Some Systemic Effects of Toxohormone

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Toxic materials released into the blood by tumors may be responsible for the deleterious effects of the tumor upon its host, or the tumor may affect the host by removing essential nutrients from the blood (9). One area of this problem intensively studied has been the effect of the tumor on the liver catalase of the host. The lowered concentration of hepatic catalase, which has been demonstrated by several technics with many types of tumors and species of animals (9), has been attributed by several investigators to the elaboration of a toxic substance by the tumor (8, 10, 13). Nakahara and Fukuoka (16) were able to isolate a fraction from tumor tissue, but not from normal tissue, which would depress liver catalase in vivo. The existence of this “toxohormone” has been confirmed by a number of investigators, who have made some progress in its purification (17).

If the concept of a cancer toxin is valid, it should be possible to reproduce further systemic effects characteristic to the tumor-bearing animal. Greenstein (9) and Begg (3) have discussed many of the changes that occur in the host during the growth of tumors, and this report will show that an extract of Walker carcinosarcoma 256 will produce in normal rats many of the effects noted in tumor-bearers.

MATERIALS AND METHODS

Toxohormone, or tumor tissue extract (TT), was prepared from the non-necrotic portions of Walker carcinosarcoma 256 as the alcohol precipitate by the method of Nakagawa et al. (15). Normal tissue preparations (NT) were made by skinning a normal Holtzman rat, removing the intestines and bones, and processing the remaining tissues in the same manner as that by which the tumor was treated for the preparation of toxohormone. The average yields from 10 TT and 4 NT preparations were 8.2 and 9.2 mg/gm of fresh tissue, respectively. These preparations were not mixed, but were assayed separately. For assay, TT and NT were dissolved in water and centrifuged at 10,000 × g for 10 minutes (less than 10 per cent was insoluble). All the materials, including the sham injections of vehicle, were given intraperitoneally to 390 normal female Holtzman rats 9–12 weeks of age, weighing between 160 and 180 gm.

Catalase activity was determined by the method of Bonnichsen et al. (4), and the units and special technics for liver and kidney were described in a previous publication (12). Hemoglobin was measured as the metcyanide derivative at 540 μg (5); the bound iron and unsaturated iron-binding capacity were determined as described by Schade et al. (19).

RESULTS

The effects of a single injection of the NT and TT preparations are presented in Table 1. The liver and thymus weights were not changed significantly 24 hours after a single injection of these materials, but both the spleen and adrenals were enlarged by the TT preparation. Both TT and NT significantly decreased liver catalase; however, the toxohormone was effective at a much lower concentration. Kidney catalase was not depressed when 300 mg. of the NT preparation was injected, but was significantly (P < 0.01) decreased by a 50-mg. dose of TT. Hemoglobin was not affected by either preparation; however, plasma iron showed a marked reduction, especially with the toxohormone material. The plasma iron was reduced without a corresponding increase in unsaturated iron-binding capacity. Since the separate preparations of TT showed no significant variation in activity, data from several preparations were combined in these tables.

A summary of the effects produced at different periods of time after injecting 100 mg. of TT is presented in Table 2. While the liver and thymus gave no response at 24 hours, there was a noticeable increase in liver weight and an involution of the thymus 2–3 days after the injection. The spleen showed a gradual increase in weight from 16 through 72 hours, and the adrenal glands increased rather abruptly at about 10 hours. The maximum effect upon liver and kidney catalase occurred 36–48 hours after injection—for hemo...
globin, 72 hours, and for plasma iron, 8–16 hours after administration.

The effects of repeated injections of toxohormone are shown in Table 3. All doses were given daily except the 30-mg. dose, which was divided into three equal parts and administered at 8-hour intervals. The NT preparations and lower doses of TT did not affect the normal gain in body weight, but TT at or above 50 mg/day significantly depressed the gain in body weight. The dry weights of the liver, spleen, and adrenals were significantly increased by injections of TT. The NT preparation at a dose of 150 mg/day also produced a slight increase in the weight of the liver and spleen; however, the toxohormone was just as effective at a dose of 1.5 mg/day. Thymus involution and anemia were also produced by TT and were especially pronounced at the higher doses. Liver and kidney catalase and plasma iron were readily depressed by small single injections of toxohormone. It was, therefore, surprising that repeated injections failed to keep them at a low concentration.

The effect upon plasma iron 8 hours after injection of small amounts of the TT and NT preparation is presented in Table 4. The toxohormone produced as much decrease in plasma iron at the 0.1, 0.5, and 1.0 mg. dose level as the corresponding 100-fold amount of NT preparation. The unsaturated iron-binding capacity was not uniformly increased by the injections; therefore, a loss in the total iron-binding capacity occurred. The TT preparation produced a marked decrease in plasma iron at 8 hours when only 0.1 mg. was administered, whereas 25–50 mg. were required to reduce the liver catalase noticeably. The plasma iron should provide a good screening test for these preparations because it can be determined rapidly and reliably.

**DISCUSSION**

Previous investigators have tested the effects of toxohormone upon the liver catalase of mice. The results of the present investigation demonstrated that toxohormone had a similar effect upon the liver catalase of the rat and, in addition,
produced many of the other systemic effects normally associated with tumor growth in the rat. The reviews by both Begg (3) and Greenstein (9) give many references showing that rats bearing transplantable tumors are anemic and have low plasma iron. The increase in weight of the liver, adrenals, and splenomegaly have also been well documented, as well as thymus involution and decreased kidney catalase. Each of these changes occurred after injections of TT.

Although it does not necessarily follow that an extract of the tumor in any way resembles the products normally being released by the tumor to the host, the observation that all these changes can be produced in normal rats by an extract of the tumor is suggestive evidence favoring a tumor toxin. Some evidence for this idea was presented by Ono, Umeda, and Sugimura (18), who found that toxohormone injections produced changes in porphyrin metabolism similar to those found in the tumor-bearing animal. Hoshizima (11) showed that toxohormone prepared from human gastric cancer induced a parallel depression of ferritin iron and liver catalase. Malmgren (14) produced an increase in the mitosis of the livers of mice by injection of a cell-free extract from the tumor, and Fukuoka and Nakahara (6) found that toxohormone induced thymus involution in normal mice but did not cause adrenal enlargement. Other evidence on cancer toxin has been adequately reviewed by Nakahara and Fukuoka (17).

While every effort was made to use only the viable portions of the tumor in the preparation of TT, it is recognized that many dead and partially autolyzed tumor cells were used. This could account for some of the differences, since normal tissue probably contained very little of this type material. However, Greenfield and Meister (7) found that fractions from the necrotic and non-necrotic areas of the Walker tumor produced depressions of liver catalase of about the same order of magnitude.

The measurement of plasma iron, which was found to be more sensitive to toxohormone than

### TABLE 3

**THE EFFECTS OF DAILY INJECTIONS OF TOXOHORMONE FOR 7 DAYS**

<table>
<thead>
<tr>
<th>Material</th>
<th>No. rats</th>
<th>Control</th>
<th>Normal tissues (NT)</th>
<th>Tumor tissues (TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>18</td>
<td>6</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Change in body wt. (gm.)</td>
<td>+12</td>
<td>+15</td>
<td>+18</td>
<td>+14</td>
</tr>
<tr>
<td>Liver*</td>
<td>1000±40</td>
<td>1085±20</td>
<td>1240±50</td>
<td>1170±40</td>
</tr>
<tr>
<td>Thymus*</td>
<td>61±3</td>
<td>55±5</td>
<td>55±9</td>
<td>68±7</td>
</tr>
<tr>
<td>Spleen*</td>
<td>73±2</td>
<td>70±3</td>
<td>88±3†</td>
<td>92±5†</td>
</tr>
<tr>
<td>Adrenals*</td>
<td>10±1</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
</tr>
<tr>
<td>Liver catalase</td>
<td>5000±200</td>
<td>5000±300</td>
<td>4700±200</td>
<td>5000±300</td>
</tr>
<tr>
<td>Kidney catalase</td>
<td>3100±200</td>
<td>2700±300</td>
<td>3000±50</td>
<td>2800±300</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14±0.2</td>
<td>13.9±0.3</td>
<td>13.5±0.4</td>
<td>13.6±0.4</td>
</tr>
<tr>
<td>Plasma iron</td>
<td>246±10</td>
<td>278±42</td>
<td>229±25</td>
<td>207±18</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
† Significantly different from control, P < 0.01.

### TABLE 4

**EFFECT OF TOXOHORMONE ON PLASMA IRON**

(8 Hours after Injection)

<table>
<thead>
<tr>
<th>Material</th>
<th>No.</th>
<th>Amount (mg.)</th>
<th>Plasma iron (µg. per cent)</th>
<th>Unsaturated iron-binding capacity (µg. per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0</td>
<td>281±4*</td>
<td>73±12</td>
</tr>
<tr>
<td>Tumor tissue (TT)</td>
<td>7</td>
<td>0.01</td>
<td>247±23</td>
<td>85±18</td>
</tr>
<tr>
<td>Normal tissue (NT)</td>
<td>12</td>
<td>6</td>
<td>64±6</td>
<td>118±14</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
was liver catalase, appeared to be a valuable test system for further purification of toxohormone or for detecting its presence in other tissues of the tumor-bearer. The failure of the tumor extract to maintain plasma iron and catalase at a low level after repeated injections deserves further investigation. It has been shown by Adams and Roe (2) that repeated doses of hydroxylamine and of butter yellow produced catalase responses similar to those obtained with a single dose, i.e., a depression followed by a rise to normal. In contrast, Adams (1) found that repeated doses of tumor homogenate produced a sustained depression in liver catalase activity.

**SUMMARY**

The administration of an extract of the Walker carcinosarcoma 256 produced changes in normal rats similar to those found in rats bearing the Walker tumor. The alterations which occurred after a single or repeated injection of toxohormone were: anemia, decreased plasma iron, depressed activity of the liver and kidney catalase, increased weight of the liver, spleen, and adrenals, and thymus involution. The relationship of these findings to the concept of a cancer toxin was discussed.

Plasma iron was found to be 250-500 times as sensitive to toxohormone as liver catalase and therefore may prove valuable in the testing for toxohormone activity.

**REFERENCES**

Some Systemic Effects of Toxohormone

Ralph F. Kampschmidt, Mabelle E. Adams and Thomas A. McCoy

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