Further Studies on the Effects of Pituitary Growth Hormone (STH) on C3H Mice Bearing a Transplanted Mammary Adenocarcinoma

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The administration of pituitary growth hormone (STH) to C3H mice bearing transplanted mammary adenocarcinoma has been shown to cause an increase in the tumor and animal weights when the mice were sacrificed at the end of a 27-day injection period (5). It was also observed that the animals receiving STH appeared generally healthier than did their respective controls at the time they were sacrificed.

In view of these interesting observations it appeared desirable to study the effect of prolonged administration of STH (2, 3) on survival time, tumor weight, and body growth in C3H mice bearing transplanted mammary tumors.

EXPERIMENTAL

Series I

Methods.—Forty C3H mice approximately 3 months of age were divided into two equal groups. The animals were weighed, and uniform fragments of a relatively slowly growing mouse mammary adenocarcinoma, approximately 1 mm. in diameter, were implanted by cannula into the dorsal subcutaneous tissue. The experimental group of twenty mice received daily injections of purified STH, and the control group of twenty mice was given daily injections of saline. Injections were begun on the 3d day (D + 3) after implantation with an initial daily level of 0.6 mg. This dose was doubled at approximately 10-day intervals until the daily dose of 2.4 mg. was reached on D + 23. This level was maintained until the end of the experiment. Tumors of animals dying during the experiment were excised and weighed within a few hours of each animal’s death. The animals were necropsied on D + 50, the last injection being made at noon the previous day.

Tumors failed to grow in three animals in the control group and two animals in the experimental group. These animals were excluded in calculating the mean tumor weights.

Results.—The index of effectiveness of the purified STH on the total body weight of the experimental mice is shown by the statistical comparison to their respective controls in Table 1. Although the mouse is much less sensitive to STH than the rat, the organ weight changes are comparable to those in the rat (4); these findings will be reported more fully in a later paper.

The greater increment in total body weight observed in the STH-injected animals compared to that of their controls appears to be due to nontumorous tissue, since the control group showed a mean tumor weight of 12.08 ± 1.1 gm., while the STH-injected group had a mean tumor weight of only 4.87 ± 0.76 gm. (Table 2). The apparent increase of nontumorous tissue of the STH animals over their respective controls is further demonstrated by comparing the mean total body weight minus the tumor weights in both groups (see Table 1).

The mean total body weight increment of the STH-treated animals was 15.2 gm. over the 50-day period, while the mean total body weight increment of the controls was 11.9 gm. (Table 1). The STH-injected animals did not continue their rapid total body weight gain in the interval between D + 42 and D + 50. This cessation of rapid gain was probably due to a development of insensitivity to the dose of STH which had been continued on the same daily level since D + 23.

As reported in an earlier note (5), the tumor-bearing STH-injected mice appeared to be in better health than their controls. Although the pres-
ent experiment was not designed to determine the effect of STH on longevity in such mice, it was possible to compare the survival times in the animals dying before the termination of the experiment. Seven controls died before D + 50 after a mean interval of 31.2 ± 2.6 days, as compared to six STH-injected mice which died after a mean interval of 41.7 ± 1.5 days (Table 2).

**Methods.**—Uniform fragments approximately 1 mm. in diameter of a relatively slowly growing mouse mammary adenocarcinoma were implanted by cannula into the dorsal subcutaneous tissues of 34 3-month-old male CSH mice. The mice were divided into two equal groups, one receiving STH and the other being the saline-injected controls. The injections were begun on the day of tumor implantation (D day) with an initial level of 1 mg STH/day, and were raised by 2 mg. at approximately weekly intervals until the final daily dose of 14 mg. was reached. Injections were given once daily for the 1st month; from D + 32 until the termination of the experiment, they were given twice daily. The mice were weighed periodically during the experiment, and tumor measurements were made by calipers on D + 45, D + 76, and D + 87. Although caged together at first, the mice were isolated when their tumors became large. The tumors were excised and weighed within a few hours after the death of the animals.

**Results.**—The transplant failed to grow in three mice in each group, and the data were compiled on two groups of fourteen animals each. The non-tumor-bearing mice were similarly injected for 3 months, however, and served as further controls on the effect of STH. The three saline-injected mice without tumors showed a mean body weight increment of 5.5 gm. after 90 days, as compared to 19.7 gm. in the three STH-injected tumor-free animals.
fore, the heavier weight of the STH-injected mice during this period was due to nontumor tissue. Four mice of the control group and five of the experimental group showed the presence of gross metastases at death. These metastases were not sufficiently large or extensive to render invalid the comparison of the main tumor masses.

SERIES III

The results of the two previous series appeared to show a discrepancy with the findings in an earlier study from this laboratory (5). It was decided to repeat the study with a relatively fast-growing mammary adenocarcinoma and to follow the growth rate by mean diameter measurements with calipers in an attempt to determine whether the observed differences could be reconciled.

Methods.—Uniform fragments approximately 1 mm. in diameter of a relatively fast-growing transplantable mammary adenocarcinoma were implanted by cannula into the dorsal subcutaneous tissues of 33 C3H mice at approximately 3 months of age. Thirteen mice were injected daily with STH, and twenty mice were used as controls. Injections were begun on the 7th day after tumor implantation (D + 7) with an initial level of 1.0 mg/day. The daily dose was augmented, as is shown in Chart 1, until a final daily dose of 6.0 mg. was reached. Mice were weighed, and mean tumor diameters were determined with calipers at 5-day intervals beginning on D + 17. Necropsy was performed on all mice on D + 35, and all tumors were excised and weighed.

Results.—As may be seen in Table 5, the STH regimen was very effective in stimulating a body weight increment. The experimental mice showed a 50 per cent increase in body weight independent of tumor weight, whereas the controls remained stationary in this regard.

Although there was no statistically significant difference between the size of the tumors, either by weight at necropsy or by caliper measurements during the experiment, there appeared to be a trend for the experimental tumors to grow faster at the beginning of the experiment and for the control tumors to catch up and grow faster toward the termination of the experiment (Table 6).

DISCUSSION

There may be an explanation in Series III for the apparent discrepancy between the results of the first two series herein reported and those of the
earlier work (5). The main difference in the first two series and the earlier work is the time of comparison of the tumors of the experimental and control groups. In the earlier work, the tumors were compared on D + 27, at which time the STH-injected animals had significantly larger tumors (5). In the present study when tumors were compared on D + 50 in Series I, or D + 76 and D + 87 in Series II, the tumors of the control groups were significantly larger than the tumors of the STH-injected groups. Chart 1 demonstrates that in Series III the tumors of the STH-injected animals up to the time of measurement on D + 22 increased in size at a faster rate than the tumors of the controls. After this period of rapid growth the increase in mean diameter of tumors became less evident and then appeared to stop. The control tumors, however, continued to increase in size at the same rate during the later period.

It has previously been recognized in this laboratory that the dose of STH must be periodically increased to overcome the insensitivity developed by the mice to this hormone (5). In all of the experiments in this study, the step-ladder dose regimen was necessary in order to maintain a steadily rising growth curve. Although the somatic tissue continued to respond to STH, the tumor tissue did not, even at the highest dosages.

Ball et al. (1) showed that Carcinoma 256 grew more slowly in hypophysectomized rats, suggesting the possibility that the growth of some neoplasms is influenced by the normal functioning of the pituitary, including the secretion of growth hormone. It might be postulated that the growth rate of a transplanted tumor is at first influenced by the combined effect of endogenous and exog-

![Chart 1](chart1.png)

**TABLE 5**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN TOTAL BODY WEIGHT D + 7 (gm.)</th>
<th>MEAN TOTAL BODY WEIGHT D + 17 (gm.)</th>
<th>DIFFERENCE BETWEEN MEAN TOTAL BODY WEIGHT D + 7 TO D + 17 (gm.)</th>
<th>MEAN TOTAL BODY WEIGHT D + 17 MINUS TUMOR WEIGHT (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline-injected)</td>
<td>21.8 ± 0.5*</td>
<td>27.2 ± 0.8</td>
<td>5.4</td>
<td>21.2 ± 1.9</td>
</tr>
<tr>
<td>Experimental (STH-injected)</td>
<td>23.1 ± 0.9</td>
<td>36.0 ± 2.0</td>
<td>13.9</td>
<td>22.8 ± 1.3</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† n = Number of mice in each group.
‡The values in parentheses are the P values of Fisher obtained when the experimental groups are compared with their respective controls.

**TABLE 6**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN TUMOR DIAMETER D + 17 (mm.)</th>
<th>MEAN TUMOR DIAMETER D + 22 (mm.)</th>
<th>MEAN TUMOR DIAMETER D + 47 (mm.)</th>
<th>MEAN TUMOR DIAMETER D + 87 (mm.)</th>
<th>MEAN TUMOR WEIGHT D + 17 (gm.)</th>
<th>MEAN TUMOR WEIGHT D + 22 (gm.)</th>
<th>MEAN TUMOR WEIGHT D + 47 (gm.)</th>
<th>MEAN TUMOR WEIGHT D + 87 (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline-injected)</td>
<td>7.8 ± 0.58*</td>
<td>12.5 ± 0.74</td>
<td>17.1 ± 1.12</td>
<td>22.5 ± 1.16</td>
<td>6.0 ± 0.90</td>
<td>12.5 ± 0.74</td>
<td>17.1 ± 1.12</td>
<td>22.5 ± 1.16</td>
</tr>
<tr>
<td>Experimental (STH-injected)</td>
<td>11.1 ± 1.6</td>
<td>14.9 ± 1.9</td>
<td>16.0 ± 1.65</td>
<td>17.1 ± 2.49</td>
<td>3.1 ± 1.1</td>
<td>14.9 ± 1.9</td>
<td>16.0 ± 1.65</td>
<td>17.1 ± 2.49</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† n = Number of mice in each group.
‡The values in parentheses are the P values of Fisher obtained when the experimental groups are compared with their respective controls.
enous STH. That the tumor develops an insensitivity to STH after 3–4 weeks of injection need not be surprising in light of the consistent finding of a pronounced concomitant general somatic refractoriness. Stress should therefore be placed not so much on the success in maintaining a growth response in mice to STH for 3 months, but rather on the development of a remarkable refractoriness that necessitated a 14-fold increase in dosage in order to accomplish this. Any attempt to explain why the tumor is a more sensitive indicator of this refractory phase than other tissue or organs would move one into the realm of conjecture. One possibly relevant finding in the response of normal mice to STH (to be reported) is that the testes and their accessory organs showed a weight loss, whereas all other organs showed a weight gain.

**SUMMARY**

The administration of growth hormone to C3H mice bearing a transplantable mammary adenocarcinoma produced a significant increase in body weight, provided the dose of hormone was increased periodically to compensate for a refractoriness developed towards it.

In contrast to a previously reported experiment, however, the tumors were significantly smaller than those of the saline-treated controls at the end and during the latter part of the experiment. Repetition of the experiment showed that STH appears to cause an acceleration in the growth rate of the tumor for periods up to 27 days. After this stage the effect seems to be reversed, despite continued increase in somatic growth.

Emphasis is placed upon the refractoriness to STH shown in mice by the necessity of increasing its dosage to relatively heroic amounts. The transplanted tumors, especially the more slowly growing ones, proved to be more sensitive in reflecting this refractoriness than the somatic tissues.

**REFERENCES**

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