The Growth-inhibitory Action of Heparin on the Ehrlich Ascites Tumor in Mice*

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The effectiveness of heparin as a mitotic inhibitor in vitro has been shown on marine eggs (8), on tumor tissue (6, 17), and on tissue culture (2, 5). The use of an ascites tumor in these experiments has made possible the demonstration of the mitotic inhibitory activity of heparin on tumors in vivo. Balazs and Holmgren (1) reported a growth-inhibitory activity of heparin on tumor tissue by showing an increase in survival time of animals when heparin treatment was begun immediately following tumor transfer. Negative effects on tumor growth were obtained by Shear (15) and Kreisler (11). These latter results may be attributed to the technics employed, which involved in one case toxic doses, and, in another, intravenous injection of the heparin.

The results reported in the present work clearly show that heparin is a mitotic inhibitor in the ascites tumor in vivo and consequently checks tumor growth. There is further indication that such activity is not of necessity followed by an increase in survival time.

MATERIALS AND METHODS

The Ehrlich ascites tumor used in these experiments was obtained from the Department of Pathology of the University of Pennsylvania Medical School through the kindness of the late

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Lederle Laboratories, Pearl River, N.Y., and Upjohn Co., Kalamazoo, Michigan, supplied the heparin used for experimental purposes.

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The mice, obtained from Rockland Farms, were males weighing between 15 and 25 gm. They were maintained on a diet of Purina Laboratory Chow and water ad libitum and received occasional dietary supplements of lettuce and oatmeal.

The tumor was transferred by withdrawal of the neoplastic exudate into a syringe and subsequent injection of 0.2 ml. (approximately 10⁷ cells) of the suspension into the peritoneal cavity of another host animal. Transfers were made every 7 days, at which time the tumor was relatively free of blood and nontumor cells. The percentage of tumor takes varied with the number of tumor cells injected (10) and the chromosome complement of tumor and host (7). There were approximately 90–100 per cent takes in the C57BL mice. In the C57BR mice, the percentage of takes varied from 75 to 90 per cent, showing a greater range of response among groups.

Mice with 4–7-day tumors were given intraperitoneal injections between 10 A.M. and noon of 0.2 ml. of a 100 mg. per cent solution of heparin in saline. Control mice received 0.2 ml. of saline. The animals were sacrificed at hourly intervals for 4 hours following injection with the heparin. The tumor was withdrawn into a heparinized syringe, and a drop of tumor exudate was placed on each of three slides. By tilting the slides, excess tumor was removed, and a single layer of cells remained. Fixation of cells was accomplished by placing a drop of Nissenbaum’s fixative (14) on the wet smear. The slides were then stained with iron-hematoxylin and mounted in balsam. A total of 1000 cells was counted for each tumor sample and the number of cells in stages of mitosis from late prophase until late telophase recorded. For 60 control animals, the mean mitotic index was 1.89 per cent ± 0.10 S.D. Measurements were made approximately 170 hours following inoculation with tumor. The error for this method of counting is analyzed by Klein (10).
RESULTS

Mitotic index values.—Table 1 gives the mitotic index values for a single injection of heparin in C57BL mice. The mean control value was 1.34 percent ± 0.57 S.D., as compared with 0.34 percent ± 0.38 S.D. for the treated animals. For C57BR mice the values were 1.9 percent ± 0.33 percent.

TABLE 1
THE EFFECT OF A SINGLE INJECTION OF A HEPARIN SOLUTION* ON THE MITOTIC INDEX OF 7-DAY EHRlich ASCITES TUMORS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hours between injection and smear</th>
<th>Mitotic index (per cent)</th>
<th>Heparin-treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
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<tr>
<td>3</td>
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<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0.6</td>
<td>0.6</td>
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</tr>
<tr>
<td>5</td>
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<td>0.0</td>
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</tr>
<tr>
<td>8</td>
<td>2-3</td>
<td>1.1</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
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<td>2-3</td>
<td>1.1</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
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<td>0.9</td>
<td>1.9</td>
<td></td>
</tr>
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<td>2-3</td>
<td>0.0</td>
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<tr>
<td></td>
<td>2-3</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean values:

- **Group A**: C57BL mice
  - Treated: 20
  - Control: 22

- **Group B**: C57BR mice
  - Treated: 8
  - Control: 7

*0.2 mg. in saline.

S.D. and 0.8 per cent ± 0.51 S.D. for controls and treated, respectively, as indicated in Table 2. The control means of the two groups (C57BL and C57BR) were compared by applying the Student-Fisher "t" test after the method described by Mode (18). The value of the deviate was 2.5 for 27 degrees of freedom. This value of the deviate indicates that the control groups differ from one another significantly at the 1 per cent level of significance. For this reason, the control groups were not pooled. Comparisons of treated and control mean indices were made independently for the two different strains of mice. Comparison of means was also made for a series of experiments in which repeated doses of 0.2 ml. of the heparin solution were given on the 5th, 6th, and 7th days. Smears were made 2 hours following the last injection.

In summary, the results showed that the reduction in the mitotic index following a single injection of 0.2 mg. of heparin was at the highly significant 0.5 per cent level. Repeated injections failed to produce a significant change in the mitotic index values under the experimental conditions.

Tumor volume.—Tumor volume was determined in several series of experiments following repeated injections with a heparin solution, as indicated on Table 3. The tumor was withdrawn into a graduated syringe and the volume measured directly. To insure complete collection of the tumor the peritoneal cavity was rinsed several times with a known amount of saline. The ratio of cells to ascitic fluid was determined by centrifugation at the rate of 2,500 r.p.m. for a period of 20 minutes in 1-ml. Wintrobe hematocrit tubes whenever possible. Hematocrits were always between 25 and 40 per cent. In all cases, only those animals with apparent tumors were chosen as experimental subjects. Table 3 gives the results of these measurements. The tumor volume was reduced by 40–50 per cent of the control volumes in all experimental groups.

Survival time.—One of the simplest screening technics used to test the effectiveness of a test compound is the measure of survival time following treatment with the test material. Table 4 indicates the results of heparin treatment with variations in the time of application and the dose administered. The average survival time for all
treated animals was 12.5 days. For all controls the average was 11.5 days. In each individual experiment, the ratio of the survival time between treated and controls approximated unity.

**DISCUSSION**

Heilbrunn (9) advanced the theory that an agent which could keep the protoplasm fluid and thereby prevent the mitotic gelation would be the most effective as a mitotic inhibitor. It was on this basis that the anticoagulant heparin was tested on the tumor in these experiments.

Heparin is a mucopolysaccharide most commonly known for its action as a blood anticoagulant and for its hemorrhage-producing properties. That its effectiveness as a mitotic inhibitor in vivo is not primarily due to hemorrhage, but to direct action on the cells themselves, is suggested by the fact that it exerts the same activity in vitro. There is evidence that Shear's polysaccharide also produces a direct action on the tumor cells prior to any detectable hemorrhage (4).

With regard to the negative effects on survival time, it is suggested that the products of a regressing tumor may actually hasten the death of the animal by shock. This proposition is supported by Zahl (17) in his work with Shear's polysaccharide.

The similar effects produced by heparin and Shear's polysaccharide on tumors indicate that further experimentation on heparin and related compounds might yield a more potent agent. The presence of one such related substance has been reported in a study (3, 16) showing that there is positive metachromasia in the follicular fluid of the ovaries of the sow. Substances producing the metachromatic color when stained with toluidine blue are the mucopolysaccharides (heparin, hyaluronic acid, chondroitin sulferic acid, etc.) and the nucleic acids. The implication is that the color obtained in the follicular fluid is due to the presence of a mucopolysaccharide rather than nucleic acid. Experiments of a preliminary nature show that a metachromatic material separated from the follicular fluid of cow ovaries is active in causing regression of the ascites tumor (12).

**SUMMARY**

Heparin acts as a mitotic inhibitor on the Ehrlich ascites tumor in vivo, as shown by a highly significant decrease in the mitotic index following the single but not the repeated injections. This reduction in the mitotic index is reflected by tumor regression to the extent of a 40–50 per cent decrease in tumor volume. The experimental conditions employed produced no effect on the survival time of the treated animals. It is suggested that heparin exerts its effects directly on the tumor cells rather than indirectly following hemorrhage.

**REFERENCES**


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