TUMOR INDUCTION AND TUMOR GROWTH IN HYPOPHYSECTOMIZED MICE

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Whereas numerous though often conflicting papers have been written on the relationship between tumor growth and the pituitary in general, only scanty data are available on the subject of tumor induction and tumor growth in hypophysectomized 1 animals. Since, with the exception of two papers, all the reports dealing with this subject have been published in this Journal, we will content ourselves with a brief summary of the conclusions which have been reached.

Ball and his associates have studied the influence of hypophysectomy on the growth of 1:2:5:6-dibenzanthracene sarcoma and grafted Walker carcinoma in the rat. They were the first (1, 2) to show that the growth rate of these tumors is markedly slower in hypophysectomized animals, and they further established that this retardation is directly dependent upon the absence of pituitary secretion (5, 7).

McEuen and Thomson, in an excellent paper (3), likewise come to the conclusion that hypophysectomy retards but does not prevent the growth of grafted Walker carcinoma, a conclusion incidentally confirmed by Franseen and McTiernan (6). McEuen and Thomson, however, are of opinion that the retardation is due not directly to lack of pituitary hormones, but to the ensuing cachexia. They observed the same retardation of tumor growth in grafted animals subjected to starvation.

Reiss, Druckrey and Hochwald (4), in a paper published simultaneously with that of the last-mentioned authors, report experiments carried out on 250 hypophysectomized rats. The tumor used was the Jensen sarcoma. The conclusions of this paper may be summarized as follows.

(a) If hypophysectomy is performed in a tumor carrier, the tumor grows much more slowly from the time of the operation onwards. In some cases it stops growing altogether or even regresses.

(b) If the grafting is performed after hypophysectomy, the tumor attains a diameter of 1.0 to 1.5 cm. and then in many cases regresses.

(c) When growth hormone is given to the hypophysectomized tumor bearer the tumor continues to grow, growth being proportional to the dose of hormone.

(d) The regression of the graft is hastened if the hypophysectomized animal is given folliculin.

(e) Extirpation of the middle or posterior lobe alone furnished no conclusive results. (The authors think themselves warranted in concluding from

1 Hypophysectomy designates surgical extirpation of the hypophysis. Experiments performed on animals with a view to suppressing pituitary function more or less completely, either by irradiation or administration of folliculin, are open to criticism, and are not discussed in this paper.
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FIGS. 1 AND 2. SKULL OF A NORMAL MOUSE (LEFT) AND SKULL SHOWING REMOVAL OF BONE PANEL (RIGHT)

The inset shows the hook used in cutting out the bone. A separate photograph of this with scale is shown in Fig. 4.

these experiments that the growth of tumors depends on the existence of an "Inkretmilieu," which may be one of the substrata of the "Tumorkrankheit."

As appears from the above review of the literature, the data available on the subject of the influence of hypophysectomy on tumor growth are scanty. All the experiments have been carried out on the rat. Since there is no technic which allows the operator to bring the whole gland of this animal into view before extirpating it, there is always a chance of performing an incomplete operation. Fragments of the gland may be left and be discovered only when histologic examination of serial sections of the skull base is made. For this reason and others, rats are not ideal material for such experiments.

One of us (8) has developed a technic in the mouse which allows the removal of a large panel of bone at the base of the skull (Figs. 1–3). The entire gland is thus extirpated under direct visual control. Since the publication of this technic, we have further simplified it in that we use a single hook (Fig. 4) for the operation instead of two, as formerly. This method dispenses with the necessity of serial cutting of the skull base, making experiments on a large scale easily possible. The operative mortality is low and the period of survival is eight to twelve months, exceptionally fourteen. This technic was used in the experiments described below, a preliminary report of which was published in 1937 (9).
The following experiments were carried out on $F_1$ hybrids. The mothers of the animals belonged to the albino strain "O20 Leeuwenhoekhuis," which has been produced by breeding brother to sister for twenty generations. The fathers belonged to the dilute brown Murray-Little strain. Animals of both sexes were used. As no difference was observed in the results, no further mention of sex will be made in this report.

Some of the animals had been hypophysectomized at the age of four to seven weeks, one to six months before the experiments were begun. After the operation the body weight decreased slightly, thereafter remaining constant for the rest of the animal's life. These two phenomena gave objective evidence of the thoroughness of the hypophysectomy, previously demonstrated by adequate exposure of the gland at the time of operation and finally controlled in some instances by serial sections of the skull base.

The mice were given a diet of milk, barley, hemp and canary seed, as well as the standard diet of laying hens (containing cod-liver oil, yeast and corn sprouts).

**Experiments with Transplanted Tumors**

The tumors used were T95379, T95548, and T95391, which are spontaneous mammary carcinomas occurring in females of the dilute brown Murray-Little strain. They had been transplanted respectively 81, 68, and

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**Fig. 3. Series of Removed Bone Panels**

Scale in half millimeters

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103 times in animals of the same strain, as well as in different F₁ hybrids of which the father was invariably a dilute brown. The grafting was done by injecting an emulsion of the tumor in saline under the skin of the back on the right side. As was to be expected from theoretical deductions, the grafts succeeded in every case.

Experiment A: Tumor T95379: (1) Nine animals, about three months of age, which had been hypophysectomized five weeks before, were grafted.
(2) Eight normal control animals of the same age were also grafted. These animals, of course, weighed more at the time of grafting than the hypophysectomized animals.

Experiment B: Tumor T95548: Three series of animals, as in experiment A, were injected with tumor emulsion. As tumor T95548 grows more slowly than T95379, the animals were allowed to live twenty-two days after grafting.

Experiment C: Tumor T95379: (1) Eight mice, about seven months of age, which had been hypophysectomized six months before, were grafted.
(2) Six normal control mice, three to four months of age, were also grafted.

Experiment D: Tumor T95391: (1) Five mice, about eight months of age, which had been hypophysectomized seven months before, were grafted.
(2) Two normal control mice, about four months of age, were grafted.

The results of these four series of experiments are presented in Table I. The weight in grams represents the average weight for each group of animals.

Discussion: In all 31 hypophysectomized mice the tumors grew. In 13 of these animals the hypophysis had been extirpated at least six months before the experiment, which eliminates the possibility of a postoperative action of the hormones. There were no differences in tumor growth and structure either grossly or microscopically between the hypophysectomized animals and the controls. Equal numbers of mitotic figures were observed in both series of tumors.
Table I: Experiments with Transplantable Tumors

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total number</th>
<th>Body weight before graft (gm.)</th>
<th>Final body weight (gm.)</th>
<th>Final tumor weight (gm.)</th>
<th>Increase of final tumor weight during experiment</th>
<th>Percentage of final tumor weight to original body weight</th>
<th>Percentage of final tumor weight to final body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>9</td>
<td>13</td>
<td>13</td>
<td>0.9</td>
<td>0%</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td>Controls same age</td>
<td>8</td>
<td>25</td>
<td>25</td>
<td>1.5</td>
<td>0%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Controls same weight</td>
<td>8</td>
<td>12.5</td>
<td>17</td>
<td>1.6</td>
<td>40%</td>
<td>13%</td>
<td>9%</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>9</td>
<td>14.5</td>
<td>16</td>
<td>1.0</td>
<td>11%</td>
<td>7%</td>
<td>6%</td>
</tr>
<tr>
<td>Controls same age</td>
<td>8</td>
<td>24</td>
<td>27</td>
<td>1.2</td>
<td>13%</td>
<td>5%</td>
<td>4%</td>
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<tr>
<td>Controls same weight</td>
<td>8</td>
<td>12.5</td>
<td>22</td>
<td>2.2</td>
<td>76%</td>
<td>17%</td>
<td>10%</td>
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<td>8</td>
<td>14</td>
<td>13</td>
<td>0.35</td>
<td>—</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>?</td>
<td>28</td>
<td>0.78</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
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<td>0.53</td>
<td>—</td>
<td>4%</td>
<td>4%</td>
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<tr>
<td>Controls</td>
<td>2</td>
<td>?</td>
<td>29</td>
<td>0.42</td>
<td>—</td>
<td>—</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

The percentage figures in the last two columns of Table I show that the tumors were relatively larger in the controls of the same weight than in the hypophysectomized mice or in the controls of the same age for experiments A, B and C. This does not hold true for experiment D, but since only 2 controls were used in this experiment this figure may not be representative for the group.

Except in experiment B, the body weight of the operated animals did not increase during the experiment. The increase of weight noted in group B is so slight that, apart from possible errors in weighing, it can be attributed to the growth of the tumor.

In adult animals the weight of the tumor was approximately proportional to the body weight. In other words, the actual weight of the tumor had the same relationship to the body weight in the hypophysectomized animals as in the control animals of the same age. As was to be expected, the rate of tumor growth was much greater in the series of younger controls.

Conclusions: (1) These experiments, with three types of transplantable tumor, show that the tumors grow perfectly well in animals hypophysectomized one to seven months previously.

(2) Comparison of the figures showing the final weight of the tumors in the hypophysectomized and in the control mice shows retardation of growth in the operated series. When these figures are considered in relation to the respective body weights of the grafted mice, however, there is no evidence of a difference in rate of growth between the hypophysectomized and the control mice of the same age.
(3) The fact that the grafted tumors grew perfectly well in hypophysectomized mice leads to the conclusion that the influence of the pituitary is certainly not qualitative. If any influence exists, it can be only quantitative in nature.

**Experiments with Induced Tumors**

In another series of experiments 3-4 benzpyrene in 0.5 benzol was used as the carcinogenic agent. The animals were F1 hybrids, described above.

*Experiment E:* At the beginning of this experiment the animals were three months old. Eight of them had been hypophysectomized four weeks previously. Eight others were used as controls; their weight was therefore much greater than that of the hypophysectomized animals. The skin of the neck was touched three times a week by a glass rod dipped in the benzpyrene solution. The hair was not removed before application of the benzpyrene. After a few weeks, however, the hair disappeared from the area of application in both series of animals. In both the controls and hypophysectomized animals there was later a regrowth of hair, more rapid in the former than in the latter. Periods of loss of hair alternated thereafter with periods of regrowth.

A papilloma was observed 259 days after the beginning of the experiment in one of the hypophysectomized mice. A carcinoma, without a preliminary papilloma, was observed in another mouse of this series at 300 days. The hypophysectomized animals died between nine and ten months after the beginning of the experiment, that is ten to eleven months after hypophysectomy.

Each of the control animals developed a papilloma within 54 to 126 days from the onset of the experiment, and a carcinoma within 141 to 255 days.

*Experiment F:* The following mice were used in this experiment.

1. Fifteen mice of about seven months of age that had been hypophysectomized at the age of six weeks.
2. Seven mice of about five months of age, which had also been hypophysectomized at six weeks.
3. Twelve control animals which at the beginning of the experiment had the same weight as the animals of Groups 1 and 2. These animals were then about six weeks old.
4. Twelve control mice, between three and six months of age. These were, of course, much heavier than the mice of Groups 1 and 2.

Since the animals of Groups 3 and 4 gave the same experimental results, no differentiation between them will be made.

In none of the animals in experiment F was the hair removed. Two drops of the carcinogenic agent (3:4-benzpyrene in 0.5 per cent benzol) were dropped from a dropper upon the skin of the neck, twice weekly. Since this solution evaporates quickly, a large portion of the benzpyrene remained in the hair and did not come in contact with the skin. After baldness had occurred only one drop of the carcinogenic agent was given.

The results of experiment F are given in Charts I and II. The hypophysectomized mice are represented in Chart I and the control mice in Chart II. The animals are arranged from above downward according to the order of appearance of the papillomas. In one animal (number 19) a carcinoma developed without a preliminary papillomatous stage. This animal's position in the table is determined by the date of appearance of the carcinoma.

In the control series all of the animals developed papillomas between 44 and 100 days, and carcinomas of the skin between 93 and 184 days.

Eight of the hypophysectomized animals died between 90 and 188 days without showing any tumor. The first four of these belonged to Group 1. Their death occurred before any of the other hypophysectomized animals showed a papilloma. These animals had survived hypophysectomy eight and a half months.

Fourteen of the hypophysectomized mice developed a tumor. Thirteen of them de-
developed a papilloma between 108 and 184 days; 10 of them a carcinoma between 121 and 190 days. In one of these 10 tumors histologic differentiation between papilloma and carcinoma was difficult. The 9 others showed the typical histologic picture of skin carcinoma.

Animals 24, 23 and 30, which developed papillomas between 187 and 192 days after the beginning of the treatment, died nine and a half months after hypophysectomy without carcinoma.

The following four animals did not develop a tumor. No. 22, dying 177 days after the beginning of the experiment and nine and a half months after hypophysectomy; No. 23, dying 188 days after the beginning of the experiment and eleven and a half months after hypophysectomy; No. 8, dying 143 days after the beginning of the experiment and ten and a half months after hypophysectomy; No. 27, dying 153 days after the beginning of the experiment and eight and a half months after hypophysectomy.

**Discussion:** The skin tumors induced by 3:4-benzpyrene developed in general much earlier in normal than in hypophysectomized mice. A number of the hypophysectomized mice did not develop a tumor. But since many of those that did develop a tumor showed it only a few days before death, it is
likely that, had these non-tumor animals lived longer, a tumor would have appeared. The average survival period of the hypophysectomized mice treated with benzpyrene was ten to twelve months. This time was evidently too short to allow all animals to develop a tumor.

The difference in result between experiments E and F, i.e. the low percentage of tumors in the hypophysectomized mice in E and the high percentage in F, notwithstanding the fact that in E the benzpyrene had been applied three times and in F twice a week, can be explained by the difference in the method of application of the carcinogenic agent. In Group F, the carcinogenic agent was dropped on the neck. In Group E it was applied with a glass rod, and therefore in this group there was a possibility that the solution did not always come in contact with the skin when the hair was abundant. This is substantiated by the observation that the tumors of the control series of ex-
Experiment F developed more rapidly than those of experiment E. In F the papillomas developed in an average of 75 days and the carcinomas in 127 days, while in E the papillomas developed in 104 days and the carcinomas in 207 days.

**Conclusion:**

1. These experiments prove that skin tumors can be induced in hypophysectomized mice.
2. With regard to the response to carcinogenic agents, the difference between hypophysectomized mice and controls is not of a qualitative nature. It is entirely quantitative.

**SUMMARY**

In this experimental study the influence of the hypophysis on tumor induction and tumor growth was studied in mice.

When the genetic constitution of the mice was compatible, transplantation was successful in every case. The tumor grafts grew more slowly in hypophysectomized animals than in controls of the same age, but the relation of the final tumor weight to the body weight of operated and control animals of equal age was the same.

Papillomas and carcinomas induced by 3:4-benzpyrene appeared markedly later in the hypophysectomized mice than in the controls.

With regard to the growth of tumor transplants and the response to carcinogenic agents, no qualitative difference was found between hypophysectomized mice and controls. The observed differences were entirely of a quantitative nature.

**LITERATURE**