The Role of Thyroid Function and Food Intake in Experimental Ovarian Tumorigenesis in Mice*  

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INTRODUCTION

Granulosa-cell tumors, luteomas, and mixed-cell tumors occurred in intrasplenic ovarian grafts in castrated rats (1), mice (18), and rabbits (16). When castrated female mice bearing intrasplenic ovarian grafts were placed in parabiosis with intact female litter-mates, the ovaries and uteri of the intact female mice were heavier, indicating an increased output of gonadotrophic hormones by the castrated mice with intrasplenic grafts (15). By the same technic, x-rayed male and female rats produced increased amounts of gonadotrophic hormones (12, 19), and ovarian tumors of the above-mentioned types have occurred after the x-radiation of mice (8). It is almost certain that the action of increased amounts of one or both of the pituitary gonadotrophic hormones is responsible for the induction of these ovarian tumors. Alteration of the type or amount of gonadotrophic hormones produced or the responsiveness of the ovary to these hormones should affect ovarian tumorigenesis.

Thyroidectomy resulted in a diminution of pituitary gonadotrophic potency (7) and desiccated thyroid power in an increased gonad stimulating potency of the pituitary gland in rats (4, 18). The administration of thyroid hormone diminished the ovarian weight response to gonadotrophins in rats, even when they were hypophysectomized, and thyroidectomy enhanced the ovarian weight response (11).

Caloric restriction resulted in a lowered production of gonadotrophic hormones (6).

The present experiment was designed to test the effects of altered thyroid function and caloric restriction on ovarian tumorigenesis.

MATERIALS AND METHODS

One hundred and nine F₁ hybrid mice (C57 female × CBA male and the reciprocal cross) were castrated, and all or a portion of one ovary was transplanted as an autologous or homologous graft into their spleens at from 37 to 71 days of age. The median ages of mice in the various groups described below ranged from 38 to 49 days.

The mice were immediately started on the experimental diets. Twenty-four dietary control mice were fed fox chow with meat meal (Purina). Twenty-four mice were rendered hyperthyroid by adding 0.2 per cent desiccated thyroid (Armour) to this diet, and 24 mice were made hypothyroid by the addition of 0.2 per cent thiouracil. Inanition was induced in 37 mice by food restriction to 66 per cent of normal. Sixteen of these mice were fed ad libitum after 4-7 months on the restricted diet. Food was supplied in covered glass jars with a hole in the lid just large enough to admit a mouse's head. All groups were kept in an air-conditioned room at 72°—74° F., and they were weighed about every 2 weeks, except for one 2- and one 4-month interval. Vaginal smears were taken only from the 2d to the 3d and from the 10th to the 11th months after beginning the experiment. The mice died or were killed with illuminating gas from 11 to 29 months after the experiment was begun. The graft, metastases when present, liver, adrenal glands, kidneys, thyroid glands, and the genital organs were weighed or measured, fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

OBSERVATIONS

Body Weight

The thyroid-fed mice had the greatest starting weight and maintained this advantage for 6 months. The starting weight of the thyroid-fed mice averaged 24.5 gm.; of the dietary controls, 23.4 gm.; thiouracil-fed mice, 22 gm.; and the inanition group, started 4 months later, weighed 19 gm. From 6 to 17 months after transplantation, body weights of hyperthyroid and dietary control mice were not significantly different, both ranging from 29 to 33 gm.; after 17 months the hyperthyroid mice again averaged slightly heavier (Chart 1).
Thiouracil-fed mice maintained a nearly constant weight of 26 gm.; they consistently weighed less than the hyperthyroid or control mice. Partially starved mice weighed from 18 to 22 gm. except at 13 months after transplantation, when they were fed ad libitum for 2 weeks because of generally poor health and high mortality. Two weeks after re-instituting the inanition diet, their average weight had diminished from a 25.8-gm. maximum to less than 20 gm.

There was a very rapid weight gain in the group of mice fed ad libitum after 3-7½ months' inanition; it amounted to a 38 per cent increase over the initial weight within 3 days. A slower rate of growth followed this initial spurt, and in 5 months the average weight, 29.5 gm., for mice in this group was equal to that of the controls. Some of these refed mice remained alive for a much longer period of time than the mice in any other group. Their average weight at autopsy was 29 gm., and exceeded that of the dietary controls, 27 gm.; thyroid-fed, 26.5 gm.; thiouracil-fed, 25 gm.; and the partially starved mice at 18 gm.

**Intrasplenic Ovarian Grafts**

The ovarian grafts ranged from about 0.1 to 2.5 cm. in greatest diameter. Some grafts were embedded in the spleen; others projected as a polypoid mass with a pedicle or sessile attachment to the spleen. They contained both solid and cystic areas. The solid tumor tissue was yellowish and firm or fibrous, alternating with areas of a lighter, grayish color and a more friable consistency. Cystic blebs filled with viscid, dark red or clear, light yellow serous fluid were present over the surface of the tumor. On cross section, the cysts were present throughout the tumor, sometimes comprising almost the total mass. In a few of the grafts the solid portion was present as a papillary or polypoid growth within a fluid-filled cyst.

Ovarian tumors occurred in sixteen of 21 mice fed the control diet; three of twenty thyroid-fed mice; ten of 21 thiouracil-fed mice, five of twelve mice with caloric restriction; and twelve of fourteen mice fed ad libitum after inanition (Table 1).

Several types of ovarian tumors occurred; cellular morphology and organization were the chief diagnostic criteria. There were 29 granulosa-cell tumors, fairly evenly divided as to the sex of the hosts. These tumors were identical in appearance to those previously described (Fig. 1). Many ovaries with granulosa-cell tumors contained large or small areas of cells with granular peripherally located chromatin (deficiency cells, Figs. 4 and 5). These are similar to theca and stroma cells of hypophysectomized mice or after chronic estrogen administration. Cells intermediate between granulosa and those of the theca deficiency type occurred in many tumors. Such cells were frequently associated with signs of necrosis and cellular degeneration. Many of the cells in contact with bone spicules had typical cartwheel nuclei.

Mixed tumors occurred in eleven female mice and one male mouse. Luteomas were present in two

<table>
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<tr>
<th>Group</th>
<th>Dietary controls</th>
<th>0.2 percent thyroid</th>
<th>0.2 percent Thiouracil</th>
<th>Inanition</th>
<th>Inanition-refeeding</th>
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</table>

* Granulosa-cell tumor.
spicules occurred in such degenerating areas in two
tumors in dietary control and thiouracil-fed mice. Osteocytes and osteoblast-like cells were present
(Fig. 8).

One thyroid-fed mouse contained a cystic graft measuring 18 × 8 × 7 mm. Inside the fluid-filled
cyst there was a polypoid tumor mass which was also seeded onto the inner surface of the thin cyst
wall. The multilobar graft consisted predominantly of faintly eosinophilic cells arranged in a single-
layered epithelium varying from cuboidal to tall columnar ciliated. The deeper parts of the tumor
contained the same type of epithelial cells arranged in tubular form. The remainder consisted of
lucent cells, which formed the main portion of the tumor deep to the epithelium. Small fibro-
blastic cells with scanty cytoplasm formed the third cell type; they were most abundant deep to
but adjacent to the epithelial layer. Large vacuoles were present in the cells of the epithelium (Figs.
6 and 7). This tumor is being called, descriptively, a papillary cystadenoma.

NonTumorous Grafts

Ovarian tumors failed to occur in 42 mice. Sixteen of these were thyroid-fed, eleven were thiouracil-fed, seven were calorically restricted, five were dietary controls, and four were fed ad
libitum following inanition. In eight mice there was no sign of the ovarian graft. Ten mice had
vascular adhesions. The germinal epithelium or epithelial ingrowths were absent in eight thyroid-
fed and two thiouracil-fed mice. The nontumorous graft of one control mouse had no epithelial in-
growths despite the presence of a surface epithelium (Table 2).

The nontumorous ovarian grafts in eight mice were small and embedded in splenic tissue. An
ovarian surface epithelium (germinal) was present on a portion of some of these grafts. Epithelial in-
growths appeared to arise from this epithelium and to penetrate the ovarian stroma. These in-
growths were tubular and had a low cuboidal epithelium. The cells had darkly-staining nuclei
and little cytoplasm. Small or large fluid-filled cysts lined by the same type of epithelium were
frequently present. The cells forming the walls of these cysts often contained hemosiderin. Macrophage-like cells were present in and adjacent to this epithelium. Some small fluid-filled cysts
surrounded by this epithelium contained macrophages. More critical evidence of the transforma-
tion of the pluripotential germinal epithelium into macrophages was the demonstration of macrophages embedded in or surrounded by cells of these germinal epithelial ingrowths. These
macrophage-like cells were sometimes lined up in epithelial fashion, in all stages of transformation;
some contained cytoplasmic inclusions characteristic of phagocytic cells (Fig. 8).

Adrenal Glands

The adrenal glands of the hyperthyroid mice averaged nearly twice as heavy as those of the con-
trols, thiouracil-fed, and inanition-refed groups: 13.4 mg. as compared to 6.8—7.2 mg. The adrenal
glands of the inanition group weighed 6.2 mg. (Table 3). Two control mice that failed to de-
velop ovarian tumors and had vascular adhesions from graft to body wall, with large uteri, had
deadenals as large as the average thyroid-fed mouse.

Adrenal cortical hyperplasia occurred in mice of every group. Chart 2 shows the degree of
hyperplasia, according to a grading system based on the proportion of adrenal surface involved, the
depth of involvement, and the types of cells comprising the hyperplastic area. Both adrenal
glands usually showed about the same degree of hyperplasia. Advanced hyperplastic changes oc-
curred in five dietary control mice and in one inanition-refed mouse.

Thyroid-fed and thiouracil-fed mice showed


equal but small degrees of adrenal cortical hyper-

plasia, and the inanition group only slightly more.

The inanition-refed mice showed marked cortical hyperplasia.

Production of Hormones

Estrogen.—The criteria of an estrogenic effect

on the uterus were increased weight, enlargement

of the lumen and of the epithelial lining cells with

care from low to high columnar, stromal edema,

and hypertrophy of the muscle layer. Estrogenic
effects were present in some mice of every group
except the inanition group (Table 3). Judging from
uterine weight, the thyroid-fed mouse with an
ovarian tumor showed an estrogenic effect smaller
than the average of the other groups. Uteri of
the control mice were next in size; the thiouracil-
fed group had heavier uteri, and mice of the
inanition-refed group had the largest uteri.

TABLE 2

CHARACTERS OF NONTUMOROUS OVARIAN GRAFTS

<table>
<thead>
<tr>
<th>No.</th>
<th>No. germinal</th>
<th>No. Adhesions</th>
<th>No. Sign of Growth</th>
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<tr>
<td>with</td>
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<td></td>
<td>graft</td>
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<tr>
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</tr>
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<tr>
<td>Inanition-refeeding</td>
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</table>
Vaginal smears.—Vaginal cornification was present in six of eighteen dietary control mice from 2 to 3 months after transplantation. Only three of these showed cornified smears at 9–10 months, and one had a granulosa-cell tumor. Of eleven mice that did not show cornified smears, seven had ovarian tumors at autopsy.

Four thyroid-fed mice presented vaginal cornification and cycling from 10 to 11 months. All had vascular adhesions between the graft and the body wall. None had an ovarian tumor at autopsy.

Androgen.—There was no evidence of androgenic hormone production in any mouse, as determined by the weight and morphology of the seminal vesicles and prostate or the morphology of the epithelium of Bowman’s capsule in the kidney.

Thyroid glands.—Twenty-four of the thyroid glands were examined, mostly from the thiouracil-fed group; these had an average weight of 31.9 mg. in three mice with ovarian tumors and 24.5 mg. in seven mice without tumors. This difference

<p>| TABLE 3 |</p>
<table>
<thead>
<tr>
<th>AVERAGE BODY AND ORGAN WEIGHS OF CASTRATED MICE WITH INTRASPLENIC OVARIAN GRAFTS, WITH AND WITHOUT OVARIAN TUMORS</th>
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<tr>
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<tr>
<td>Ovarian tumor</td>
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<tr>
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<tr>
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<tr>
<td>0.2 percent Thio-</td>
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<tr>
<td>lar-feeding</td>
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<tr>
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<tr>
<td>No ovarian tumor</td>
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<tr>
<td>Dietary controls</td>
</tr>
<tr>
<td>0.2 percent Thyroid</td>
</tr>
<tr>
<td>0.2 percent Thio-</td>
</tr>
<tr>
<td>lar-feeding</td>
</tr>
<tr>
<td>Inanition</td>
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</table>

**TABLE 4**

<table>
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<th>ESTROGENIC HORMONE OR PROGESTERONE PRODUCTION IN MICE WITH OVARIAN TUMORS</th>
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<tbody>
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<tr>
<td><strong>No</strong></td>
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<tr>
<td>Dietary Control</td>
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<td>0.2 percent Thyroid</td>
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* Progesterone effects were present only in the presence of estrogenic effects.

in weight may be due in part to the delay of as long as 1 week between the time mice were taken off the diet and the time of autopsy. The thyroid gland weight of three thyroid-fed mice averaged 4.3 mg. The large thyroid glands were hyperplastic with occasional small papillary cystadenomas. The parathyroid glands appeared normal. No thyroid tissue was seen in the lungs grossly or on test sections; serial sections were not made.

Liver.—The average weight of the livers ranged from 1.6 to 1.8 gm. in all except the inanition group, which averaged 1.1 gm., and eleven thyroid-fed mice without ovarian tumors, which
tumor the normal liver tissue was compressed, a biliary system. Around the periphery of the filtration of moderate degree occurred in some of cells and did not have an orderly arrangement with them was any estrogenic hormone produced. The failed to develop ovarian tumors, and in none of them was any estrogenic hormone produced. The thyroid-fed mice may be attributed to a diminution of thyroid hormone on the ovary; this might also be responsible for the occurrence of the serous cystadenoma in one thyroid-fed mouse in the present experiment.

There was a slight but significant reduction in the tumor incidence in the thiouracil-fed mice. This may have been the result of a diminished ovarian responsiveness to gonadotrophins, as discussed in the preceding paragraph. The body weights of the thiouracil-fed mice were midway between the weights of the normal and the food-restricted mice. Inanition rather than the thyroacil or the hypothyroid state per se might have been responsible for the inhibition of tumorigenesis in this group.

Inanition resulted in some inhibition of tumor production. The occurrence of four tumors in this group is evidence that some gonadotrophin was being produced. The terms “functional hypophysectomy” or “physiological hypophysectomy” are therefore only partially correct when applied to the effect of inanition. The rapid development of the granulosa-cell tumors that occurred after mice in the inanition group were fed ad libitum is evidence of the presence of a low gonadotrophic hormone secretion rate in mice fed two-thirds the ad libitum food intake.

The malignancy of one of the tumors in the present experiment was shown by the presence of liver and lung mestastases in the host. Mitotic figures were frequent in some tumors that had an anaplastic appearance. Eight of the 24 tumors that were transplanted subcutaneously grew. Three of the eight tumors that were transplanted successfully to mice of the same hybrid stock showed a high incidence of lung mestastases (unpublished data).

Partial inhibition of adrenal cortical hyperplasia occurred in thyroid-fed, thiouracil-fed, and food-restricted mice, and partial inhibition of ovarian tumorigenesis resulted from the same experimental conditions. These data support the concept that an increased output of gonadotrophic hormone is responsible for both adrenal cortical hyperplasia and ovarian tumorigenesis. The greater adrenal weights but diminished adrenal hyperplasia of the thyroid-fed mice indicate that adrenocorticotrophic hormone is not responsible for this hyperplasia (5).

In the thiouracil-fed group, hepatomas occurred in five of ten mice without ovarian tumors but in only one of ten ovarian tumor-bearing mice. The presence of ovarian tumors in this group was associated in every mouse with the presence of large amounts of an estrogenic hormone in the peripheral circulation and even larger amounts going directly to the liver from the intrasplenic tumor; there was no estrogen production in the
nontumor-bearing mice. The inhibitory effect of ovarian tumors on hepatoma formation might be a result of the estrogenic hormone produced by these tumors, especially if an increase in thyrotrophic hormone is responsible for the production of hepatomas in thiouracil-fed mice (9); however, thiouracil appears to act directly as a trophic hormone on the liver (10); it may, like other trophic hormones administered in excess and over a long period of time, finally result in a tumor of the target organ.

Scrotal hernias occur in intact male mice given estrogenic hormones for long periods of time (2). Their occurrence was associated with the maintenance of testes of nearly normal size and the formation of an interpubic ligament (caused by prolonged estrogen administration), resulting in an alteration of muscle attachments and an increased diameter of the inguinal rings (9). Four scrotal hernias occurred in castrated male mice with intrasplenic ovarian grafts in the present experiment; a scrotal hernia occurred in one intact male mouse with a rigid pubic symphysis and a subcutaneous granulosa-cell tumor transplant. It must therefore be concluded that the presence of an interpubic ligament and the presence of testes are not essential for the occurrence of scrotal hernias in mice, but they appear to enhance their development.

SUMMARY

1. One hundred and nine hybrid mice (CC1) were castrated, and in each an ovary was grafted into the spleen. The mice were then placed on special diets. Eighty-nine of the mice were examined from 11 to 29 months after transplantation.

2. Granulosa-cell tumors, mixed tumors, or luteomas occurred in sixteen of 21 dietary control mice, one of twenty mice fed 0.2 per cent desiccated thyroid powder in the diet, ten of 21 fed 0.2 per cent thiouracil in the diet, five of twelve restricted to two-thirds of the ad libitum after 4½–7½ months on the restricted diet.

One tumor, of a type previously undescribed, and one tubular adenoma occurred in two female mice fed the desiccated thyroid. The total incidence of ovarian tumors in the thyroid-fed mice was significantly lower than that of any other group.

3. Adrenal cortical hyperplasia occurred in mice of every experimental group. It was most extensive in dietary control and inanition-refed mice.

4. An estrogenic hormone and progesterone were produced in sufficient quantities to cause changes in the uterus despite their passage through the liver, by ovarian tumor-bearing mice in all the experimental groups except the underfed mice; their uteri showed no effects of either hormone. Progesterone effects were present only in mice which showed evidence of estrogen production. Estrogenic effects on the uterus were present in mice without ovarian tumors in association with adrenal cortical hyperplasia.

5. Hepatomas occurred in five of the ten thiouracil-fed mice which did not have ovarian tumors but in only one of the ten such mice with ovarian tumors. The uteri of the ovarian tumor-bearing mice revealed marked estrogenic effects, which were absent in the mice without ovarian tumors. Estrogenic hormone may have inhibited this tumorigenic action of thiouracil.

6. Scrotal hernias occurred in four castrated male mice bearing intrasplenic ovarian tumors.

REFERENCES


14. Mettes, J., and Chandrasheker, B. The Effect of Induced Hyper- and Hypothyroidism on the Response to a Con-


Fig. 1.—Granulosa-cell tumor of diffuse type with many large cystic cavities (X508).

Fig. 2.—Luteoma. Note the areas resembling a tubular adenoma; also, the abundant foamy cytoplasm and the well defined cell boundaries (X508).

Fig. 3.—Granulosa-cell tumor; degenerative changes, including bone formation, are marked (X508).

Fig. 4.—Granulosa-cell tumor. Note the chromatin arranged in clumps at the nuclear membrane (X960).
Fig. 5.—Granulosa-cell tumor of follicular type; note the presence of cartwheel nuclei (×960).

Fig. 6.—Papillary cystadenoma showing the tall columnar, ciliated and vacuolated epithelium with nuclei just beneath the free surface (×254).

Fig. 7.—Papillary cystadenoma showing more of the luteinized stromal cells present in this tumor (×254).

Fig. 8.—Wall of a small ovarian cyst. The macrophage-like lining cells contain granular material (×960).
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