Tumor Development in Adrenalectomized Rats Given Inoculations of Aged Tumor Cells after Surgical Stress*

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SUMMARY

Inoculation of Walker 256 tumor cells after surgery yielded tumors in 51.3 per cent of 78 sham-adrenalectomized rats and in only 35.1 per cent of the 168 rats not operated upon, confirming previous findings that operative stress increased host susceptibility to inoculated tumor cells. In the 69 adrenalectomized rats tumor incidence was 66.7 per cent, although adrenalectomy retarded the growth rate of tumors by approximately one-half. In the second experiment, involving re-operation 6 weeks after adrenalectomy, the above results were confirmed: tumor incidence was 64.3 per cent in adrenalectomized, 47.7 per cent in sham-adrenalectomized, and 22.6 per cent in rats not operated upon.

These results suggest that adrenal glands do not contribute to the decrease of resistance to tumor implantation seen after surgery. This deleterious effect of operations was shown to last less than 4 days.

It has been shown recently (5, 15, 16, 22) that surgical stress decreases the resistance of experimental animals to inoculated tumor cells. We have no proof that this decreased resistance to tumor cells is sustained by the human being after operation, but we suspect it occurs in a moderate number of patients. This possibility is supported by the fact that most surgeons (6, 9) have observed an occasional tumor which revealed very invasive tendencies shortly after operation, and metastasized rapidly, thus resulting in death much earlier than expected. Under such circumstances it is obvious that cancer cells must have disseminated from the original tumor before it was removed, but they had not yet grown to a detectable size.

Buinauskas et al. (5) have shown that the incidence of tumors developing from Walker 256 cancer cells inoculated subcutaneously into rats was about twice as high in animals having a celiotomy at the time of inoculation as in control animals not having operation. Similar results have been obtained by Moore (16). Lewis and Cole (15) and Schatten (22), working independently with different tumors in mice, found an increased incidence of pulmonary metastases when a tumor-bearing extremity was amputated 21 days after inoculation of cells. However, amputation of the well leg did not increase the number of pulmonary metastases in either group. Schatten and Kramer (23) noted no effect of anesthesia alone.

Hyperactivity of adrenal glands in response to stress (24) could be responsible for the phenomenon discussed above. Only a few reports have dealt with growth of tumors after adrenalectomy (4, 11, 12, 21, 25). The recent investigators, Ingle and Baker (11) and Talalay et al. (25), found in adrenalectomized rats a retardation of tumor growth by approximately one-half. How-
ever, in none of these investigations were the tumors inoculated immediately following surgical stress.

**MATERIALS AND METHODS**

Female albino rats of the Holtzman strain were used. Two experiments were performed, representing the sum of several experimental series. Each series consisted of adrenalectomized, sham-adrenalectomized, and control rats not operated upon. In the first experiment a single operation was performed. In the second experiment adrenalectomized and sham-adrenalectomized animals were re-operated (sham-adrenalectomized) after a 6-week rest period.

Adrenalectomized rats drank 1 per cent saline fortified with 5 per cent Sustagen\(^1\) and 1 per cent glucose; the sham-adrenalectomized and rats not operated upon received 5 per cent Sustagen in 1 per cent glucose solution.\(^2\) In the first experiment there was an additional group of controls not operated upon which received plain water to determine any effect of Sustagen on tumor development.

**Operative technic.**—Intraperitoneal 0.32 per cent nembutal, 92 mg/kg body weight, was used for anesthesia. Adrenalectomies and sham-adrenalectomies were performed on alternate rats. Operations were made particularly stressful for maximal effect. A dorsal midline skin incision was made two inches long. Muscles and fasciae on each side were incised for one inch, parallel and medial to the twelfth ribs. The adrenal glands with peri-adrenal and adjacent peri-renal tissue were removed (but not removed in the sham-adrenalectomized rats), the intestines were massaged gently, and the wound was left unsutured for 45 minutes. The skin was then closed with a continuous #20 cotton suture.

**Preparation of aged cell suspensions** of the Walker 256 carcinosarcoma (5, 8) (as modified during this study).—Three firm, non-necrotic tumors were used for each cell suspension. Strict sterile technic was employed. Chemically clean apparatus was used.

After the tumors were excised and trimmed, they were placed on glass shells, together with 5 ml. of saline each, and cut into fine particles with curved iris-scissors. The resulting suspensions were filtered through a wire-gauze filter (80 wires per square inch, doubled) to eliminate clumps of cells. The combined suspension was stored at 72°–75° F. for 6 or 12 hours, to reduce the incidence of tumors in control animals to below 100 per cent. In the first experiment tumor cells were aged for 6 hours and inoculated immediately, or 48 hours, or 96 hours, after operation. In the second experiment the cells were aged for 12 hours and inoculated immediately after the second operation.

The cells were counted shortly before inoculation. Eosin, 1:2,000, was used as diluting fluid in a white blood cell pipette. All cells stained pink were disregarded in the count as nonviable. The final suspension contained 25,000 cells/ml. One ml. of this suspension was inoculated subcutaneously into the anterior abdominal wall. The percentage of rats developing tumors and the growth rate of tumors were evaluated. Rats were examined regularly to establish the length of the latent period of tumors (from inoculation to becoming palpable). Tumor-free rats were retained for 3 months from the time of inoculation.

**RESULTS**

**Incidence of Tumors**

**First experiment** (Table 1).—In groups given inoculations immediately after operation, a significantly higher percentage of sham-adrenalectomized rats developed tumors as compared with control rats (P = 0.006). The incidence was even higher in adrenalectomized animals. In animals given inoculations after 48 hours, small differences were still apparent but were not statistically significant (P > 0.05), except for the difference between adrenalectomized and rats not operated upon (P = 0.01). No appreciable difference in tumor incidence was observed in groups given inoculations 96 hours after operation.

**Second experiment** (Table 2).—This experiment served to confirm the first. Adrenalectomized re-operated rats showed a significantly higher incidence of tumors than did sham-adrenalectomized re-operated rats (P = 0.04), and the latter had more tumors than did controls not operated upon (P = 0.02).

**Tumor Growth**

While adrenalectomy as compared with sham-adrenalectomy resulted in increased tumor incidence, it retarded growth of tumors. This was in spite of an equal average latent period (Table 3). Similar retardation of growth of tumors in...
adrenalectomized rats was seen in the second experiment (Table 2).

DISCUSSION

Subcutaneous inoculation of Walker tumor cells may be analogous to implantation of cancer cells in the wound during an operation, which leads to recurrence of cancer in some patients. Surgery is still the best method of treatment of cancer whatever deleterious effect the operation itself may have. However, if the stress of operation does result in a decreased resistance to their cancer in a certain percentage of patients, and if one could find the agent responsible for this tumor-promoting effect of operations (5, 6, 9, 15, 16, 22) and other forms of stress, it might be possible to remove or neutralize this factor and thus improve the ultimate “5-year survival” rate. If adrenal glands were the responsible agent one would expect the percentage of tumors developing in adrenalectomized rats to be less than in sham-adrenalectomized rats. In this study, the incidence

4 S. Gines and E. Trevino (Dept. of Surgery, University of Illinois, Chicago, Illinois): Effect of Anesthesia on the “Take” of the Walker 256 Tumor (to be published).

5 J. D. Griffith, Increased “Take” of Walker 256 Tumor in Rats Following Hypothermia and Cold Stress. Presented at the Surgical Forum of the American College of Surgeons, 1959 (to be published).

TABLE 1

TUMOR INCIDENCE IN ADRENALECTOMIZED RATS

<table>
<thead>
<tr>
<th>Time of inoculation</th>
<th>Adrenalectomized</th>
<th>Sham-adrenalectomized</th>
<th>Not operated upon controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. rats</td>
<td>Rats with tumor (per cent)</td>
<td>Total no. rats</td>
</tr>
<tr>
<td>Immediately after operation</td>
<td>69</td>
<td>66.7</td>
<td>78</td>
</tr>
<tr>
<td>48 hours after operation</td>
<td>35</td>
<td>51.4</td>
<td>43</td>
</tr>
<tr>
<td>96 hours after operation</td>
<td>31</td>
<td>38.7</td>
<td>33</td>
</tr>
</tbody>
</table>

* Given inoculations subcutaneously of 25,000 tumor cells aged for 6 hours.
† The incidence of tumors in the control rats drinking Sustagen was the same as in the controls drinking water; therefore, combined results are presented in this table.

TABLE 2

TUMOR INCIDENCE AND GROWTH IN ADRENALECTOMIZED RE-OPERATED RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no. rats</th>
<th>Rats with tumor (per cent)</th>
<th>Latent period of tumors (days)</th>
<th>Rate of tumor growth (gm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomized, re-operated</td>
<td>56</td>
<td>64.3</td>
<td>32</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Sham-adrenalectomized, re-operated</td>
<td>48</td>
<td>47.8</td>
<td>35</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Controls (anesthetized)</td>
<td>37</td>
<td>22.6</td>
<td>34</td>
<td>0.38 ± 0.04</td>
</tr>
</tbody>
</table>

* Given inoculations subcutaneously of 25,000 cells aged for 12 hours, immediately after second operation.

TABLE 3

GROWTH OF TUMORS IN ADRENALECTOMIZED RATS

<table>
<thead>
<tr>
<th></th>
<th>Adrenalectomized</th>
<th>Sham-adrenalectomized</th>
<th>Controls (not operated upon) drinking Sustagen</th>
<th>Controls (not operated upon) drinking water</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumors</td>
<td>76</td>
<td>67</td>
<td>55</td>
<td>52</td>
</tr>
<tr>
<td>Latent period (mean day of appearance after inoculation)</td>
<td>14.4</td>
<td>14.3</td>
<td>14.7</td>
<td>13.8</td>
</tr>
<tr>
<td>Mean weight of tumors at time of sacrifice</td>
<td>6.8</td>
<td>11.5</td>
<td>11.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Mean rate of tumor growth (grams per day)</td>
<td>0.76 ± 0.03</td>
<td>1.30 ± 0.09</td>
<td>1.27 ± 0.08</td>
<td>1.33 ± 0.10</td>
</tr>
<tr>
<td>Mean weight gain of rats (per cent initial body weight per day)</td>
<td>1.70</td>
<td>1.53</td>
<td>1.72</td>
<td>1.76</td>
</tr>
</tbody>
</table>

* Given inoculations subcutaneously of 25,000 tumor cells aged for 6 hours, within 4 days after operation.
of transplanted tumors was significantly higher in adrenalectomized than in sham-adrenalectomized rats. This suggests that a factor other than adrenal glands is responsible for the deleterious tumor-promoting effect of surgical stress (hypothalamus, hypophysis, ovaries, testes, and thyroid gland are being studied and will be the subject of subsequent reports). Extensive studies on hormone-tumor relationship (10, 14) have often yielded contradictory results, particularly in the case of cortisone. Several workers have reported enhancement of metastatic growth of experimental tumors following cortisone treatment (1, 2, 3, 7, 19); others have found no significant change (23, 25). Heterotransplantation is facilitated by cortisone (26). On the other hand lymphoid neoplasia is usually inhibited by cortisone not only in animals but also in man (18). It is possible that the physiological response of adrenal glands to stress is protective against tumors, but adrenal insufficiency and excess of adrenal hormones are both harmful to the host, except in the case of lymphoid tumors.

The apparent paradox of increased tumor incidence and decreased tumor growth in the adrenalectomized rats emphasizes the differences inherent in the study of incidence (popularly called "take") as contrasted with the study of growth of transplanted tumors. The "alarm reaction" (24) due to an operation lasts less than 4 days and is largely over within 24 hours. It may be expected to influence establishment of tumor cells but not growth rate of tumors many days later. On the other hand, adrenal insufficiency can influence not only incidence but also growth of tumors. Furthermore, any acquired immunological response would affect later growth rather than the early development of tumors.

The special diet used after adrenalectomy achieved normal weight gain in rats without the stress of forced feeding and without steroid replacement, thus avoiding the introduction of additional variables. Sustagen had no direct influence on tumors.

Aged Walker tumor cells were used to achieve an incidence of tumors in control rats below 100 per cent, so that tumor enhancement as well as tumor inhibition could be studied. Following the inoculation of even 1,000 fresh cells, tumors tend to develop in all the rats.

ACKNOWLEDGMENTS

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REFERENCES


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