more than 2 years postoperative without
evidence of recurrent disease.

We should note that we found CPK activities from prostates
removed at autopsies within 8 hr of death generally low and
highly variable as compared with resected prostates (Table 3).
Incubation of the high-speed supernatant from 4 different trans-
urethrally resected tissues for 3 or 6 hr in a 4° ice bath resulted
in no loss of activity, whereas incubation of the supernatant
for 10 or 20 min at 56° resulted in total loss of activity. If individual
chips from the same resected prostate were incubated at 37°
and then the preparation of the high-speed supernatant, 75% of
the initial CPK activity remained after a 3-hr incubation, 57%
after a 6-hr incubation, and only 22% after a 9-hr incubation.
Incubation of the chips at 4° prior to the preparation of the
high-speed supernatant resulted in no loss of activity when the
incubation was 3 or 6 hr and an 18% loss of activity when the
incubation lasted 9 hr. Data concerning variation in CPK-BB
activities among different chips from the same patients are
shown in Table 4. Carcinomatous prostates tended to show a
greater average variation across patients than did hyperplastic
prostates.

The amounts of BB isoenzyme reported for the various
prostatic tissues in Tables 1 to 4 reflect the CPK activity in
fractions recovered after chromatography with the technique
of Mercer (28). For prostates with hyperplasia, total CPK
activity recovered chromatographically ranged from 75 to
112% (91 ± 9%) of the activity observed in the 31,300 × g
supernatant, and of that total activity the BB isozyme repre-

dented 98 ± 1%. For carcinomatous prostates, recovery of
total activity ranged from 76 to 121% (96 ± 13%), and of that
activity the BB isozyme represented 99 ± 2%. The analyses of
CPK-BB by column chromatography and by electrophoresis
were similar, i.e., when CPK-BB from the chromatographic
column was Electrophoresed in parallel with a standard, only
CPK-BB was detectable on the cellulose acetate.

The histochemical distribution of CPK in prostates with be-
nign hyperplasia and prostatic carcinoma varied somewhat
from that reported by Sjovall et al. (43). In agreement with
Sjovall et al., most of the histochemically demonstrable CPK
in both diseases was located in epithelial cells (Figs. 1 to 6). Also
in agreement with Sjovall et al. (43), we found the amount of
histochemically demonstrable CPK highly variable in prostates
with benign hyperplasia (Figs. 1 and 2). We found not only
variability in the intensity of the reaction from cell to cell and
from gland to gland but variability from patient to patient. In a
few patients, most of the epithelial cells stained intensely for
CPK. In other patients, the variability was marked from one
epithelial area to another. In contrast to the findings of Sjovall
et al. (43), we did not find that the histochemically demonstrable
CPK in most carcinomas "was uniform within a given specimen
in contrast to the variation of epithelial CPK activity within non-
carcinomatous tissues." In a minority of carcinomas, it was
possible to find whole microscopic fields in which most epithe-
lial cells stained strongly (Fig. 3) or weakly (Fig. 4); however,
the variability in single histological sections was great, and we
found microscopic fields that stained only faintly or not at all in
all carcinomas with intensely stained fields. All carcinomas that
stained generally weakly contained some epithelial cells that
stained with at least a moderate level of intensity (Fig. 4). Most
often, sharply contrasting patterns (Figs. 5 and 6) of staining
could be observed in different microscopic fields from the same
frozen sections, i.e., the areas that stained differently were not
widely separated or exposed to different conditions for the
histochemical procedure. Sections incubated in staining solu-
tions without exogenous substrate occasionally stained faintly.

DISCUSSION

To our knowledge, this is the first study that has shown that
prostatic carcinomas contain significantly less CPK than do
hyperplastic prostates. Benign hyperplasia arises in the peri-
urethral tissue of the prostate while prostatic cancer is thought
to arise in the peripheral portion of the gland. The lower values of
CPK-BB for prostatic cancer might be related to the different
site of origin. We used benign hyperplastic prostates for our
comparisons since this is the prostatic tissue available from
individuals of the same age.

While the number of patients in our series currently is insuf-
ficient to demonstrate a relationship between CPK-BB activity
and histological differentiation, clinical stage, or clinical ag-
gressiveness of the tumors, it is quite possible that CPK will be a
valuable compliment to the conventional pathological char-
acterizations (11, 27, 30, 31) as a prognostic indicator. Knox
(24) has suggested a correlation between the enzymatic char-
acterization of certain transplantable tumors and their biologi-
ical behavior. In discussing the dependence of the oncologist
upon histology for the recognition of and classification of
cancers, Sprouls (44) expressed her view that in the future...
... we may find biochemical evaluation more accurate than
one so dependent upon human judgment." Despite the fact
that numerous eminent tumor biologists and pathologists have
expressed similar views and despite abundant data from ex-

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time (hr)</th>
<th>Age of patient (yr)</th>
<th>Histological diagnosis</th>
<th>BB isoenzyme activity (units/mg protein)</th>
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<td>15-45</td>
<td>5.5</td>
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<td>Benign hyperplasia</td>
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<tr>
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<td>5.34 ± 1.12</td>
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<td>5.0</td>
<td>68</td>
<td>Benign hyperplasia</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Length of time between death and the removal of the specimen.

Table 3

CPK activity in prostates from autopsy

Values are given in units (μmol of ATP formed per min) per g tissue or per mg protein. The isoenzyme was separated from the supernatant of the centrifuged homogenate on DEAE-Sephadex columns prior to assay. BB isoenzyme was 98 ± 1% of CPK activity.

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peptidase. They found that the activities of both enzymes in these tumors "varied over 100-fold, with all of the highest values occurring in tumor regions with the lowest CT [calcitonin] values ..." We have already discussed the histochemical heterogeneity that we observed in the prostatic carcinomas studied by us. In addition to the heterogeneity that we observed histochemically within individual tumors, considerable variability was observed among tissues from different patients both with and without cancer. Weber (46, 47) has reviewed the extensive enzymatic studies of cancer. He and his associates have carried out some studies of human tumors and have emphasized (21) the general experience that "variation between samples was much greater for human material than for rat tissues." This greater variation is seen both in tumors and in tissues not involved by cancer.

REFERENCES


8. Eckfeldt, J., and Kershaw, M. Increased circulating creatine kinase isoenzyme BB in a patient with metastatic prostatic carcinoma gave spuriously
Figs. 1 to 6. Frozen sections of prostate stained for CPK activity according to the method of Sjovall (42). × 280.

Fig. 1. Prostate with benign hyperplasia showing variable activity for CPK. Arrowheads, examples of lightly (L) staining epithelial elements; arrows, darkly (D) staining epithelial elements.

Fig. 2. A different hyperplastic prostate. Arrowheads, areas of lightly (L) staining epithelial elements; arrows, darkly (D) staining epithelial elements.

Fig. 3. Prostatic carcinoma demonstrating mostly darkly staining epithelial cells.

Fig. 4. Prostatic carcinoma with mostly weakly staining epithelial cells. Arrows, indicate epithelial cells that stained moderately darkly (D).

Figs. 5 and 6. Prostatic carcinomas demonstrating variable activity for CPK in closely related areas. Arrowheads, lightly (L) staining epithelial elements; arrows, darkly (D) staining epithelial elements.
Decrease in Creatine Kinase in Human Prostatic Carcinoma Compared to Benign Prostatic Hyperplasia


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