A Study on the Biologic Activity of a Transplanted Granulosa-Cell Tumor in Castrate C57 Mice

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The purpose of this report is to present the results of a study on the growth of a specific transplanted granulosa-cell tumor and the progressive effects it has on the host tissues.

METHODS

Fifty-three mice, 3—4 months old, of the C57 black strain were used, 18 females and 35 males. All the animals were castrated at the beginning of the experiment and were maintained on Laboratory Chow and water ad libitum. They were weighed weekly. Eleven females and 21 males each received a transplant of a small fragment of a granulosa-cell tumor (18C57), which was introduced subcutaneously with a No. 15 gauge trocar 1—6 days following castration. The tumor used in this study was in its fifth transfer generation and showed no change from its original histological structure (Fig. 1). Seven females and fourteen males which did not receive tumor transplants were used as controls. All control animals were sacrificed 60 days after castration. Following transplantation each animal was examined daily until a palpable tumor nodule was present. Daily vaginal smears were taken from 1 week after castration until the time the animals were sacrificed. All tumor-bearing animals were sacrificed at intervals of from 62 to 100 days following transplantation. At autopsy the tumors and visceral organs were weighed and then preserved in Bouin’s fixative for histological study. Gross mounts of the mammary glands were made.

OBSERVATIONS

A tumor was considered a “take” when a distinct, firm nodule approximately 5 mm. in diameter could be palpated. The first nodule did not occur until the 18th day following transplantation. By the 28th day after transplantation 22 “takes” had occurred. At the end of the 52d day “takes” were palpated in all the animals with transplants. There was no significant difference between the onset of the tumor “takes” of the males and females, although the tumor grew more rapidly in the males than in the females.

At autopsy, the animals with tumors had dilated and slightly heavier hearts than did the controls (Tables 1 and 2).

All the livers of the tumor animals, except those from the two with the smallest tumors, were enlarged.

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<tr>
<th>TABLE 1</th>
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<td>AVERAGE ORGAN AND BODY WEIGHTS OF CASTRATED MALE MICE (C57) BEARING A TRANSPLANTED GRANULOSA-CELL TUMOR</td>
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<td>AVERAGE ORGAN AND BODY WEIGHTS OF OVARIECTOMIZED FEMALE MICE (C57) BEARING A TRANSPLANTED GRANULOSA-CELL TUMOR</td>
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<td>Uterus (mg.)</td>
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† Post-doctorate research fellow, National Institutes of Health.
‡ Post-doctorate research fellow, National Institutes of Health.

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larged and congested, and their surfaces presented a peculiar mottled appearance. This congestion and mottling was more marked in animals with large tumors, and mice with tumors over 5 gm. in weight had livers weighing 2–3 times more than those of the controls (Tables 1 and 2). Microscopically, the livers of the tumor-bearing animals showed dilatation of the blood sinusoids, varying from a slight to a very marked degree (Figs. 3–6). This progressive dilatation of the sinusoids was correlated with the increase in tumor weight. The sinusoidal dilatation set in rather rapidly and was noted in all livers except in those of one female and one male which had the smallest tumors—1.56 gm. and 1.13 gm., respectively. In all animals with tumors over 3.00 gm., moderate to extreme dilatation was noted, with most of the liver substance displaced by large, dilated blood sinususes. Although there was an increased blood volume in the tumor-bearing animals, the progressive increase in body weight was approximately equal to the increase in tumor weight (Tables 1 and 2). The spleens of these animals increased progressively in size and weight with the tumor weight (Tables 1 and 2) and microscopically showed vascular congestion. No increase in hemopoiesis was noted.

There was no significant change in the weight of the kidneys in the tumor animals as compared to those in the control animals (Tables 1 and 2). Microscopic examination of the kidneys of the castrated male control animals revealed that they were of the male type in which the greater percentage of Bowman's capsules have a parietal epithelium consisting of cuboidal cells (Fig. 7). The parietal epithelium of the remaining small percentage of capsules consisted of squamous cells. In four female control animals the number of the female type was slightly predominant, while in three female controls there was approximately an equal number of both types. The kidneys of the male and female tumor-bearing animals had capsules of the female type, with a parietal epithelium consisting of squamous cells, while only a small number of male-type capsules was seen (Fig. 8).

Histological examination of the submaxillary glands revealed that all the controls had an altered female type, which is not uncommon in castrated male and female as well as intact female mice of the C57 strain (unpublished data). This altered type is similar to that produced by injecting progesterone into castrated male and female mice (9). The lining epithelium of the terminal tubules of these altered female type glands consisted of columnar cells containing little or no secretion granules. The nucleus was situated at the basal portion of each cell (Fig. 9). Eight male animals and three female animals with tumors of less than 3.95 gm. in weight had submaxillary glands of this altered female type. All the animals with tumors weighing 3.95 gm. or more had glands which exhibited the characteristic features of the estrogen-stimulated female type described by Lacassagne and Fekete (2, 3). The lining epithelium of the terminal tubules of these glands consisted of cuboidal cells with the nucleus located in the center of the cell (Fig. 10).

No change was noted in the adrenal or pituitary glands. The X-zone of the adrenal glands was either extremely degenerated or absent. Dilatation of the cortico-medullary sinuses of the adrenal gland was not noted in any animal in this study.

The seminal vesicles and prostate glands of the male tumor-bearing animals were atrophic.

The weight of the uteri of the female animals with tumors increased progressively as the weight of the tumor increased (Tables 1 and 2). The females with the largest tumors had uteri 4–5 times heavier than those of the controls. Histologically, the uteri of the control females were of the castrate type, while the uteri of all the tumor-bearing females showed varying degrees of estrogenic stimulation (Fig. 15). The uteri of the animals with the largest tumors showed the most marked stimulation. No progesterone effect was noted. Likewise, the vaginal mucosa of these animals was heavily cornified, whereas the vaginal mucosa of the animals with smaller tumors showed only a slight increase in cell layers when compared to the atrophic epithelium of the castrated controls (Figs. 11–14).

The first estrous-type vaginal smear was obtained from a female animal 51 days after its tumor was first palpated. The animal was sacrificed at that time. Its uterus weighed 60.0 mg. and the tumor 4.30 gm. This same animal had the first cornified vaginal epithelium. In the succeeding tumor-bearing female animals it was observed that they would have an estrous-type smear for 3 or more days and then have a diestrous-type smear for a period of 5 days. Several animals were permitted to run such "artificial" cycles before being sacrificed. The uteri of these females weighed from 66.0 to 82.0 mg. Careful examination of these animals at autopsy revealed no gross evidence of ovarian tissue. It is also unlikely that there was any ovarian tissue present, as these animals previously had diestrous vaginal smears for a period of 90 days or more.

Gross mounts of the mammary glands revealed varying degrees of stimulation correlated with the tumor weight (Figs. 16–22). The mammary glands of the males with tumors weighing from 2.90 to
ficient amount of estrogenic substance to cause the female type following ovariectomy, as might be expected. Crabtree (1) found that after castrating male control mice did not retain the characteristic type upon castration, and the kidneys of the female animals did not revert to the characteristic female type by the tumor. The kidneys of the male control glands, in addition to the uterus and vagina, served as useful indicators of estrogenic secretion. Also, a close correlation existed between the size and weight of the granulosa-cell tumor and the amount of estrogenic substance secreted. Therefore, it appears that the response in both instances depends on the size and weight of the tumor. This is contrary to the findings of Geist and his co-workers (4), who studied parenchymatous lutein tumors of mice produced by roentgen radiation. They concluded that the biologic activity of these tumors, as expressed by the effect produced upon the uterus and vagina, did not depend upon the size of the tumor or the extent of luteinization.

The kidney, submaxillary and mammary glands, in addition to the uterus and vagina, served as useful indicators of estrogenic secretion by the tumor. The kidneys of the male control animals did not revert to the characteristic female type upon castration, and the kidneys of the female control mice did not retain the characteristic female type following ovariectomy, as might be expected. Crabtree (1) found that after castrating male mice there was a decrease in the percentage of Bowman's capsules lined with cuboidal cells to approximately the female level for animals of the same age, and also noted that accompanying this decrease there was a regeneration of the X-zone or androgenic zone of the adrenal. Due to the fact that the X-zone of the adrenal in all the mice of this experiment was extremely degenerated or absent, one might suspect this to be an important factor in the explanation as to why the castrated and ovariectomized controls had kidneys which tended to be or were the male type. However, this occurrence served a useful means by indicating that a tumor weighing only 1.13 gm. secreted a sufficient amount of estrogenic substance to cause the kidney to assume the characteristic female type. The submaxillary gland findings were analogous to those of the kidneys. The epithelial cells of the terminal tubules of the glands in all the controls were columnar instead of the usual cuboidal found in castrates. This occurrence also served a useful means by indicating that a tumor weighing 2.56 gm. in the female and 3.95 gm. in the male secreted a sufficient amount of estrogenic substance to cause the submaxillary glands to become the characteristic estrogen-stimulated female type.

Because the females with tumors weighing 4.43 gm. or more showed cyclic vaginal cornification, it appears that the estrogenic secretion of the large tumors was rhythmic, possibly being governed by the pituitary.

Trentin (7) made single injections of estradiol benzoate in oil into ovariectomized mice of eleven inbred strains to determine the amount necessary to cause 50 per cent of a strain to produce cornified vaginal smears. He found the mice of the C57 strain were the most sensitive; a single injection of 0.06 µg. produced the desired effect. Using this finding, one may estimate that a tumor weighing 4.80 gm. was secreting an amount of estrogenic substance with a stimulating effect approximately equal to that which 0.06 µg. of estradiol benzoate has on the vaginal mucosa.

There was no evidence that the tumor produced androgens. The hypervolemic changes associated with granulosa-cell tumors are not caused by the estrogenic substance secreted by the tumor, for experiments on sustained administration of large doses of stilbestrol and natural estrogens failed to elicit hypervolemia in mice (6). Similar changes are also seen in mice with nonestrogen-producing tumors.

DISCUSSION

A close correlation existed between the size and weight of the granulosa-cell tumor and the amount of estrogenic substance secreted. Therefore, it appears that the response in both instances depends on the size and weight of the tumor. This is contrary to the findings of Geist and his co-workers (4), who studied parenchymatous lutein tumors of mice produced by roentgen radiation. They concluded that the biologic activity of these tumors, as expressed by the effect produced upon the uterus and vagina, did not depend upon the size of the tumor or the extent of luteinization.

The kidney, submaxillary and mammary glands, in addition to the uterus and vagina, served as useful indicators of estrogenic secretion by the tumor. The kidneys of the male control animals did not revert to the characteristic female type upon castration, and the kidneys of the female control mice did not retain the characteristic female type following ovariectomy, as might be expected. Crabtree (1) found that after castrating male mice there was a decrease in the percentage of Bowman's capsules lined with cuboidal cells to approximately the female level for animals of the same age, and also noted that accompanying this decrease there was a regeneration of the X-zone or androgenic zone of the adrenal. Due to the fact that the X-zone of the adrenal in all the mice of this experiment was extremely degenerated or absent, one might suspect this to be an important factor in the explanation as to why the castrated and ovariectomized controls had kidneys which tended to be or were the male type. However, this occurrence served a useful means by indicating that a tumor weighing only 1.13 gm. secreted a sufficient amount of estrogenic substance to cause the kidney to assume the characteristic female type.

SUMMARY

1. A study of a transplanted granulosa-cell tumor in C57 castrate mice revealed a close correlation between the size and weight of the tumor and the quantity of estrogenic substance secreted.

2. It was estimated that a tumor weighing 4.80 gm. was secreting an amount of estrogenic substance with a stimulating effect approximately equal to that which 0.06 µg. of estradiol benzoate has on the vaginal mucosa of C57 mice.

3. A close correlation was also found to exist between the size and weight of the tumor and the severity of hypervolemic changes which occurred in the tumor-bearing mice.

REFERENCES

Fig. 1.—Representative histological section of the granulosa-cell tumor showing the typical architecture. Mag. ×100.

Fig. 2.—Liver of an ovariec tomized female control mouse. Mag. ×100.

Fig. 3.—Liver showing early (2+) sinusoidal dilatation from a castrated male mouse with a 3.95-gm. tumor. Mag. ×100.

Fig. 4.—Liver showing advanced (3+ to 4+) sinusoidal dilatation from an ovariec tomized female mouse with a 5.39-gm. tumor. Mag. ×100.

Fig. 5.—Liver showing marked (4+) sinusoidal dilatation with rupture of the liver capsule imminent. From an ovariec tomized female mouse with a 5.28-gm. tumor. Mag. ×100.

Fig. 6.—Liver showing extreme sinusoidal dilatation with preservation of the small bile ducts. Section from an ovariec tomized female mouse with a 6.58-gm. tumor. Mag. ×100.

Fig. 7.—Renal corpuscle from the kidney of a castrated male control mouse. Cuboidal epithelial cells are present in the parietal layer. Mag. ×600.

Fig. 8.—Renal corpuscle from a kidney of an ovariec tomized female mouse with a 6.58-gm. tumor. Squamous epithelial cells are present in the parietal layer. Mag. ×600.

Fig. 9.—Submaxillary gland from an ovariec tomized female control mouse. The terminal tubular epithelium is the altered female type. Mag. ×1,000.

Fig. 10.—Submaxillary gland from a castrated male mouse with a 6.26-gm. tumor. The terminal tubular epithelium is the characteristic female type. Mag. ×1,000.

Fig. 11.—Atrophied vaginal mucosa from an ovariec tomized female control mouse. Mag. ×450.

Fig. 12.—Vaginal mucosa from an ovariec tomized female with a 1.56-gm. tumor. The mucosa shows early signs of stimulation with an increase in cell layers. Mag. ×450.

Fig. 13.—Vaginal mucosa from an ovariec tomized female with a 4.48-gm. tumor. The thickened mucosa indicates moderate stimulation. Mag. ×450.

Fig. 14.—Cornified vaginal epithelium from an ovariec tomized female mouse with a 5.62-gm. tumor. Mag. ×450.
FIG. 15.—Castrate uterus from a female control mouse (insert) and a section of a uterus showing marked stimulation from an ovariectomized female mouse with a 4.43-gm. tumor. Same magnification. Mag. ×55.

FIG. 16.—Atrophied mammary gland from a castrated male control mouse. Mag. ×55.

FIG. 17.—Mammary gland from a castrated male mouse with a 2.90-gm. tumor. Early signs of stimulation are indicated by dilated ducts and end buds. Mag. ×55.

FIG. 18.—Mammary gland from a castrated male mouse with a 3.34-gm. tumor. The increased growth of the duct system indicates moderate stimulation. Mag. ×55.

FIG. 19.—Mammary gland showing beginning alveolar development from a castrated male mouse with a 5.00-gm. tumor. Mag. ×55.

FIG. 20.—Atrophied mammary gland from an ovariectomized female control mouse. Mag. ×55.

FIG. 21.—Mammary gland from an ovariectomized female with a 2.58 gm. tumor. Early signs of stimulation are indicated by dilated ducts and end buds. Mag. ×55.

FIG. 22.—Mammary gland showing beginning alveolar development from an ovariectomized female mouse with a 5.62-gm. tumor. Mag. ×55.


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