The Histopathology and Biochemistry of Rats Bearing an Adrenocortical Tumor

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SUMMARY

An adrenocortical tumor originating in a female Osborne-Mendel rat was transplanted to intact and adrenalectomized males and to female rats of the same strain. The transplanted tumor grew best in intact males, less in intact females, and least in adrenalectomized males. The following changes characterized the animals bearing this tumor: atrophy of zona fasciculata and zona reticularis of the adrenal cortex; a sharp reduction in urinary sodium excretion, both in intact and adrenalectomized rats; increased urinary potassium excretion; hypernatremia and hypokalemia. These findings suggest mineralocorticoid secretion by this tumor.

Atrophy of the vagina and uterus, and anestrus are among the nonspecific changes due to the presence of the tumor. Polyuria in these rats and its relief with pituitrin may indicate pituitary dysfunction.

Spontaneous carcinomas of the adrenal cortex are reported to be rare in rats (2, 5, 6). However, Snell and Stewart (10) found primary adenomas or carcinomas of the cortex of the adrenal gland in 53 of 59 inbred NIH Osborne-Mendel rats 18 months of age or older. Iglesias (6) found a spontaneous adrenocortical tumor which was transplantable and functional in a female A X C strain rat. Adrenal tumors have been found in two radiothyroidectomized rats (2), in gonadectomized rats (5), and in rats bearing estrogen pellets (3). The induction of adrenocortical tumors and their hormonal secretory activities have been extensively studied in mice (1, 11). We observed carcinomas of the adrenal cortex in four of 55 female rats of inbred NIH Osborne-Mendel strain fed a low protein diet containing p-dimethylaminoazobenzene (8). These tumors were transplantable. This paper describes the histological and biochemical changes occurring in rats bearing this tumor.

MATERIALS AND METHODS

Fifty-five female Osborne-Mendel rats were fed a low protein semi-synthetic diet containing 0.06 per cent p-dimethylaminoazobenzene for 286 days. Four of these rats developed adrenocortical carcinomas. These tumors were successfully transplanted, and one line has been continued through 25 transplant generations. This tumor in the fifth generation was transplanted subcutaneously into twelve male and twelve female Osborne-Mendel rats about 10 weeks old. An equal number of rats of comparable age, sex, and weight served as controls without tumors. All the rats were kept in the same room, in similar cages, and received the same diet and care. Each rat was weighed at the start of the experiment and at the time of sacrifice. The liver, lungs, spleen, heart, kidneys, adrenal glands, and gonads were weighed, and pieces of tissues from these and other organs were prepared for microscopic examination. Tissues were fixed in Zenker's acetic fluid, and microscopic sections were stained with hematoxylin and eosin. Some sections were stained by the periodic acid-Schiff reaction, and others with Mayer's mucicarmine stain.

The rats were examined at regular intervals for general health, and the size of the tumors was estimated by palpation and recorded. When the tumors were estimated to be about 2 cm. in diameter, daily vaginal smears were made on the tumor-bearing females, for a period of 4 weeks. A similar procedure was carried out on tumor-free control rats. An additional 50 male rats were later given subcutaneous inoculations of the same tumor. Bilateral adrenalectomy was performed on a group of male rats, through paravertebral incision under

1 Adrenalectomies were skillfully performed by Louis Stroud, whose technical assistance is acknowledged.
anesthesia produced by pentobarbital given intraperitoneally. The adrenal glands were removed intact with the adherent fat tissue, and the operation was successful in over 25 per cent of the rats. After the operation, the animals received 0.9 per cent saline in place of drinking water. One month after adrenalectomy, these rats were given subcutaneous inoculations of the same tumor. When the transplants grew and were visible as subcutaneous nodules, saline was replaced with tap water. Adrenalectomized tumor-bearing rats considered in the results are rats from which two intact adrenal glands were removed, in which tumor transplants grew, and which did not show any visible accessory adrenal bodies at necropsy. Twenty-four-hour urine samples were collected from these tumor-bearing rats at all stages of tumor growth after the tumors were visible. Urine and spleen were frequently invaded. Metastases rarely involved other organs. Figures 1 to 6 illustrate the microscopic structure of the transplanted tumor and its metastases.

The weights of experimental and control rats and rates of tumor growth are given in Table 1. Growth of the tumor as determined by its terminal weight was slower in female than in male rats, and slowest in adrenalectomized male rats (0.5 gm/wk/100 gm). The average weight of lungs, spleen, and kidneys in both male and female rats, and of liver in males, was considerably increased in tumor-bearing rats, whereas adrenal glands, ovaries, oviducts, and seminal vesicles weighed about half as much as the respective organs in control rats (Table 2).

The heart in tumor-bearing males averaged about 13 per cent heavier per animal than in

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor-bearing</td>
<td>Normal</td>
</tr>
<tr>
<td>Body wt., start</td>
<td>214±30</td>
<td>207±24</td>
</tr>
<tr>
<td>% gain in body wt.</td>
<td>27</td>
<td>92</td>
</tr>
<tr>
<td>Tumor wt.</td>
<td>44.5</td>
<td>20.4</td>
</tr>
<tr>
<td>Rate of tumor growth (gm/wk/100 gm body wt.)</td>
<td>1.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Twelve rats in each group. S.D., standard deviation.

volume, specific gravity, sodium, potassium, non-protein nitrogen, and protein nitrogen were determined on the collected urine samples. Blood for chemical and hematological study was obtained by cardiac puncture. Serum and urine sodium and potassium were determined by a flame photometric method. Nitrogen was determined by the Nesslerization method, sugar by Folin's copper reduction method, and volume of packed cells by the Wintrobe method.

**RESULTS**

This transplantable adrenocortical carcinoma grew in 100 per cent of the inoculated intact rats, both male and female. The tumor grew in only 80 per cent of the adrenalectomized male rats and had an induction period of 4 weeks, or about twice that seen in the intact male rats. Transplants metastasized to distant sites in most of the animals. Lung and liver were common sites of metastasis, and mesentery, adrenal gland, control rats. This difference was increased to 36 per cent when calculated on body weight basis.

Study of the vaginal smears revealed that all tumor-bearing female rats were in continuous anestrus, whereas control animals were in various phases of the estrous cycle during the same period.

*The tumor.*—The histologic structure of the transplanted tumors grown in male, female, and adrenalectomized male rats was essentially the same. Microscopically, the transplanted tumors were composed of either multiple small nodules (Fig. 1) or larger solid masses of tumor cells partially divided into irregular lobules by connective tissue septae (Fig. 2). The tumors were well circumscribed and usually partially encapsulated. The individual tumor cells were uniform in size but variable in shape. They were mostly polygonal, but round, oval, and irregular shapes were present. The cells were arranged in cords or in small nests, adjacent to or surrounded by capillary spaces. The cytoplasm of the cells was abundant,
granular, and acidophilic. The nuclei were round or oval, regular in size and shape, with one or two large nucleoli and irregular distribution of nuclear chromatin. Mitoses averaged two to three per high power field. In some of the cells the nucleus was stained darker or pyknotic, and the cytoplasm was homogeneous and deeply acidophilic (Fig. 3). The stroma of the tumor was indistinct. The tumors contained numerous slitlike vascular spaces and usually a few dilated sinusoidal spaces or veins. Nearly all the tumors contained small to extensive foci of necrosis with small foci of calcification in some of them. The were slightly smaller than those of the control rats. The cytoplasm was decreased in amount, was less clearly defined, and the cells appeared closer together. Some of the nuclei appeared normal, but many were stained darker, and there was a variable increase in the number of cells with pyknotic nuclei.

**Genital organs.**—There was considerable atrophy of the vagina and uterus of the tumor-bearing rats (Figs. 9, 10). The vaginal epithelium was reduced in thickness and in many areas was composed of tall columnar cells, the cytoplasm of which was periodic acid-Schiff-positive and mucin-
MULAY AND PRICE—Adrenocortical Tumor in Rats

Kidneys.—On histologic examination, the kidneys of the tumor-bearing rats revealed no specific pathologic changes. Protein casts were present in the distal convoluted tubules of nine but were absent in fifteen of the tumor-bearing animals. No casts were observed in the controls. The lesion described by Snell and Stewart (4) was found in an occasional distal tubule in but six of the tumor-bearing rats and was present in only minimal degree.

Spleen and lymph nodes.—The lymph nodes of approximately half of the tumor-bearing rats appeared normal. The remaining lymph nodes were about equally divided between those with depletion and those with proliferation of the lymphoid elements. The spleen likewise varied from normal to either atrophy or hyperplasia of the lymphoid tissue. Many of the lymph nodes and spleens contained foci of hemopoiesis. There was also decreased amount of parenchymal lymphoid tissue in the trachea, lungs, and intestines of tumor-bearing animals. The bone marrow was studied in a few animals and was hyperplastic in the tumor-bearing rats. No recognizable thymus tissue was found in the tumor-bearing rats.

| TABLE 3 |
|-----------------|-----------------|-----------------|
| SPECIFIC GRAVITY OF URINE AND DAILY URINARY EXCRETION OF ELECTROLYTES AND OF NITROGEN BY INTACT AND ADRENALECTOMIZED CONTROL AND TUMOR-BEARING RATS |
|                  | CONTROL RATS    | TUMOR-BEARING RATS | ADRENALECTOMIZED RATS |
|                  | No. observations | Mean ± S.D.         | No. observations | Mean ± S.D. | No. observations | Mean ± S.D. |
| Specific gravity | 18              | 1.054 ± 0.003      | 12              | 1.020 ± 0.002 | 22              | 1.008 ± 0.005 |
| Na (mg.)         | 104             | 19.1 ± 3.9         | 100             | 9.5 ± 3.7    | 100             | 50.8 ± 11.7 |
| K (mg.)          | 104             | 44.0 ± 5.4         | 100             | 6.3 ± 2.1    | 100             | 178 ± 21 |
| K/Na Ratio       | 104             | 2.4 ± 0.7          | 100             | 178 ± 21     | 100             | 178 ± 21 |
| Nitrogen (mg.)   | 104             | 165 ± 54           | 100             | 178 ± 21     | 100             | 178 ± 21 |

Biochemistry.—When the adrenal tumor grew to about 2 cm. or more, as determined by palpation, polyuria and polydipsia were observed in tumor-bearing rats. Twenty-four-hour urine samples were collected on 120 normal rats, and 100 tumor-bearing rats were divided into two groups, “early” and “late.” The tumors in the “early” group had been growing for less than 10 weeks and were less than 3 cm. in diameter, as estimated by palpation, whereas those in the “late” group had been growing for more than 10 weeks and were more than 3 cm. in diameter. The average urine volume for normal rats was 3.6±0.4 ml. per rat per day, for the “early” group 13.8±2.3 ml., and that for the “late” group 29.8±5.2 ml.

The results on the specific gravity of the urine and urinary excretion of electrolytes and nitrogen for intact and adrenalectomized tumor-bearing rats and tumor-free controls are given in Table 3. Tumor-bearing rats, intact and adrenalectomized, excreted in their urine less sodium and more potassium than did their tumor-free counterparts, and their K/Na excretion ratio was more than doubled. The urine of tumor-bearing rats had lower specific gravity than the urine of control rats. The urinary nitrogen excretion of the tumor-bearing rats was slightly higher than that of the controls. However, in four tumor-bearing animals with extensive metastases to lungs and liver, nitrogen excretion was 50 per cent higher.

Five tumor-bearing rats received daily intramuscular injections of 1 unit of pituitrin for 7 days. Daily urine volume of each of the rats after the treatment was reduced to about one-half of that before the treatment (Table 4).

Results of serum electrolytes and blood sugar studies are given in Table 5. The sodium and chloride concentration of the serum of tumor-bearing rats was increased, potassium concentration decreased, and the Na/K ratio was significantly higher than that for tumor-free rats. There was no...
significant difference between the fasting blood sugar concentration of tumor-bearing rats and that of the control animals. An erythrocyte volume of 15–20 per cent was noted in some tumor-bearing rats, as opposed to an erythrocyte volume of 45–55 per cent in normal tumor-free rats.

**DISCUSSION**

This transplantable adrenocortical carcinoma, although originating in female rats, thrives best in intact males, less in intact females, and least in adrenalectomized male rats. In this respect it differs from the adrenocortical tumor of the rat, reported by Cohen et al. (3), which grew best in adrenalectomized male rats, less in intact males, and not at all in females, and from the tumor reported by Iglesias and Mardones (7) which grew best in intact females. The adrenal glands of the tumor-bearing animals reported here were atrophic, a finding noted previously by Cohen et al. (2), Iglesias and Madrones (6), Snell and Stewart (10), and Mulay and Eyestone (8). In our series, however, the zona glomerulosa remained uninvolved. Atrophy of the zonae fasciculata and reticularis suggests inhibition of secretion of ACTH by the pituitary gland. The probable cause of this inhibition is secretion of corticoids by the tumor resulting in maintenance of a high blood corticoid level, thereby inhibiting ACTH secretion. The animals bearing this tumor were hypernatremic, the urinary sodium excretion of tumor-bearing rats (both intact and adrenalectomized) was sharply reduced, potassium excretion was slightly increased, and the ratio of potassium/sodium excreted was more than doubled. These findings suggest secretion of mineralocorticoids by this tumor.

All the rats bearing this adrenocortical tumor were polyuric and polydipsic, a finding noted by Snell and Stewart (10) in Osborne-Mendel rats bearing transplanted adrenocortical tumor 494, by Cohen et al. (2) in mice bearing transplanted adrenal tumor, and reported in many cases of Cushing's syndrome (9) in the human. Rats made polyuric by this adrenocortical tumor differed from those reported by Snell and Stewart (10) in that (a) polyuria in the former responded to pituitrin whereas that in the latter did not respond to pitressin; (b) no significant renal changes were found in the former whereas there were marked renal changes in the latter. Polyuria, low specific gravity of the urine, and significant reduction of polyuria by pituitrin injections suggest failure of the pituitary gland to secrete necessary hormone.

Adrenocortical tumors that are functional have been known to influence electrolyte metabolism, carbohydrate metabolism, and sexuality in both males and females. The adrenocortical tumor reported here influenced electrolyte metabolism of the host, indicating mineralocorticoid secretion. There is no evidence for secretion of sex hormones.

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**TABLE 5**

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Tumor-bearing rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No observations</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Na (meq/l)</td>
<td>21</td>
<td>144 ± 3.9</td>
</tr>
<tr>
<td>K (meq/l)</td>
<td>21</td>
<td>43 ± 0.25</td>
</tr>
<tr>
<td>Na/K ratio</td>
<td>21</td>
<td>31 ± 1.8</td>
</tr>
<tr>
<td>Cl (meq/l)</td>
<td>15</td>
<td>111.6 ± 9.2</td>
</tr>
<tr>
<td>Sugar (mg. %)</td>
<td>15</td>
<td>123 ± 10</td>
</tr>
</tbody>
</table>

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Fig. 1.—Transplanted tumor. The tumor cells are grouped in the form of small nodules. X100.

Fig. 2.—Transplanted tumor. The tumor cells form a solitary large mass. X100.

Fig. 3.—Transplanted tumor. Note the relationship of the tumor cells to the capillaries and the presence of many cells with dark staining cytoplasm and pyknotic nuclei. X290.

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All sections were stained with hematoxylin and eosin.

We are grateful to the Biometry Branch of the National Cancer Institute for statistical analysis of all results.
The purpose of this paper was to study the primary growth of leukemic cells. It was found with the help of organ assay that organs were invaded from intraperitoneal exudate. It appears from the results on peritoneal exudate that this invasion is also by blood route at the expense of the primary growth, to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It is suggested that tissue growth from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is paralleled by their trend to grow as free cells in the blood, and to infiltrate into organs is a specific characteristic of malignant cells due to their reduced adhesiveness.

The relatively slight difference between primary growth in the peritoneal exudate was overshadowed by the early massive blood-borne invasion of organs. For that reason, for this strain the role of implantation from peritoneal exudate was overshadowed by the early massive blood-borne invasion of organs. It was concluded that primary localization of tumor cells on the 6th day was higher for leukemic cells than for nonleukemic tumors, inversely to the results of their subcutaneous growth. This was attributed to their high tendency to early spread in the blood.

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Fig. 4.—Metastasis to lung. Both large and small metastatic nodules are seen. X100.

Fig. 5.—Metastasis to spleen. X100.

Fig. 6.—Metastasis to adrenal gland. X100.
tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For transition from peritoneal exudate was overshadowed by tissue growth earlier and to a greater extent than organs were invaded from intraperitoneal growth earlier and to a greater extent than organs were invaded from intraperitoneal growth.

leukemic cells into organs was the essential lethal factor. However shorter survival of all leukemic strains the difference in the amount of tumor cells in serous body fluids (Table 1). Thus, primary localization of tumor cells on the 6th day was higher for leukemias than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is paralleled by their trend to grow as free cells in the blood, and to infiltrate into organs is paralleled by their trend to grow as free cells in the blood. It appears from the results on leukemias reported above and those on other tumors (6) that both tendencies vary only quantitatively for leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs is paralleled by a difference in survival (Table e), as well as their shorter survival (Charts 1 and ~) as well as their shorter survival.

PERITONEAL EXUDATE. It appears from the results on tumors that both tendencies vary only quantitatively for all the mouse tumors investigated (leukemias). However shorter survival of all leukemic strains the difference in the amount of tissue growth 'from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is a specific characteristic of malignant cells due to their reduced adhesiveness (1).

primary growth in the peritoneal exudate indicated the role of primary growth as early massive blood-borne invasion of organs. For transition from peritoneal exudate was overshadowed by tissue growth earlier and to a greater extent than organs were invaded from intraperitoneal growth.
Fig. 7.—Adrenal gland, control male rat. Compare with Figure 8. ×100.

Fig. 8.—Adrenal gland, tumor-bearing male rat. Atrophy of the cortex, particularly the zona fasciculata and zona reticularis, is marked. Zona glomerulosa is relatively uninvolved. Compare with Figure 7. ×100.

Fig. 9.—Uterus, control rat. Compare with Figure 10. ×75.

Fig. 10.—Uterus, tumor-bearing rat. The atrophy of the wall and the submucosal fibrosis are apparent. ×75.
The purpose of this paper was to study the primary growth of leukemic cells (7). The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For transition between various strains of leukemias compared to those bearing scantier local growths factor in leukemias. However, shorter survival of suggests that metastatic infiltrative growth of all leukemic strains the difference in the amount of implantation from peritoneal exudate was overshadowed by tissue either at the site of inoculation or from the opposite tendencies to spread extensively into organs. It appears from the results on leukaemias reported above and those on other tumors either at the site of inoculation or from the results of their subcutaneous growth. It may be presumed that tissue growth 'from benign and their reduced adhesiveness (1). While the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is paralleled by their trend to grow as free cells in the blood, and to infiltrate into organs is the essential lethal factor. This was attributed to the role of primary growth as source of metastatic infiltrative growth in organs is paralleled by their trend to grow as free cells in the blood-borne infiltrative growth in organs was the essential lethal factor. For this strain the role of implantation was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from peritoneal exudate. It appears from the results on peritoneal exudate. It appears from the results on peritoneal exudate. It appears from the results on peritoneal exudate. It appears from the results on subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); the shortest survival periods in the intraperitoneal series and the longest in the flank series. It was found with the help of organ assay and their reduced adhesiveness (1).

REFERENCES

male or female. Atrophy of the female genital organs and continuous anestrus observed in these rats may be nonspecific changes due to the presence of the tumor and cannot be interpreted as evidence for sex hormone secretion. Among other symptoms reported in human cases of adrenal tumor, edema and obesity (4) were found in some tumor-bearing rats, but albuminuria and diabetes mellitus (9, 4) were absent.

The other changes described, those involving the lymph nodes, spleen, and bone marrow, are probably secondary changes brought about by the presence of the tumor, rather than being primarily the result of hormone secretion.

REFERENCES
The Histopathology and Biochemistry of Rats Bearing an Adrenocortical Tumor

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