Systemic Effects of Tumors in Rats*

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Systemic effects of tumors are regarded as the changes produced in tissues of the host which are remote from the tumor and in which no evidence of metastatic malignant cells is found. They may be anatomical, of a nature described by Parsons (17), or the type of biochemical change reviewed by Greenstein (11).

It has been suggested on histological grounds that the clinical state of malignant cachexia is due to hypofunction of the adrenal (6), and this view may be supported by certain clinical studies (18, 19, 34). In view of the number of metabolic factors known to be influenced by the adrenal cortex (30), the possibility that some systemic effects could be explained by a primary action on the pituitary-adrenal system was investigated.

Liver catalase activity was chosen for study as a well established systemic effect (1, 2, 7, 12), and adrenalectomy leads to a diminished activity of liver catalase (4). Hemoglobin and liver catalase have a common prosthetic group (29), and anemia has been related to tumor growth (28) and to the adrenal cortex (32).

The enlarged adrenal low in ascorbic acid and cholesterol which has been described in the tumor bearing animal (22, 14) would be compatible with exhaustive hypofunction of the adrenal cortex (24), particularly in conjunction with the diminished liver glycogen deposition (35) and lymph node hypertrophy (15) which have been demonstrated in tumor-bearing mice.

It was decided to study these multiple systemic effects in a single animal at different stages of tumor growth, and the rat was chosen to provide the required amount of tissue. Thymus weight was followed as an example of lymph tissue, for it was assumed to react to experimental procedures in a manner similar to lymph nodes (31).

METHODS
Young, male Sprague-Dawley (Holtzman) rats¹ were maintained on a diet of Purina Fox Chow and tap water in a room maintained between 72° and 78° F. The rats were bred in the laboratory or supplied by the Holtzman-Rolfsmeyer Company,¹ the latter rats being adapted to the animal room for at least 10 days before use. Bilateral grafting was done aseptically when the rats were approximately 6 weeks of age, a tumor suspension being used. The Walker 256 carcinoma was grafted either subcutaneously or intramuscularly, but only the latter method was used for the Jensen sarcoma. Normal male rats served as controls and were killed at the same time as tumor-bearing rats. Sixteen hours before sacrifice the rats were placed in clean cages without food but with water supplied ad libitum. At this time hemoglobin was determined on tail blood (8).

The rats received intraperitoneal injections of sodium pentobarbital in warm, normal saline at a dose level of 5 mg/100 gm body weight. Under anesthesia the abdomen was opened, the right adrenal transferred to formal saline, and the left placed in a dish of ice-cold normal saline. A second operator removed a piece of liver for glycogen determination (9). This was rinsed in ice-cold saline, blotted dry, weighed, and introduced with minimum delay into a tube of hot 30 per cent potassium hydroxide. Meanwhile, the left adrenal had been freed of adherent fat, blotted dry, sectioned with a razor blade, and weighed on a torsion balance while wrapped in cellophane. One piece was introduced into trichloroacetic acid for estimation of ascorbic acid (21). The other half was homogenized with a loose pestle in a tube containing acetone, and an equal amount of absolute alcohol was added for the extraction and determination of cholesterol (27). The remainder of the liver was ground and extracted for the estimation of liver catalase activity (12) and the thymus weighed on a torsion balance. After 48 hours' fixation, the adrenals were washed in running tap water for an hour,

¹ Holtzman-Rolfsmeyer Co., Rt. 4, Madison 5, Wis.

* Aided by grants from the Donner Foundation, the National Cancer Institute of Canada, and the Nova Scotia Division of the Canadian Cancer Society.

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Received for publication December 6, 1950.
sectioned on a freezing microtome, and stained with Sudan IV (5).

The body weights recorded are those prior to sacrifice and include both tumor and carcass weight. The tumors were measured in two diameters and are presented as the mean diameter of both measurements. Tumors were not weighed in all cases, but sufficient data are available to state that in the 20-mm. group they formed approximately 5 per cent of the body weight, in the 30-mm. group 15 per cent, and in the 40-mm. group 30 per cent of the body weight. With the sub-

### TABLE 1

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>Body weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>170 ± 4(7)</td>
</tr>
<tr>
<td>10 mm.</td>
<td>160 ± 7(16)</td>
</tr>
<tr>
<td>20 mm.</td>
<td>161 ± 7(10)</td>
</tr>
<tr>
<td>30 mm.</td>
<td>173 ± 11(10)</td>
</tr>
<tr>
<td>40 mm.</td>
<td>182 ± 12(10)</td>
</tr>
</tbody>
</table>

± Standard error of the mean. Number of observations in parentheses.

### TABLE 2

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>Adrenal weight (mg.)</th>
<th>Thymus weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4 ± 0.8(7)</td>
<td>387 ± 8.3(7)</td>
</tr>
<tr>
<td>10 mm.</td>
<td>17.5 ± 0.4(10)*</td>
<td>428 ± 5.2(12)</td>
</tr>
<tr>
<td>20 a</td>
<td>18.8 ± 0.3(10)†</td>
<td>234 ± 4.5(10)</td>
</tr>
<tr>
<td>30 a</td>
<td>21.3 ± 2.0(10)*</td>
<td>288 ± 8.6(6)</td>
</tr>
<tr>
<td>40 a</td>
<td>30.4 ± 4.0(10)†</td>
<td>156 ± 20(0)</td>
</tr>
</tbody>
</table>

± Standard error of the mean. Number of observations in parentheses.

* P < 0.05 in t test.
† P < 0.01 in t test.

In Table 4 the effects on adrenal cholesterol and ascorbic acid have been recorded. The values for cholesterol and ascorbic acid fall to 60 per cent of the control at the 40-mm. stage.

### TABLE 3

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Hemoglobin (gm/100 ml)</th>
<th>Liver catalase activity (IU/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4 ± 0.3(7)</td>
<td>1570 ± 74(6)</td>
</tr>
<tr>
<td>10 mm.</td>
<td>16.2 ± 0.8(10)</td>
<td>9070 ± 128(10)</td>
</tr>
<tr>
<td>20 a</td>
<td>11.8 ± 1.2(10)†</td>
<td>1520 ± 116(10)</td>
</tr>
<tr>
<td>30 a</td>
<td>10.7 ± 1.1(10)†</td>
<td>1352 ± 128(10)</td>
</tr>
<tr>
<td>40 a</td>
<td>8.6 ± 0.5(10)*</td>
<td>735 ± 81(10)*</td>
</tr>
</tbody>
</table>

± Standard error of the mean. Number of observations in parentheses.

* P < 0.01 in t test.
† P < 0.05 in t test.

Histological examination revealed a loss of sudanophilia from the adrenals of the tumor-bearing rats. The loss of sudanophilia paralleled the loss of cholesterol from the adrenal.

### TABLE 4

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>Adrenal cholesterol (mg/100 mg)</th>
<th>Adrenal ascorbic acid (mg/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.65 ± 0.52(7)</td>
<td>0.415 ± 0.018(7)</td>
</tr>
<tr>
<td>10 mm.</td>
<td>4.16 ± 0.38(10)</td>
<td>0.572 ± 0.018(10)*</td>
</tr>
<tr>
<td>20 a</td>
<td>3.79 ± 0.24(10)†</td>
<td>0.594 ± 0.017(10)†</td>
</tr>
<tr>
<td>30 a</td>
<td>3.25 ± 0.45(10)</td>
<td>0.514 ± 0.029(10)†</td>
</tr>
<tr>
<td>40 a</td>
<td>2.97 ± 0.44(10)*</td>
<td>0.538 ± 0.027(10)†</td>
</tr>
</tbody>
</table>

± Standard error of the mean. Number of observations in parentheses.

* P < 0.05 in t test.
† P < 0.01 in t test.

The Walker 256 carcinoma at an early stage of growth reduces the food intake of the host, and as the tumor increases in size the carcass loses weight (16). The suggestion has been made that this may be a factor in the loss of liver catalase activity in the light of the known effects of starvation on liver catalase (7). Experiments have been reported which indicate that in the force-fed tumor-bearing...
rat no loss of carcass weight occurs, but systemic effects are present (3). Thus, failure to record food consumption in this study does not render the results invalid.

The present experiments are not in agreement with the statement that tumors forming 5 per cent of the body weight cause a diminution in catalase activity (11) but do agree with observations that half the liver catalase activity is lost from animals bearing tumors of 15–30 per cent of the body weight (7). The catalase effect appears to be biphasic, as has been reported by Greenstein in rats (19) but not noted by Adams, who found an asymptotic relation between tumor weight and percent inhibition of catalase in mice (1).

The earliest significant effect produced by a tumor in this investigation is the hypertrophy and loss of ascorbic acid from the adrenal at the 10-mm. stage, followed by anemia at the 20-mm. stage. That the degree of anemia is a consistent and reproducible finding in tumor-bearing rats is suggested by the fact that comparable groups of rats bearing the Walker carcinoma yielded hemoglobin values of 8.34 ± 0.49, 8.50 ± 0.40, and 8.03 ± 0.44 gm/100 ml at intervals of 6 months.

It was suggested that diminished liver catalase activity and anemia might be associated with hypofunction of the adrenal cortex in the tumor-bearing rat. Anemia has been produced by adrenalectomy in mice (32), and it has been demonstrated that adrenalectomy lowers liver catalase activity in the rat (4). But the diminution in catalase activity in the rat from which the adrenals are removed is only half as great as in the tumor-bearing animal. Thus, it is unlikely that hypofunction of the adrenal cortex is a major factor in the production of anemia and loss of liver catalase activity in the tumor-bearing rat.

The observed involution of the thymus in the tumor-bearing rat might be regarded as indicative of hyperfunction of the adrenal cortex (25) or inanition (20). Extrapolation to another species should be done with caution, but thymus involution and lymph node hyperplasia have been observed in tumor-bearing mice (15, 22) and appear to be independent of the pituitary (23). This throws some doubt on the interpretation of the present data, and further studies should be done to investigate the response of lymph nodes as well as the thymus to the presence of a tumor in the rat, and the role of the adrenal (39).

The results obtained from the study of adrenal weight, adrenal ascorbic acid and cholesterol, and sudanophilia might be interpreted as following the pattern of the stress reaction as exemplified by the Type III adrenal response of Sayers (24), on an extended time scale. This could be the result of an initial stimulation of the adrenal cortex being maintained and leading to eventual cortical exhaustion.

The progressive fall in adrenal cholesterol and ascorbic acid would suggest that the host was approaching the stage of adrenal cortical failure (24). In the absence of the determination of peripheral effects known to be influenced by the secretions of the adrenal cortex these changes in the adrenal are difficult to interpret. The determination of liver glycogen in rats starved for 10 hours gives such low results, and the data are so variable, that these experimental findings have not been presented. A plot of mean per cent change does indicate an initial increased storage of glycogen, with a diminution in the amount of liver glycogen in the terminal state. This problem should be studied with the use of intraperitoneal glucose (35).

It is probable that a state of adrenal cortical deficiency does occur in the tumor-bearing animal in the terminal stages of cancer, but further experimental substantiation is required. All the systemic effects cannot be explained on the basis of this deficiency, and some mechanism must be responsible for the many factors known to be altered in the tumor-bearing host.

As the result of the present and related investigations it is necessary to explain how the presence of a tumor at a remote site brings about a stimulation of the adrenal cortex, a diminution in the amount and activity of hemeproteins, and an involution of the thymus. Neither hormonal nor nutritional explanations seem to be adequate.

In his original studies on liver catalase activity in tumor-bearing rats, Greenstein suggested that "the effect of the transplanted tumor on the liver catalase is elicited by a toxic substance produced in the tumor and carried by the blood to the liver" (18). A recent abstract reports that such a substance can be extracted from a tumor (10). Adams favors the release of some substance from a tumor as an explanation of the diminished liver catalase activity of mice (1). A consideration of the systemic effects of tumors may lead to the revival of the concept of a cancer toxin and stimulate further research along this line.

A claim for specificity in relation to malignant tumors cannot be made for the observed systemic effects, and no explanation is available to account for these characteristic effects in tissues remote from a tumor. At the same time, they are manifestations of the profound metabolic changes produced in the host, and, as such, it is felt that they play an important part in the fatal outcome of cancer.
SUMMARY

Tumor-bearing rats exhibit enlargement of the adrenal with loss of ascorbic acid and cholesterol, atrophy of the thymus, diminution in liver catalase activity, and progressive anemia.

The thesis that rats bearing large tumors are in a state of hypofunction of the adrenal cortex requires further substantiation, and in any event such a state would not explain the observed systemic effects.

ACKNOWLEDGMENTS

The author is indebted to Dr. H. P. Rusch of the McArdle Memorial Laboratory for donor rats carrying the Jensen sarcoma and the Walker 256 carcinoma, to Dr. G. B. Mider for technical assistance. The work was begun during the tenure of a British Council Scholarship at the Sir William Dunn School of Pathology, University of Oxford.

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