Effects of a Growth Inhibitor and Other Factors on the Tissue Cathepsins of Tumor-bearing Rats*

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It has been known from the work of Maver and Dunn (5) and others (a review of the earlier work is given in [5]) that the presence of certain tumors causes an increased catheptic activity in various host tissues. These observations have been confirmed and extended by Zamecnik and Stephenson (10, 11), who demonstrated that only certain of the catheptic enzymes were thus affected. The effect of the presence of a tumor on the over-all catheptic reaction of the host tissues has again been confirmed here, and additional studies have been made concerning the effect on the cathepsins of such factors as starvation, protein depletion, and the administration of 4-dimethylaminostilbene hydrochloride, a substance indicated by Hadlow, Harris, Kon, and Roe (3) to be a potent tumor growth inhibitor.

EXPERIMENTAL

The animals used were Maguran or Sprague-Dawley male rats. The Walker carcinoma 256 was the only tumor used; it was carried separately in each strain of rat by successive subcutaneous implantations.

Animals were treated with the stilbene compound as follows: Beginning with the fourth day after the implantation of the tumor, the stilbene compound was injected intraperitoneally, on alternate days; the amount was 50 mg. per kilogram of body weight, and the concentration was 100 mg. per milliliter of propylene glycol. A total of five injections was given, and the animals were killed by decapitation 24 hours after the last injection. The desired tissues were then removed, immediately frozen in a bath of dry ice and ether, and kept under deep freeze until use. Control experiments showed that no loss of activity was incurred by the freezing.

RESULTS AND DISCUSSION

Results are generally expressed on the basis of units of catheptic activity per milligram of tissue protein. Since the Robinson and Hogden biuret method for the determination of tissue protein is inaccurate when applied to tissues with a high fat content, due to turbidity in the color solution, the liver data are also given on the basis of activity per gram of tissue, although this expression appears to

1 All protein-depleted and all tumor-bearing animals were obtained from Dr. John W. Green, to whom grateful acknowledgment is made.

2 After completion of the work described herein, a convenient means was found (2) of circumventing this difficulty in the biuret method, by chilling and filtering off the fat.
be statistically less consistent than that based on tissue protein.

Table 1 presents the catheptic activity of the tumors of rats treated in various ways. In each case Student's $t$ values (4) were calculated. For the population sizes indicated in the tables, a $t$ value of 2.5 or greater is considered significant, although in those few cases where only two or three animals were used, it is difficult to attribute great significance to the figures, no matter what the $t$ value.

The following may be noted from this table: (a) Treatment with the stilbene compound appreciably increases the catheptic activity of the tumor ($t = 4.8$) as does starvation ($t = 2.7$); and (b) treatment of a starved animal with 4-dimethylaminostilbene hydrochloride increases the catheptic activity ($t = 4.3$) to a degree representing approximately the sum of the two individual effects; thus the effect of the chemical is not due to inanition.

Table 2 presents data on the cathepsin of rat spleen and liver. From this table the following may be observed: (a) Implantation of a tumor causes an increase in the catheptic activity of the spleen ($t = 3.1$) and of the liver ($t = 2.5$, on protein basis); (b) if the animal is starved, this effect of the tumor on the spleen is retained ($t = 4.3$), but the effect on the liver is lost ($t = 0.3$); (c) treatment of the fed, tumor-bearing animal with the stilbene compound is without significant effect on either spleen ($t = 1.0$) or liver ($t = 1.0$), although the apparent tendency is toward a reversal of the effect of the tumor implantation; and (d) treatment of the starved, tumor-bearing animal with the stilbene compound causes a significant decrease in the spleen catheptic activity ($t = 3.0$) but not in the liver catheptic activity ($t = 0.7$); in the latter case, in fact, the tendency is toward an increased activity.

Any explanation of these results must be tempered by the finding of Maver, Dunn, and Greco (6) that not all tumors cause a significant increase in the catheptic activities of host tissues. The data presented here, however, indicate that the Walker carcinoma 256 does increase the catheptic activity of the host's liver and spleen, and that treatment of the tumor-bearing animal with the tumor inhibitor tends to reverse this effect, although the tendency is statistically significant only in the case of the spleen of the starved rat.

**SUMMARY**

1. The catheptic activity of rat spleen, liver, and tumor (Walker carcinoma 256) has been determined under the influence of a variety of treatments.

2. In confirmation of earlier work, it is shown that the presence of this tumor causes an increased catheptic activity in the host tissues.

3. Treatment of tumor-bearing animals with 4-dimethylaminostilbene hydrochloride, a potent tumor growth inhibitor, increases the catheptic activity of the tumor; it also decreases the catheptic activity of the spleen of the starved, tumor-bearing animal with the tumor inhibitor.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Catheptic activity per milligram of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maguran</td>
<td>No treatment</td>
<td>7</td>
<td>1.13 ± 0.07*</td>
</tr>
<tr>
<td></td>
<td>Treated with DAS</td>
<td>6</td>
<td>1.72 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Starved</td>
<td>4</td>
<td>1.80 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Starved; treated with DAS</td>
<td>3</td>
<td>2.46 ± 0.35</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Catheptic activity per milligram of spleen protein</th>
<th>Catheptic activity per milligram of liver protein</th>
<th>Catheptic activity per gram of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maguran</td>
<td>No treatment</td>
<td>6</td>
<td>1.17 ± 0.08*</td>
<td>0.57 ± 0.08*</td>
<td>75.0 ± 4.6*</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>7</td>
<td>1.77 ± 0.18</td>
<td>0.81 ± 0.09</td>
<td>94.4 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>CA; DAS</td>
<td>6</td>
<td>1.55 ± 0.14</td>
<td>0.68 ± 0.09</td>
<td>88.3 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>DAS</td>
<td>7</td>
<td>1.39 ± 0.44</td>
<td>0.88 ± 0.09</td>
<td>109 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>PG</td>
<td>3</td>
<td>1.21 ± 0.32</td>
<td>0.75 ± 0.12</td>
<td>79.5 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>Starved</td>
<td>4</td>
<td>1.10 ± 0.21</td>
<td>0.75 ± 0.09</td>
<td>105.3 ± 20.9</td>
</tr>
<tr>
<td></td>
<td>CA; Starved</td>
<td>4</td>
<td>2.30 ± 0.21</td>
<td>0.76 ± 0.07</td>
<td>88.0 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>CA; DAS; Starved</td>
<td>5</td>
<td>1.67 ± 0.09</td>
<td>0.87 ± 0.13</td>
<td>98.0 ± 9.0</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>CA</td>
<td>5</td>
<td>2.01 ± 0.18</td>
<td>0.57 ± 0.11</td>
<td>67.3 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>CA; Protein depleted</td>
<td>3†</td>
<td>3.05 ± 0.62</td>
<td>0.64 ± 0.08</td>
<td>67.0 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Protein depleted</td>
<td>4</td>
<td>4.33 ± 0.32</td>
<td>0.31 ± 0.11</td>
<td>58.3 ± 5.0</td>
</tr>
</tbody>
</table>

*Data presented are mean activity and standard error of the mean.
† Three rats included in liver experiment; only two rats in spleen experiment. CA = host bears Walker carcinoma 256. DAS = treated with 4-dimethylaminostilbene hydrochloride. PG = treated only with propylene glycol (solvent for the DAS).
bearing rat, and has a tendency, not mathematically significant, to decrease also the catheptic activity of the liver and spleen of the fed, tumor-bearing rat.

4. Starvation increases tumor catheptic activity.

5. Treatment of a starved animal with the stilbene compound further increases the tumor's catheptic activity.

REFERENCES


Effects of a Growth Inhibitor and Other Factors on the Tissue Cathepsins of Tumor-bearing Rats

Robert N. Feinstein


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