A Transplantable Functional Ovarian Tumor Occurring Spontaneously in a Rat*

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Although the experimental induction of functional ovarian tumors in laboratory rodents, both by irradiation and by intrasplenic transplants, has been extensively studied, there are few records of spontaneous functional tumors in these animals. Spontaneous ovarian tumors of any variety are uncommon in rats. Bullock and co-workers (2, 4) found only six ovarian tumors in 14,038 female rats observed. We have been unable to find any report in the literature of a spontaneous functional ovarian tumor in the rat.

In the mouse, spontaneous ovarian tumors are also unusual. Slye and co-workers (8) observed only 44 ovarian tumors in 22,000 mice. Although Cloudman (3) referred to solid, spontaneous ovarian tumors, seen in the mice at Jackson Memorial Laboratory, as granulosa-cell tumors, Woolley, who is at the same laboratory, stated that none of these tumors showed evidence of function.1 We have been able to find only two reports (Gardner et al. [6] and Strong et al. [9]) of spontaneous functional ovarian tumors occurring in mice.

It is the purpose of this paper to report a spontaneous functional ovarian tumor in the rat. This tumor displayed a high degree of endocrine activity and has grown readily on serial passage.

RESULTS

The tumor occurred in an adult female rat of the AXC line 9935. The animals of this line were originally obtained from Drs. Dunning and Curtis of the Detroit Cancer Institute and were maintained, in New Orleans, by line breeding. The animal had been set aside for timed pregnancy studies, and vaginal smears had been taken daily for 162 days. She had been caged with a male for the last 147 days of this period. The animal did not become pregnant, despite the occurrence of a vaginal plug. Cycles were never regular, diestrous smears predominating. During the last 43 days of observation, there was an unbroken sequence of diestrous vaginal smears. A mass was palpated in the flank, and the animal was killed with ether. The rat weighed 196 gm. at this time. A solid tumor measuring $2.6 \times 1.9 \times 1.9$ cm. (Fig. 1) had replaced the left ovary. There was congenital absence of the right horn of the uterus, a fairly common developmental anomaly in this line. The remaining horn was normally developed. The right ovary appeared atrophic.

The ovarian neoplasm was well encapsulated, and there was no evidence of local invasion or metastatic spread. It contained two cysts (8 and 5 mm. in diameter, respectively) which were filled with clear, yellow fluid. The tumor was composed of two types of tissue—one firm and brown, the other softer and almost white.

Histologic observations.—The ovarian tumor was of the granulosa-cell type (Fig. 4), with an admixture of large lutein cells. Although granulosa cells were most conspicuous, theca cell areas could also be identified. Such histologic admixtures are also seen in induced neoplasms in rodents as well as in human ovarian tumors. The granulosa cells were arranged in varying histologic patterns: diffuse, trabecular, and papillary. Follicle-like structures, both large and small, were present. Some of the large follicle-like structures reproduced the normal architecture of the follicle to a remarkable degree, with a histologically distinct zone of theca cells.
FIG. 1.—AXC rat, dissected at necropsy, showing spontaneous tumor of left ovary. The tumor (upper portion of photograph) has been sectioned and laid open. Arrow, lower, points to atrophic right ovary. There is congenital absence of right uterine horn.

FIG. 2.—Photograph of portion of cleared whole mount of mammary gland from AXC rat (above) bearing spontaneous ovarian tumor. The degree of mammary proliferation is equal to that of a term (20-day) pregnancy. ×12.

FIG. 3.—Photograph of portion of cleared whole mount of mammary gland from a control AXC rat at twentieth day of pregnancy. For comparison with Figure 2. ×12.
surrounding the granulosa layer (Fig. 5). When stained for reticulum with silver, the theca cell layer showed a delicate reticulum about each cell, while the granulosa showed none—much as in the normal follicle.

The lutein cells were intimately intermingled with the granulosa cells but were strikingly different, histologically (Figs. 6, 7). The lutein cells had a diameter 4 or 5 times greater than that of the granulosa cells. The former had abundant, intensely acidophilic, granular cytoplasm, with a varying amount of vacuolization. They were arranged in interlacing cords within masses of granulosa cells; in some areas they were the predominant cell. The nuclei were more spherical, vesicular, and slightly larger than those of the granulosa cells, which were ovoid and hyperchromatic.

The granulosa cells had numerous mitoses, but none was noted in the lutein cells. It was not possible to ascertain whether the lutein cells were derived from granulosa or theca cells. Cells whose appearance suggested transitions to large lutein cells were seen among both granulosa and theca cells.

Cystic spaces containing blood or protein-rich fluid were abundant. Fibrosis, necrosis, granulation tissue, and foci of pigmented macrophages were conspicuous in and around the tumor.

Histologically, the uterus showed definite evidence of estrogen stimulation, although the response was probably not a purely estrogenic one. The vagina, showing extensive mucification of the epithelial cells, was similar to that seen in pregnancy. The mammary glands showed microscopic as well as gross evidence of proliferation, indistinguishable from that of late pregnancy. No significant microscopic changes were noted in the thyroid, adrenals, pituitary, skeletal muscle, spleen, kidney, liver, or thymus. Unfortunately, the right ovary was lost in the process of sectioning.

**Transplants.—** The original tumor was transplanted into six adult male and seven adult female rats of the same AXC strain. It was transplanted subcutaneously into two intact and two castrated male animals, and two other castrated males received intrasplenic grafts. Subcutaneous transplants were performed in three females. Four females, of which two were spayed, had intrasplenic transplants. In all six of the males and in three of the seven females the grafts grew progressively.

A total of 98 grafts (of which 92 were successful) were made over three transplant generations. The sites of transplantation, the number of successful transplants, and the gonadal status of the recipient animals are recorded in Tables 1 and 2. As indicated in Table 2, the number of successful transplants was greater in male recipients. A similar influence of sex has been observed in the transplant of induced granulosa-cell tumors in mice (1).

The histologic appearance of the tumor transplants in three serial passages did not differ significantly from the original tumor. In some the lutein cells were diminished in number, whereas occasional transplanted tumors showed a predominance of lutein cells. The granulosa cells reproduced the pattern of the initial tumor.

Only one metastasis was observed. This occurred in a castrated female rat in which the tumor had been implanted subcutaneously in the right axillary region. A bulky tumor grew at the site of transplantation, and a metastatic nodule in the cortex of the left kidney was observed at necropsy. On histologic examination, the metastatic nodule was composed of both granulosa and lutein elements.

Bone formation appeared in the stroma of several of the transplanted tumors. Such metaplastic bone has also been noted in transplants of experimentally induced granulosa-cell tumors (7). Histochmical studies of alkaline and acid phosphatase on several of the tumor transplants showed abundant alkaline phosphatase and little acid phosphatase in the granulosa cells of the tumor. It is likely that the production of bone in the transplanted is related to the high alkaline phosphatase content of the tumor.

**Evidence of hormonal production.—** The functional character of the original tumor is indicated...
by the mammary gland proliferation (equivalent to that of late pregnancy), comparable changes in the histology of the vagina, and irregular cycles as determined by vaginal smears. Diestrous smears were maintained for the 43 days before necropsy, and the animal failed to become pregnant.

There is little doubt that the tumor produces estrogens. Transplants of the tumor in spayed females resulted in pronounced proliferative changes in the mammary glands as well as (in some) diestrous smears after a period of diestrus.

In several rats bearing transplants of this ovarian tumor for over 6 months, chromophobe adenomas of the pituitary developed. In two of these mammary adenocarcinoma also developed. Although spontaneous mammary carcinoma is rare in this strain, it is readily induced by estrogen (5). The occurrence of both pituitary chromophobe adenomas and mammary carcinomas is corroborative evidence of estrogen production by the tumor.

Subcutaneous tumor transplants in male rats produced pronounced atrophy of the testes (Fig. 8). The histologic appearance of the testes in these animals was comparable to that following hypophysectomy, presumably due to the inhibitory action of estrogen on the release of pituitary gonadotrophin. In addition, biologic assays for gonadotrophin were carried out on the pituitaries of three castrated rats bearing tumor transplants. Assays were accomplished by grafting such pituitaries into immature female rats. These preliminary experiments indicate that the presence of the transplanted tumor prevents the rise in pituitary gonadotrophin which ordinarily follows castration.

In males (intact and castrate) which received transplants of the tumor, the accessories showed a complex pattern which could not be interpreted as a pure estrogen effect. Seminal vesicles and coagulating glands resembled those of castrates to some extent, although the epithelium was much more abundant than that seen in the castrate state. Furthermore, there was none of the fibrous hyperplasia of the stroma that is seen with a purely estrogenic effect. The possibility remains that the tumor produces a hormone in addition to estrogen. However, a single attempt to demonstrate progesterone in a tumor-bearing animal, by the deciduoma test, yielded negative results.

### SUMMARY

A report of a spontaneous estrogen-producing ovarian tumor in an AXC rat is presented. The tumor was of the granulosa-cell type with an admixture of large lutein cells. It has been successfully transplanted over three transplant generations in male and female, intact and castrate rats. A single metastasis was observed, occurring in the kidney, and composed of both granulosa and lutein cells. There is abundant evidence for estrogen production by the tumor both in the original animal and in animals bearing transplanted tumors. Other hormonal production, although likely, has not been demonstrated conclusively. Pituitary adenomas were seen in rats bearing transplants of the tumor. In two of these animals mammary carcinoma also developed. Histochemical study indicated abundant alkaline phosphatase in the granulosa cells of the tumor. This may account for the oc-
occurrence of metaplastic bone in some of the tumor transplants.

REFERENCES

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