A Transplantable Ovarian Papillary Adenocarcinoma of the Rat with Ascites Implants in the Ovary

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The occurrence of spontaneous ovarian tumors in laboratory rodents is not frequent as far as can be ascertained from the literature. Slye et al. (1) found, among 22,000 mice, 44 with spontaneous ovarian tumors. Most of the tumors were benign papillary adenomas, while four were primary malignant ovarian tumors. The authors also mentioned, in a review of the literature, eight reports of tumors arising in the ovaries of mice. Other investigators reported spontaneous granulosa-cell tumors of the ovaries in mice (2, 3) and rarely, ovarian teratomas (4). At the National Cancer Institute, three malignant teratomas of the ovaries of spontaneous origin have been found in strain C3H adult female mice. Ovarian tumors are rare in mice and rats. Bullock et al. (5), and Curtis et al. (6) found six spontaneously occurring ovarian tumors, equally divided between carcinomas and sarcomas, in 14,638 female rats of five different strains. The rats were over 19 months of age, and the tumors were distributed as follows: Fischer strain—one carcinoma and two sarcomas; August strain—one carcinoma and one sarcoma; Copenhagen strain—one adenocarcinoma. No ovarian tumors were found in rats of the Marshall and Zimmerman strains. The three ovarian carcinomas were papillary cystadenocarcinomas. Ratcliffe (7) reported three ovarian carcinomas in 295 tumor-bearing female rats of the Wistar strain. The 295 rats with spontaneous neoplasms represented 6.7 percent of the total female population of the colony. Animals having ovarian tumors averaged approximately 17 months of age.

Recently, Iglesias et al. (8) reported a transplantable functional ovarian tumor in a strain AXC rat. The tumor was of the granulosa-cell type mixed with large lutein cells.

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Extensive work has been done on ovarian tumors that were induced in mice by irradiation (9-12) and in mice and rats by intrasplenic ovarian grafts (13-18), and many of these tumors were transplanted (19-21). The experimentally induced ovarian tumors were nonfunctional tubular adenomas and functional granulosa-cell tumors and luteomas. The benign and malignant papillary ovarian neoplasms, which are the commonest ovarian tumors of women, have not been thoroughly investigated in experimental animals. With the exception of a short gross and microscopic description by Curtis et al. (6) of three papillary cystadenocarcinomas in rats, neither detailed studies nor transplantation of this type of ovarian tumor have been made.

The occurrence of a mucinous papillary adenocarcinoma in the ovary of a 9-month-old Osborne-Mendel rat is reported here. The tumor has been successfully transplanted by subcutaneous, intraperitoneal, and intracranial routes. At the present time, the tumor is in its 25th transplant generation, and it is carried on in two lines: 1) intraperitoneally by tumor ascites fluid injections, and 2) subcutaneously by tumor-tissue transplants. The tumor arose apparently spontaneously in an untreated animal. The mother of this tumor-bearing rat, however, had been fed 2-acetylaminofluorine during lactation. This paper deals with a description of the original tumor, its histopathologic characteristics, certain transplantation experiments with it, and the production of large ovarian tumors by it following intraperitoneal inoculation.

**ORIGINAL TUMOR**

**Gross description.**—At autopsy the peritoneal cavity of the rat was found to be filled with bloody fluid. A tumor mass measuring 5.2 × 4.3 × 3.6 centimeters, with a round and slightly lobulated smooth surface, involved the right ovary and oviduct (fig. 1). It was pinkish-white in color, and soft in consistency. In cross section, the tumor was found to be solid, with large necrotic and hemorrhagic zones in the central part (fig. 2). The left ovary was normal in size and shape and appeared grossly to be free of tumor. A soft mass of polypoid growth, 0.5 centimeters in thickness and grayish-red in color, was spread across the visceral and parietal peritoneal wall, the omentum, and the mesentery. Metastasis had occurred to the abdominal and mediastinal lymph nodes, the liver, and both lungs. On the surface of both lungs were grayish-red nodules of pinhead size and slightly larger. Three of these were seen on the right lung and four on the left. Two nodules of similar size and color were found in the right lobe of the liver.

**Histologic methods.**—The tissues of the original tumor and the transplants were fixed in Zenker's acetic acid fluid, 10 percent formalin, and Carnoy's fluid, and embedded in paraffin. Histologic sections were stained with hematoxylin and eosin, and by the Masson trichrome technique. Best's carmine method for glycogen, the periodic acid-Schiff reaction (PAS), Mayer's mucicarmine, and Wilder's silver impregnation
method for reticulum were also employed. Smears from the ascitic fluid were stained by hematoxylin and eosin or by the Papanicolaou stain.

**Microscopic description.**—The tumor showed a variable pattern. Some areas showed solid groups of cells, some had an alveolar or cordlike structure, and others showed a definite adenomatous and papillary arrangement (fig. 3). Very often all these patterns could be seen in the same microscopic field.

The tumor was surrounded by a thick vascularized connective tissue capsule, which at the pedicle penetrated part of the tumor and divided it. This connective tissue showed a lymphocytic- and granulocytic-cell infiltration with focal hemorrhages. The stroma of the tumor was formed by a fine fibrillar network that was more prominent at the periphery of the tumor where a solid pattern was often found. A remarkable feature of the tumor was the formation of an acidophilic, homogeneous material. This material seemed to be a secretory substance produced by tumor cells which occasionally revealed the presence of cytoplasmic acidophilic granules or round or oval-shaped masses within vacuoles. Moreover, some early papillary formations consisted of goblet-type cells (fig. 4). The tumor cells appeared to be surrounded by this substance, and the fibrillar stroma was often obscured by it. Occasionally, the homogeneous material was so extensive that the glandular formations or individual cells were isolated in it. This material contained no glycogen. With the mucicarmine stain the homogeneous material stained pinkish-red in some areas, and was metachromatic (toluidine blue). This substance in both intracellular and extracellular locations was reddish in color in preparations with periodic acid-Schiff reaction. It will be designated as “mucoid” substance until further histochemical studies may determine its nature more exactly.

Variation in size and shape of the tumor cells seemed to be correlated with variation in the supporting stroma. In the denser portions of the tumor with abundant stroma, the cells were usually small with a round nucleus, scant and ill-defined cytoplasm, and a coarse, stippled pattern of the chromatin. In the areas with loose stroma, the cells were larger and contained the acidophilic homogeneous material described above. In papillary formations, they were cuboidal or flat, and arranged in a single row. Hyperchromatic and distorted nuclei, and cells in mitotic division, were also seen. Large necrotic zones showed eosinophilic “ghost” cells or granular debris.

In a well vascularized area of the tumor near its capsule, several foci of multinucleated syncytial cells with hyperchromatic nuclei and deeply stained cytoplasm were found. These cells were located adjacent to or within blood sinusoids, and close to them cuboidal cells with faintly stained cytoplasm were seen. Other areas consisted of rather solid portions occasionally forming glandlike spaces lined by large epithelioid cells.

The histology of the tumor closely resembled that of a papillary adenocarcinoma of the ovary, with conspicuous formation of a mucoid material.
probably secreted by the tumor cells. The tumor implants in the peritoneal cavity, as well as the tumor metastases in the lymph nodes, liver, and lungs showed the same microscopic characteristics as the primary tumor. Tumor emboli have been found microscopically in the branches of the pulmonary artery in the lungs.

The left ovary was examined in serial section, and although it appeared to be normal on gross examination, microscopically a very small polypoid growth with a narrow pedicle was seen arising from its surface near the hilus (fig. 5). The growth was composed of nests of clear cells with scant granular cytoplasm, surrounded by a few spindle-shaped cells and a very delicate reticulum. The arrangement of the cells was adenomatous or tubular, with formations in some areas reminiscent of rudimentary gonadal structures (anovulatory ovarian follicles and male seminiferous tubules) (figs. 6 and 8). Some glands or tubules which were dilated showed infolding or invaginations, with a tendency toward papillary formation (fig. 7). On the surface of the polypoid growth, the so-called "germinal" epithelium appeared to be invaginated and to be in direct continuity with the adenopapillary formations (fig. 5). The scant stroma around adenomatous or follicle-like formations displayed the characteristics of ovarian stroma. The structure of the polypoid growth showed clearly that it derived from the left ovary in which it arose, and probably from its "germinal" epithelium. No metastases from the malignant neoplasm of the right ovary were found in the left ovary.

TRANSPANTATION EXPERIMENTS

Transplants.—Tissue from the original tumor was immediately transplanted into female rats of the same strain, subcutaneously by grafts, and intraperitoneally by injecting the ascitic fluid.

Transplantation experiments were carried out by serial passages in female rats of the same strain (Osborne-Mendel). A few rats of other strains (Sprague-Dawley, Marshall-520, and Wistar) were also used. The tumor was transplanted only occasionally to male rats. It was transplanted subcutaneously, intraperitoneally, and intracranially. For subcutaneous and some intraperitoneal transplants, the trocar method of implanting tumor tissue was used. Ascitic fluid containing tumor cells was injected intraperitoneally in most of the peritoneal transplants. Three newly born Sprague-Dawley rats were injected intracranially with 0.5 cc. of ascitic fluid. Following are the results of the twelve first-transplant generations.

Results of the transplantation experiments.—The tumor was transplanted subcutaneously into 31 Osborne-Mendel rats (29 females and 2 males), and grew successfully in 27 females and in both males (93.5% of the animals). This was the strain of rat in which the tumor originated. Intraperitoneal transplants were made successfully in 31 of 35 female rats (88.6%). These intraperitoneal transplants were tissue transplants in 9 of the rats (all successful), and ascitic fluid transplants in 26 of the rats
(22, or 84.6% successful). The ages of the hosts varied from 36 to 223 days at the time the transplants were made.

Growth of transplants in the Osborne-Mendel rats.—Successful transplantation was obtained at all ages, without any significant differences in the rate of growth. A significant difference was observed in the survival of tumor-bearing rats after transplantation depending upon the site of transplantation. In those with subcutaneous transplants, the average time of death was 68 days after transplantation. The subcutaneous transplant appeared as a small palpable nodule about two weeks after transplantation, reaching a size of 3 to 4 centimeters within four or five weeks. It killed the animal nine or ten weeks after transplantation. Metastases to regional lymph nodes or to the lungs were occasionally seen.

In rats with peritoneal transplants, the average survival time was 40 days. In these animals, swelling of the abdomen due to ascites and tumor was noticed at about two weeks after transplantation. The swelling increased, and the animal died in about six weeks. At autopsy, usually a large soft polypoid mass was found to involve the omentum, with papillomatous nodules spreading throughout the mesentery and peritoneal wall. The polypoid mass was implanted on the capsules of the liver and spleen, and metastases to the abdominal and mediastinal lymph nodes, pancreas, liver, spleen, and lungs often developed.

The selective localization in the ovary of the tumor tissue growing after intraperitoneal transplants was strikingly consistent. The ovaries were involved in 83.9 percent of 31 female rats receiving intraperitoneal transplants that grew. Bilateral implantation in the ovaries was found in 22 rats, and in 4 animals only one ovary appeared to be involved. The size of the ovarian tumors varied from a few millimeters to 3 or 4 centimeters in their largest dimensions, and in one instance a tumor (fig. 9) approximately the same size and form as the original one developed. Others were not seen grossly, but in microscopic sections of the ovaries, early growths in the saccular space or in the corpus luteum were found (figs. 13, 14, and 15).

Transplantation in newborn and adult rats of other strains (Marshall-520, Wistar, and Sprague-Dawley).—Intraperitoneal transplants were successful in 5 of 6 adult female Marshall-520 rats, with bilateral ovarian implantation resulting in two of them. Of the intraperitoneal transplants in four adult female Wistar rats, the tumor grew in one rat, and only in the peritoneal cavity. Seven 1-day-old Sprague-Dawley rats received transplants with ascitic fluid—two female and two male rats intraperitoneally, and three male rats intracranially. All of these animals grew normally, and developed tumors. They were killed within 39 to 48 days after transplantation. Both female rats developed bilateral ovarian tumors from the implants. From the intracranial implants in rats, tumors proliferated on the meninges of the brain (fig. 10), with characteristic papillary formation. In one of these, the neoplasm invaded the bones at the base of the skull.
Miscroscopic findings in transplanted tumors.—In the early growths on the serosal surfaces (peritoneum and meninges), readily apparent damage to the coeliac lining cells could be seen (22). The cells were swollen and showed degenerative changes and necrosis. The exfoliated serosal surface was in places covered by tumor cells, which produced excrescences or large papillomatous masses (fig. 11), sometimes infiltrating the subserosal connective tissue. The tumor infiltration extended sometimes into the parenchyma of organs beneath the visceral serosa. Metastases in the lymph nodes (fig. 12), the abdominal organs, and in the lungs were found microscopically quite often in organs that had appeared to be normal upon gross examination. The omentum became extensively involved in the rat, probably due to a falling of the tumor mass because of gravitational factors, such as occurs in pelvic peritoneal implantation of tumor tissue in man. A serosanguineous effusion was the result of the serosal carcinomatosis.

By careful study of the sections from the tumor transplants, it could be ascertained that the growth in the ovary was an implantation. In early implants, the ovary upon gross examination appeared to be normal. Microscopically, however, small clusters of tumor cells and minute adenopapillary growths could be seen in the space of the ovarian capsule, suggesting that this locus may be a favorable culture medium for implants (figs. 13 and 14). Evidently soon thereafter, the ovary itself was involved. Generally, a corpus luteum was apparently the first to be invaded, and after that the luteal cells were replaced by the adenopapillary growths (fig. 15). In later stages, the tumor completely infiltrated the functional parenchyma, which still maintained the shape of the ovary and of the individual corpora lutea. It is probable that the tumor cells reached the ovary through the small opening in the ovarian sac which is a characteristic structure of the mouse and of the rat (23, 24). This phenomenon of “organotropy” of tumor cells toward the ovary of the host, as in the Krukenberg ovarian tumor, is the subject of another study with this tumor in comparison with other transplantable tumors of rats.

The ascitic fluid.—The ascitic tumor fluid was hemorrhagic, containing a large number of tumor cells that appeared singly or in clusters of two to twelve cells. The cells, varying in size and shape, were round with deeply stained nuclei. The cytoplasm was basophilic in some cells, and neutrophilic in others; and in still others, it was acidophilic with intracytoplasmic vacuoles and secretion droplets.

DISCUSSION

The primary ovarian tumor reported here showed through its serial passages fundamentally the same pattern as the original tumor. There was no important change that could be accounted for by age or strain differences of the hosts, the number of passages, or by the place of transplantation. The giant cells and syncytium-like tissue found in a few foci of the original tumor were not seen in any of the serial passages of the tumor transplants.
The tumor did not show any hormonal-secretory activity. The endometrium of the tumor-bearing rats was always found to be normal. In animals with intraperitoneal transplants of the tumor, the endometrium was found to be in a resting phase, with no hyperplasia of mammary gland tissue. Hypervolemia, which accompanies some granulosa-cell tumors of the mouse, was not found. The vaginal smears of some rats revealed irregularities of endometrial cycle, with tendency toward a permanent state of diestrus that could be accounted for by spontaneous castration due to involvement of the ovaries by the tumor. However, the same type of estrus cycle was observed in some animals having intraperitoneal implants without involvement of the ovaries. The testes of male rats bearing the tumor did not show any changes. Active spermatogenesis with no increase of interstitial cells was found.

It is important to note that the tumor grew very well on the serosal surfaces (peritoneum, meninges, bursa-ovarii), where rich growth of papillary formations was seen. The adenopapillary pattern of the tumor, its secretion of a mucoid substance, and its tendency to papillary serosal implantation, resemble the features of pseudomucinous papillary cystadenomas and papillary adenocarcinomas of the human ovary.

Primary bilateral ovarian growths are not infrequent (25). The small polypoid growth found microscopically in the left ovary of the original rat was a second primary histologically benign ovarian neoplasm which apparently derived from the germinal epithelium. It was a so-called tubular adenoma with a tendency toward papillary growth and formation reminiscent of rudimentary gonadal structures. It is worth mentioning that in mice treated with X ray, the altered ovaries (26, 27) and the subsequently developing ovarian tumors (19, 28) showed similar structures, and that embryologic studies indicate that the surface epithelium of the ovary is pluripotent (29).

It may be that the malignant tumor of the right ovary originated, like the polypoid growth in the left ovary, in the germinal epithelium and differentiated into tissue components resembling derivatives of the müllerian duct (30) with the formation of adenopapillary structures consisting of epithelial cells that secrete a mucoid substance.

**SUMMARY**

An ovarian papillary adenocarcinoma was found in a 9-month-old Osborne-Mendel rat. The main characteristics of the tumor were its definite adenopapillary pattern, with a conspicuous mucoid substance, its predilection for implanting in the ovary after intraperitoneal transplantation, and its high degree of malignancy. The tumor showed no evidence of hormonal secretory activity. It proved to be readily transplantable both subcutaneously and intraperitoneally to serosal surfaces, and readily transplantable in the same strain of rat with some success in other strains. The tumor is believed to be derived from the germinal epithelium.
REFERENCES


FIGURE 1.—Original ovarian tumor at the time of autopsy. Tumor implants can be seen in the omentum and mesentery, and small nodules (tumor metastases) appear in the lower lobe of the left lung.

FIGURE 2.—Ovarian tumor and attached uterine horn. ¾ natural size.

FIGURE 3.—Original ovarian tumor, showing the papillary and glandular pattern, with strands of mucoid-hyaline material. × 100
Plate 39

Figure 4.—Adenopapillomatous growths consisting of goblet cells. Hematoxylin and eosin. $\times 290$

Figure 5.—Small polypoid growth in the left ovary of the tumor-bearing rat. Neoplastic adenomatous, tubular, and papillary formations are attached to the ovary by a narrow pedicle. Wilder's silver impregnation method for reticulum. $\times 96$
Figure 6.—Area of polypoid growth in the left ovary with tubular and adenomatous structures, some of which resemble rudimentary ovarian follicles, surrounded by cellular ovarian stroma. Hematoxylin and eosin. × 290

Figure 7.—Area from the same section as shown in figure 6. Cordlike and tubular structures can be seen. Dilated formations show papillary invaginations. Hematoxylin and eosin. × 215

Figure 8.—Tubular structure of the polypoid growth in the left ovary resembling an aspermatic male tubule. Hematoxylin and eosin. × 315

Figure 9.—Large spherical tumor of the left ovary and a smaller tumor of the right ovary removed from a rat possessing intraperitoneally transplanted ascites fluid. Below the tumors, the ovaries and uterine horns of a normal tumor-free rat of the same strain and age are shown for comparison. ¾ natural size.
Figure 10.—Papillary proliferation of the tumor on the meninges of the cerebellum of a Sprague-Dawley rat which had received an intracranial implant of tumor ascites fluid. Hematoxylin and eosin. × 100

Figure 11.—Tumor nodule on the peritoneal surface of a rat which received a transplant intraperitoneally. Thin strands of the mucoid substance, among the glandular formations, stained purplish red and appear black. Periodic acid-Schiff reaction. × 210
FIGURE 12.—Tumor metastasis in the subcapsular sinus of a mediastinal lymph node. Note clusters of tumor cells resembling glandular structure (upper cluster). Below this, the black mucoid substance can be seen inside a number of the cells, and at the right it can be seen as distorted ropelike strands between cells. Periodic acid-Schiff reaction. \( \times 430 \)

FIGURE 13.—Adenopapillary tumor growths in the free space of the ovarian capsule. At the upper right, a tumor nodule with characteristic adenopapillary structure may be seen. The ovary itself was not invaded by the tumor. Hematoxylin and eosin. \( \times 40 \)
FIGURE 14.—Adenopapillary growths within the ovarian capsule between a fimbria of the oviduct (right) and a corpus luteum of the ovary (left) in a rat that possessed an intraperitoneal transplant. Hematoxylin and eosin. × 235

FIGURE 15.—Ovary invaded by the tumor tissue. Evidence of partial replacement of luteal cells may be seen in center. Hematoxylin and eosin. × 75