THE EFFECT OF THE ANTERIOR PITUITARY HORMONES ON THE GROWTH OF MOUSE SARCOMA

OTTO F. KREHBIEL, M.D., CUSHMAN D. HAAGENSEN, M.D., AND HERMA PLANTENGA, PH.D.

(From the Institute of Cancer Research of Columbia University, Francis Carter Wood, M.D., Director)

It has been suggested by H. Zondek, B. Zondek, and Hartoch (1) that the so-called ovarian follicle ripening component of the anterior pituitary hormone (Prolan A) may act to hinder tissue growth in general, and particularly the growth of tumors. To test this hypothesis, these authors carried out a series of experiments in which anterior pituitary hormone containing a mixture of both the follicle ripening component (Prolan A) and the luteinizing component (Prolan B) was administered to mice inoculated with transplantable carcinoma. The tumor used was the Frankfurt strain of Ehrlich mouse carcinoma, which had never regressed spontaneously in 1019 inoculations.

The plan used by the authors was to give their mice frequent and exceedingly large doses of the hormone. The injections were begun on the day following inoculation with the tumor and were continued for twenty-two days. The tail vein was used for all but the last 4 to 6 injections, which were given subcutaneously. From 0.1 to 0.2 c.c. of the solution of the hormone was injected daily. Each cubic centimeter contained from 500 to 1000 rat units. The maximum daily dose was therefore 200 rat units. On the 23d day the animals were killed and the tumor removed. The results can be summarized as follows:

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Number of Animals</th>
<th>Average Weight of Tumor in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>274</td>
<td>1.65</td>
</tr>
<tr>
<td>Controls treated with other hormones (thyroxin, adrenalin, insulin and grape sugar, hypophysin, folliculin)</td>
<td>750</td>
<td>1.55</td>
</tr>
<tr>
<td>Prolan*</td>
<td>405</td>
<td>0.2</td>
</tr>
<tr>
<td>Boiled prolan</td>
<td>64</td>
<td>1.5</td>
</tr>
<tr>
<td>Ashed prolan</td>
<td>24</td>
<td>1.55</td>
</tr>
<tr>
<td>Tumors treated with prolan, then removed and grafted into other animals. No further treatment. One transplantation</td>
<td>128</td>
<td>0.24</td>
</tr>
<tr>
<td>Same: 2 transplantations</td>
<td>124</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* In a series in which Prolan A alone was used the results were essentially the same.

The authors concluded that their experiments showed that transplanted mouse carcinoma can be inhibited by injections of Prolan.

Cannavo (5) has reported that independently he carried out an experiment much like that of Zondek and his collaborators, using the same Ehrlich mouse carcinoma. He treated "more than a hundred" mice, but does not present the results in detail. He concluded that treatment with Prolan retards the growth of grafted tumors.
Shortly after the publication of the findings of Zondek and his collaborators, Gross (2) reported that, in a series of experiments which antedated those of the above authors, he had administered anterior pituitary hormone to mice which had been inoculated with transplantable sarcoma. He gave comparatively small doses, only 10 rat units per day for from six to twelve days. He concluded that the hormone in such small doses slightly accelerated tumor growth. Gross did not present the details of his experiments.

Wiesner and Haddow (3) have recently tested the effect of administering bovine anterior pituitary extract as well as a gonadotropic preparation of pregnancy urine to rats inoculated with Jensen rat sarcoma. The animals were given six injections of these substances during the ten days prior to inoculation, and fourteen doses during the fifteen days following inoculation. Wiesner and Haddow do not venture to state in terms of mouse or rat units the potency of the hormones which they employed; their doses were comparatively large, however. In 24 treated animals no inhibitory effect, such as has been reported by Zondek, was observed. Indeed, the gonadotropic hormone from pregnancy urine seemed to stimulate the growth of the tumor slightly.

In view of this conflicting evidence, it seemed advisable to repeat this experiment. An attempt was made to follow as exactly as possible the technic used by Zondek and his collaborators. E. R. Squibb & Sons, through their director Dr. John F. Anderson, courteously undertook to prepare an anterior pituitary hormone which resembled as closely as possible that used by Zondek and his collaborators. It is called Follicute and is said to contain both the so-called follicle ripening and luteinizing components. It is in the form of a dry powder, each gram containing from 3000 to 15,000 mouse (Zondek-Aschheim) units, depending upon the particular lot of the hormone. A fresh aqueous solution was made up daily from this powder, since the hormone is said to be very labile in aqueous solution.

Zondek and his collaborators gave daily injections containing between 50 and 200 rat units per day. Our preparation of the hormone was calibrated in terms of mouse units rather than rat units. The relative values of mouse and rat units of anterior pituitary hormone are imperfectly known. It has been suggested that these units are approximately equal. If, however, the standard ordinarily applied in this type of biological work were used, namely that 1 rat unit equals 4 mouse units, the dose given by Zondek and his collaborators would amount to from 200 to 800 mouse units per day. A few preliminary trials showed us clearly that our preparation of the hormone when given in these relatively enormous doses was very toxic for mice, and that 200 mouse units per day was as much as a white mouse weighing 20 gm. would tolerate intravenously. Our experiments were therefore carried out with a dosage corresponding at least to the lower limits of the dose used by Zondek and his collaborators. Even with this dosage three-quarters of our animals succumbed to the toxic effects of the hormone, as will be shown in detail subsequently. The manufacturers made repeated ef-
forts to supply a less toxic and more concentrated preparation of the hormone. They were successful in increasing the concentration very materially, but the toxic effect still continued to be very marked for the high dosage used.

Mouse sarcoma 180 was selected as the most suitable tumor upon which to test the effect of the hormone. This polyhedral-cell sarcoma, which has been transplanted at this Institute for twenty years, is remarkable for the uniformity with which it grows. In a total of 23,457 inoculations done in this laboratory during the period mentioned, it has given 98.4 per cent of takes, and but 0.0034 of spontaneous regressions. These facts make it the most reliable tumor with which we are familiar for testing the therapeutic effect of materials claimed to inhibit the growth of cancer. Young white mice weighing from 15 to 20 grams were inoculated subcutaneously in the lateral abdominal region with 0.025 gm. of an emulsion of this tumor. On the following day the injections of the hormone were begun in the tail vein. A daily dose of 200 mouse units was given intravenously by this method for as long as could be kept up. When the intravenous method failed, the hormone was given subcutaneously.

Five series of experiments were carried out, in each of which there were twelve treated mice and twelve untreated controls. The rates of growth of the tumors plotted at weekly intervals for three weeks are shown in the accompanying charts. As is customary in such charts, ulceration of the tumor is indicated by a white area in the black outline of the tumor and death is shown by a cross.

Results

Experiment 1 (Series 180/354C): The mice were given the hormone intravenously for the first four injections. One mouse died on the fourth day. It was then necessary to continue the experiment with a new lot of the hormone. Since this did not dissolve readily enough in water to make it possible to give the dose of 200 mouse units intravenously, it was given subcutaneously. Four of the mice died the following day. In view of the great toxicity of the preparation, further treatment was discontinued. The mice in this group each received, therefore, a total of only 1000 mouse units of the hormone. The four treated animals which survived the twenty-one-day period of observation were not in good condition. Their tumors, as shown in the accompanying chart, were smaller than those of the controls. This difference, however, was not greater than that which would be expected in sick as compared with healthy animals.

Experiment 2 (Series 180/355J): Following the first injection, which was given intravenously, the mice did not look well. They had rough coats, and were apathetic and had rapid respiration. Two of them died five hours after the injection had been given. The next day a number of the mice had diarrhea. Nevertheless, it was possible to give the remaining animals, except for one which died of pneumonia on the sixth day, a total of twelve injections. Except for a few failures,
eleven of these injections were given intravenously, the last dose being given subcutaneously. These mice, therefore, each received a total of 2400 mouse units of the hormone. The four animals which survived the twenty-day period of observation had tumors which were about the same size as those of the controls, although the general condition of the treated animals was not as good as that of the controls.

Experiment 3 (180/3584): Except for mouse No. 12, which died of pneumonia on the second day, these mice received intravenous doses on the first six consecutive days. At the end of that time they did not look well, and treatment was omitted for a day. The following day the
hormone was given intravenously. The next day three mice were dead and the rest appeared to be in poor condition. It seemed advisable to give no treatment on this day. On the next, the tenth day after treatment had been begun, the mice were given another intravenous dose, with the result that three more were found dead the following day. The remaining five mice were nevertheless treated for seven days more, mostly subcutaneously. At the end of the twenty-one-day observation period the four mice remaining alive bore tumors which approximated in size those of the control group. These four had received a total of 3000 mouse units of the hormone. Their general condition had suffered considerably from the treatment.
Experiment 4 (180/360E): Four of the mice in this group were found dead the day after their first injection, which was given intravenously. Treatment having been suspended for a day, a second intravenous injection was given on the following day, with the result that three more mice succumbed. The remaining five mice were given seven intravenous injections on successive days. Three more then died, and further treatment was discontinued. The one treated mouse which survived the twenty-day observation period had a tumor which was very large. He had received a total of 1800 mouse units of the hormone.
Experiment 5 (180/364B): On the assumption that the dose given in the previous experiments had been disproportionately large in terms of the weights of the mice, the animals in this experiment were weighed each day and the dose calculated in terms of 200 mouse units per 20 grams of weight. At the beginning of the experiment the mice weighed between 14 and 18 grams. Two died following the first injection, which was intravenous. One day’s rest from treatment was given and the injections continued for nine days more. Almost all were given intravenously. The mice continued to die off as the injections were con-
tinued, so that on the twelfth day, when the last injection was given, only three mice remained alive. These weighed 13, 14, and 15 grams, respectively, and their general condition was poor. One of these mice died on the following day, so that but two survived the fourteen-day period of observation. One of these had a large tumor, the other a tumor which was slightly smaller than the average of those in the controls. The former had received a total of 1400 and the latter a total of 1600 mouse units of the hormone.
DISCUSSION

Out of the total of 60 treated animals but fifteen survived the period of observation. Of these fifteen, seven had tumors which were slightly smaller than the tumors in the controls, while the remaining eight had tumors which were as large or larger than those in the controls. Since the condition of all the treated animals suffered as a result of the injections, it would be expected that their tumors would be rather smaller than those of the untreated controls. These experiments fail to show, however, any specific and marked inhibitive action of anterior pituitary hormone on tumor growth as claimed by Zondek and his collaborators.

It should be noted that it has been impossible to demonstrate an inhibitive action of anterior pituitary hormone on tumor growth despite the use of doses of the hormone which are so enormous in terms of the amount of the hormone normally excreted in the adult human being that all calculations become relatively meaningless. Katzman and Doisy (4) have calculated that the average daily prolan excretion of adult males is 8 mouse units and that of adult females 10 mouse units. Yet 200 mouse units a day did not inhibit tumor growth in mice. These facts should discourage any attempts to use anterior pituitary hormone in the treatment of human cancer.

References

2. Gross, Ludwik: Zur Frage des Einflusses der Hypophysenvorderlappengeschlechts-