IV. A STUDY OF THE INFLUENCE OF NUTRITIONAL FACTORS ON WALKER TUMOR 256 IN RELATION TO THE EFFECT OF HYPOPHYSECTOMY

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(McEuen and Thomson have previously observed the influence of degrees of starvation on the rate of growth of Walker tumor 256 and compared their results with the same tumor in hypophysectomized animals. They concluded that starvation may retard tumor growth as much as hypophysectomy. From the data presented, however, there was some question as to the validity of this conclusion. We have attempted to restudy the question, using a technic of feeding which we believe increases the accuracy of the experiment.

METHODS

Male rats about four months of age (150–180 grams) were inoculated with Walker tumor 256. The donor tumors were second, third, and fourth generation transplants in our hands from a tumor-bearing female of generation 154–B obtained through the courtesy of Dr. F. C. Wood of the Institute of Cancer Research of Columbia University. Solid tissue grafts were implanted by the trocar method into the left axillary region, through a skin incision in the groin. Ten days to two weeks later the animals were paired prior to removal of the pituitary in one mate. The operations were performed through a neck incision, pains being taken to avoid the nasopharynx. Completeness of removal was judged by the development of genital atrophy, and at death by direct inspection of the pituitary fossa from above.

In the first experiment the animals were paired on the basis of the same litter origin, while in the others body weight and tumor size were the determining factors. Each pair was placed on an experiment designed to control food intake. In the earlier experiments this took the form of paired feeding, the normal tumor-bearing animals receiving during twenty-four hours an amount of powdered stock diet equivalent to that consumed by the hypo-

1 This investigation has been made possible by a grant from the International Cancer Research Foundation.
physectomized mate during the preceding day. Later this was replaced by the more accurate method of gavage, using the following formula:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Parts by Weight</th>
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<tbody>
<tr>
<td>Powdered whole milk</td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
</tr>
<tr>
<td>Harris yeast extract</td>
<td>15</td>
</tr>
<tr>
<td>Water</td>
<td>q. s. to 300</td>
</tr>
</tbody>
</table>

The ingredients were first emulsified by vigorous stirring in a small quantity of water heated on a water bath, and then diluted to volume.

The appropriate quantity of the warm mixture was drawn into a hypodermic syringe and administered through a small firm-walled rubber tube suitable for passing through the esophagus. The hypophysectomized animals received during the experimental period 1 c.c. of this formula twice daily for each 40 grams of body weight at the beginning of the experiment, when tumor weight was negligible. The amount fed to the controls was adjusted so that their body-weight plus tumor-weight remained roughly equivalent to that of the hypophysectomized group. This resulted in the controls receiving approximately \( \frac{3}{4} \) the food given the hypophysectomized animals, a distinct restriction of voluntary intake, while the latter received a total average throughout the experimental period of more than they would have voluntarily consumed.

Tumor measurements were made in three planes twice weekly with a low-friction pincer-type caliper. Volumes and weights were calculated from these data as outlined by Schrek (9).

All animals were given Rubin-Krick (10) salt solution in place of drinking water because of the beneficial results on hypophysectomized animals noted in other experiments. The animals in Experiment 4 received 0.1 c.c. of adrenal cortex extract daily unless tumor necrosis was marked, under which circumstances the dose was repeated.

**Results**

In the paired feeding experiments where the hypophysectomized animals did not receive adrenal cortex extract a rapid reduction of food intake was noted, which resulted in death after a short period of observation. The charts of these experiments are presented as confirmatory evidence of the results found in the stomach-tube experiment in which a significant group of animals was observed for three weeks after hypophysectomy.

In all four studies the tumors of the hypophysectomized animals grew at a much slower rate than those of the controls even though in the paired feeding experiments they were larger at the time of operation. Table I shows the change in mean tumor volumes for the various groups of animals. Even though a representative number of individuals could be observed for only ten days after hypophysectomy in the paired feeding experiments, the curves of tumor size for the operated rats, while originally above the control curves, cross to the lower side. In Experiment 4, in which the rats were observed for three weeks after operation the control tumors were 3.3 times as large as

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2 Supplies of the extract were kindly furnished by the Upjohn Co., Kalamazoo, Mich.
TABLE I: Average Tumor Volumes* in Cubic Centimeters

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of Animals</th>
<th>Days after Inoculation</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>16 20 24 26 34 37</td>
</tr>
<tr>
<td>EXPERIMENT 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls, full diet, 16 tumors</td>
<td>10</td>
<td>0.46 2.16 10.76</td>
</tr>
<tr>
<td>Hypophysectomized, 14 tumors</td>
<td>9</td>
<td>0.56** 1.74 2.55 2.74</td>
</tr>
<tr>
<td>Controls, paired,†† 15 tumors</td>
<td>9</td>
<td>0.19 1.04 2.61 4.01 7.76 9.43</td>
</tr>
<tr>
<td>EXPERIMENT 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>8</td>
<td>0.29** 1.13 1.42 1.79</td>
</tr>
<tr>
<td>Controls, paired</td>
<td>9</td>
<td>0.21 1.68 2.56 3.50</td>
</tr>
<tr>
<td>EXPERIMENT 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>10</td>
<td>0.62** 1.61 2.22 3.02</td>
</tr>
<tr>
<td>Controls, paired</td>
<td>10</td>
<td>0.26 1.37 3.54 6.22</td>
</tr>
<tr>
<td>EXPERIMENT 4 (gavage)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>13</td>
<td>0.14** 0.89 1.22 2.24 3.34 5.12 7.19</td>
</tr>
<tr>
<td>Controls, paired</td>
<td>21</td>
<td>0.22 1.22 2.61 5.07 8.04 13.42 26.79</td>
</tr>
</tbody>
</table>

* Calculated from group-average geometrical mean diameters by Schrek's formula: 

\[ V = 0.5236d^3 \]

† Data based on individual tumors, not on animals.

* Hypophysectomy performed at this time.

†† These animals were given the same amount of food as that eaten by their hypophysectomized partners until the latter died; they were then continued on half of the average food per rat consumed by the fully fed controls.

The changes in tumor weights and the significance of the differences between the hypophysectomized and control groups in the stomach-tube experiment are shown in Table II. There was no significant difference between the groups at the end of four days; in fact the ratio between the tumors was not only less than at the time of operation (1.37 compared with 1.52) but the
ratio of difference to the probable error of the difference was less (1.4 compared with 2.4). By the end of a week, however, the control tumors were 2.14 times as large as those of the hypophysectomized animals and the difference between the two was 3.98 times the probable error of the difference. The change was therefore abrupt, occurring during the last three days of the first week after hypophysectomy. From this time on there was an increase in the difference both of relative size and significance.

The observation made by other investigators that limitation of food intake affects the rate of growth of tumors is borne out by the data for Experiment 2,

![Graph showing ratio of tumor weight to total weight in two groups.](image)

<table>
<thead>
<tr>
<th>Days after Inoculation</th>
<th>10</th>
<th>12</th>
<th>16</th>
<th>19</th>
<th>23</th>
<th>26</th>
<th>30</th>
<th>33</th>
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<tr>
<td>M</td>
<td>150</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Table I.** The growth of the tumors in normal rats receiving only half the food of their paired controls is definitely below that of the latter, but much above that of the hypophysectomized group. Food limitation has only a minor influence when compared with hypophysectomy.

The insignificance of general nutrition in the hypophyseal influence on tumor growth is further illustrated by the curves in Fig. 1 which represent the mean total weight of animal plus tumor, and of the animal only. The somatic tissues of the control animals decreased in weight at an accelerating rate; those of the hypophysectomized group, on the other hand, lost at a steadily decreasing rate. In consequence, although the mean weight of the animals without pituitary exceeded that of the controls by only 3 grams at
the beginning of the experiment, their normal body tissue weighed 25.7 grams more at the end. Even the weight of somatic tissue plus tumor exceeded the corresponding weight of the controls by 5.4 grams at the end.

Schrek (9) has shown that Walker tumor 256 grows at such a rate that the geometrical mean diameter, determined by taking the cube root of the product of the three dimensions of the tumor, increases as a straight linear function. The slope of this line is quite constant for different transplants. In Fig. 2 we have plotted the mean diameters for the various groups in our

![Figure 2: Mean Tumor Diameters for Four Experimental Groups](image)

The upper graphs show the results of paired feeding and of food restriction in intact tumor-bearing animals. —○— = full-fed controls; —⊕— = paired-fed controls, later restricted to half rations; —— —— —— = hypophysectomized animals. The lower graph shows tumor growth in stomach tube fed groups. ——— = controls; ——— ——— = hypophysectomized animals. The time of hypophysectomy is indicated by arrows. All curves have been extended from the first point of observation to the base line.

four experiments. Normal groups show a practically straight line function, although in Experiment 2 there seems to be a slight tendency for the slope to decrease after restriction of food intake. The hypophysectomized groups showed normal slopes before they were operated upon. Within a week after operation, however, the rate of increase of the mean diameter showed a relatively abrupt change. It continued then as a straight line function but with a slope only about half that of the earlier part of the curves or of the normal controls. This is seen in all four experiments but is most definite in the last, in which the hypophysectomized rats were followed sufficiently long to give a good series of points.
DISCUSSION

It is clear from these results that nutritional deficiency as judged by a restricted weight of functional body tissue is not an adequate explanation of the retarded tumor growth rate in hypophysectomized animals, as assumed by McEuen and Thomson. In the stomach tube feeding experiment reported here, the normal animals bearing tumors were so restricted in food intake that their total weights (body weight plus tumor weight) were similar to those of the hypophysectomized group. Under these circumstances the tumor growth rate may have been somewhat slowed as compared with that of the fully fed controls in other experiments, but the difference between the restricted controls and the hypophysectomized animals was still great. The amount of food transformed into tumor tissue in the controls was several times larger than that transformed into tumor in the hypophysectomized group, while that used in maintaining functional tissue was considerably less. There is, therefore, a change in the differential distribution of metabolites between body and tumor which requires explanation.

It can be concluded from our experiments that, while the changes observed following hypophysectomy do not prove a greater sensitivity of the tumor metabolism to pituitary secretion, they do indicate that the absence of the pituitary produces a greater effect on tumor tissue than on the remaining body cells.

The graphs of the mean diameters are important in indicating certain facts regarding the mechanism of pituitary influence. The change after removal of the hypophysis is not a gradual one due to atrophy of some gland, for in that case the curve after the operation would be a smooth one. Instead there is a sharp bend followed by maintenance of the new slope. This would indicate that the effect is a direct one due to the sudden removal of the pituitary secretion, and that in the absence of the latter the relative function of the different tumor cells remains the same but the whole process continues at a much slower rate. If this were not true we would expect the curve of the mean diameters to vary from a straight line in the same way that it does in Schrek's studies of tumors which cause host immunization (11). The cells of the tumor are therefore all simultaneously affected by pituitary removal. Further evidence bearing out this point will be presented in a subsequent paper.

In addition to the inferential evidence from the type of growth curve, that the tumor effect is directly dependent on the absence of pituitary secretion, there is direct experimental evidence that the influence is not due to the atrophy of the thyroid gland or adrenal cortex which follows pituitary removal. In our first experiments (2) the hypophysectomized rats were fed adequate amounts of thyroid gland, yet their tumors grew at a much slower rate than those of the controls. In the present study we supplied an active extract of the adrenal cortex in doses sufficient to maintain totally adrenalectomized rats in normal condition. This increased the length of life of the hypophysectomized tumor-bearing animals but did not prevent the retardation of tumor growth. Atrophy of the adrenal cortex is therefore not responsible for the effect, although it does decrease the length of life of the tumor-bearing hypophysectomized animals.
The researches of Lathrop and Loeb (12) and of Lacassagne (13) have shown that estrin, which specifically stimulates the mammary gland under normal conditions, will also affect the development and growth of mammary carcinoma. Since removal of the pituitary gland stops the production of this hormone, it might be inferred that the effects on the Walker tumor, originating from mammary tissue, were due to lack of this stimulus. However, fibrosarcomas originating in subcutaneous connective tissue after injections of 1:2:5:6-dibenzanthracene are also affected by hypophysectomy (5). The pituitary influence cannot, therefore, be looked upon as any specific organ relationship, but as a general effect on various types of tumor tissue.

**SUMMARY**

1. The growth of transplanted Walker carcinoma 256 was much slower in hypophysectomized male rats than in paired controls receiving the same amount of food.

2. When both control and hypophysectomized animals were fed by stomach tube so that the total weight of both groups remained approximately equal, the tumors of the control group were 3.3 times as large as those of the group without pituitary at the end of three weeks.

3. Since in stomach tube experiments the controls lost as much body weight as the hypophysectomized rats while the tumors grew larger, there must have been a definite difference in the distribution of metabolites between tumor and body cells in the two groups.

4. The change in tumor growth was relatively abrupt after pituitary removal, and was not a progressive change such as would result from atrophy of some dependent organ.

5. All cells of the tumor must have been equally affected, since the subsequent tumor growth was of the same type as during the control period, but slower.

6. In previous work we have shown that the thyroid secretion is not a factor. The present study indicates that the adrenal cortex can also be excluded as an explanation of the effects observed.

**REFERENCES**