Studies of Cholinesterase Activity

VI. The Depression of Serum Cholinesterase Activity by a Locally Implanted Human Tumor in the Guinea Pig*

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SUMMARY

The study of glioblastoma multiforme in the anterior chamber of the guinea pig eye provided an unusual opportunity to observe the effect of a malignant but locally confined neoplasm on a serum enzyme system. Biochemically, these tumor-bearing guinea pigs were distinguishable from normal controls by a significant depression of serum cholinesterase activity. This observation substantiated the previously reported clinical phenomenon that many human patients with nonhepatic carcinoma had lowered cholinesterase activity in the absence of lowered serum albumin. The implications of this observation are discussed.

Patients with advanced carcinoma often have an abnormal depression of serum cholinesterase activity (7-9, 15). Some investigators have speculated that these low values observed in carcinoma patients were secondary to involvement of the liver (the site of cholinesterase production) or were associated with the nonspecific defects of protein synthesis as seen in chronic disease states (3, 8). Other studies seemed to link depressed serum cholinesterase activity with lowered serum albumin levels, indicating that alteration of serum albumin was responsible for the observed changes in the enzyme studied (2, 3, 10, 11). More recently it has been shown that the statistical intercorrelation between serum albumin and cholinesterase activity (13, 14) is relatively weak and that altered protein synthesis is by no means the sole factor in depression of this enzyme. Transplantation of human tumor into the anterior chamber of the guinea pig eye afforded a means of further evaluating the effect of a local tumor on serum cholinesterase activity in the laboratory animal.

MATERIALS AND METHODS

Serum cholinesterase activity was determined before and at intervals following tumor transplantation, with the final value assayed 7 weeks after the operative procedure. Serum proteins were analyzed 7 weeks after the time of transplantation. The enzyme and protein studies were carried out at corresponding times in the control animals which had not been operated upon.

Animals were weighed at the time of each cardiac puncture. Post-mortem examinations were performed at the completion of the study. Permanent histologic specimens were prepared from all eyes.

Cholinesterase activity1 was determined by the method of de la Huerga, Yesinick, and Popper (1) as described by Wetstone et al. (12). Only male animals2 were selected to avoid the known depressive effect3 of estrogenic substances on this enzyme's activity. Total protein, albumin, and globulin levels were determined by the method of Kingsley (5). Paper electrophoresis was performed by the method of Durrum on the Spinco Model R electrophoretic apparatus with the analytrol densitometer.

One-millimeter fragments of histologically intact human glioblastoma multiforme4 were im-

1 Normal adult range, 150-305 units/ml serum.
2 Supplied by Cedars Rabbitry, West Haven, Connecticut.
3 Unpublished data.
4 Kindly supplied by Dr. Harry S. N. Greene, Yale University School of Medicine, New Haven, Conn.
planted in the anterior chamber of the right eye by the method of Greene (4). A common donor was used for ten animals. All animals operated upon, as well as a control group not operated upon, were observed regularly for evidence of systemic illness, eye infection, or disturbance of growth. Such abnormal animals were excluded from the study.

Diet consisted of liberal quantities of standard Purina Laboratory Chow, supplementary leafy vegetables, and water. No animal was given systemic medication at any time during the experiment.

Blood for enzyme and protein studies was obtained by cardiac puncture with the use of a Vacutainer tube with a 1½-inch #22 disposable needle. Five ml. of nonhemolyzed blood was permitted to clot and the serum separated immediately after withdrawal from the animal.

RESULTS

Fifty per cent of the implant-bearing animals used in this study showed active tumor growth as determined by gross inspection and subsequent microscopic examination. Within each group of ten guinea pigs receiving tumor from a common donor there were animals with as well as without active tumor growth. There was no evidence of extraorbital spread of tumor in any animal with an implant.

Grossly the tumor appeared as a glistening, vascularized pink mass in the anterior chamber of the eye. It was usually possible to determine, within 3 weeks after implantation, which tumors would proliferate. Six to 8 weeks after transplantation, the anterior chamber was filled with tumor in the positive animal. The cut surface of the tissue was gelatinous, greyish-white, and could be stripped without difficulty from the overlying vascularized cornea. Rarely an area of necrotic tissue was present in the center of the removed specimen. When examined microscopically the tissue consisted of numerous pleomorphic glial cells in random pali-sades with occasional to frequent mitotic figures. The margins of the tumor often showed round-cell leukocytic infiltration. Tumor implants which failed to proliferate were resorbed or replaced by fibrous tissue.

The animals were divided into three groups: Group I, the controls not operated upon; Group II, the animals which had been operated in whom the tumor did not proliferate; and Group III, the

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>MEAN CHANGE IN SERUM CHOLINESTERASE AND OTHER PARAMETERS IN CONTROLS NOT OPERATED UPON (I), NONTAKES OPERATED (II), AND TUMOR TAKES OPERATED (III)</strong></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NO. ANIMALS:</strong></td>
<td>74</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Initial cholinesterase*</td>
<td>136.5</td>
<td>156.2</td>
<td>158.7</td>
</tr>
<tr>
<td>Final cholinesterase</td>
<td>125.9</td>
<td>138.4</td>
<td>108.0</td>
</tr>
<tr>
<td>%Δ cholinesterase</td>
<td>-7.8</td>
<td>-11.4</td>
<td>-31.9</td>
</tr>
<tr>
<td>σ†</td>
<td>19.5</td>
<td>28.3</td>
<td>36.0</td>
</tr>
<tr>
<td>P&lt;</td>
<td>0.025</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Initial weight‡</td>
<td>568</td>
<td>547</td>
<td>597</td>
</tr>
<tr>
<td>Final weight</td>
<td>644</td>
<td>687</td>
<td>706</td>
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<tr>
<td>%Δ weight</td>
<td>+14.6</td>
<td>+23.9</td>
<td>+18.3</td>
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<tr>
<td>Total protein§</td>
<td>5.5</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.2</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Globulin</td>
<td>2.3</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>NO. ANIMALS:</strong></td>
<td>20</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>Albumin§</td>
<td>2.67</td>
<td>2.83</td>
<td>2.72</td>
</tr>
<tr>
<td>α1 Globulin</td>
<td>0.15</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>α2 Globulin</td>
<td>1.23</td>
<td>1.61</td>
<td>1.29</td>
</tr>
<tr>
<td>β Globulin</td>
<td>0.77</td>
<td>0.75</td>
<td>0.77</td>
</tr>
<tr>
<td>γ Globulin</td>
<td>0.71</td>
<td>0.53</td>
<td>0.62</td>
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* Serum cholinesterase activity, units/ml.
† Standard deviation.
‡ In grams.
§ Colorimetric in grams per cent.
# Paper electrophoresis (durrum) fractions.
animals in whom the transplanted tumor grew. Each group of animals exhibited a significant fall in serum cholinesterase activity during the period of observation (Tables I and II). Group I animals showed a mean decline of 11 units; Group II, 18 units; and Group III, 51 units.

Each group showed a significant increase in mean weight. There was no statistically significant difference in weight gain between any of the three groups, nor was there a significant correlation between cholinesterase level and weight in these animals.

The animals in Group II had an elevated serum albumin on colorimetric assay, as compared with the other two groups. Paper electrophoretic fractionation of individual animals’ serum revealed a decrease in α1 globulin and an increase in α2 globulin in the tumor-free animals which had been operated upon, as compared with the other two groups (Table I).

DISCUSSION

The study of serum cholinesterase following transplantation of glioblastoma multiforme into the anterior chamber of the guinea pig eye afforded a method of observing enzyme changes in the presence of a malignant but locally confined neoplasm. Histologically confirmed tumor growth occurred in 50 per cent of the implant-bearing animals. The histologic features are similar to those described by Greene (6). That the nontake animals could not be distinguished from the normals with respect to their serum cholinesterase activity indicated that neither the operative procedure nor the reaction to foreign tissue affected the enzyme under study.

The tumor-bearing animals displayed a fall in cholinesterase activity 3-4 times that observed in the other groups. This depression occurred without associated changes in general health or normal pattern of weight gain or hematocrit and in the absence of depressed serum albumin levels. These observations confirm the previous clinical studies in which a fall in serum cholinesterase was seen in patients with local carcinoma, and support the concept that this depression is not merely a reflection of altered albumin synthesis, as had previously been reported.

Although the depression of cholinesterase in the tumor-bearing animals was clearly shown, the mechanism of this decline remains obscure. Whether the tumor directly or indirectly suppresses hepatic synthesis of this enzyme, utilizes it in its metabolism, or produces an anticholinesterase-like substance is open to speculation. The finding of the decrease in α1 globulin and the elevation of α2 globulin in those animals that rejected the tumor raises the possibility that these changes are associated with an immunologic response to the implanted tumor. Both the enzyme and other protein changes are the subject of continued study.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>“t”</th>
<th>P&lt;</th>
</tr>
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<tbody>
<tr>
<td>I-II</td>
<td>1.16</td>
<td>.80 (&gt;0.25)</td>
</tr>
<tr>
<td>I-III</td>
<td>5.77</td>
<td>.001</td>
</tr>
<tr>
<td>II-III</td>
<td>4.73</td>
<td>.001</td>
</tr>
</tbody>
</table>

TABLE 2

Statistical Comparison of Cholinesterase Depression between Groups

REFERENCES

3. ———. Relationship between Serum Cholinesterase and Serum Albumin. Ibid., pp. 72-91.
Studies of Cholinesterase Activity. VI. The Depression of Serum Cholinesterase Activity by a Locally Implanted Human Tumor in the Guinea Pig

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