Morphology, Secretion, and Transplantability of Ten Mouse Ovarian Neoplasms Induced by Intrasplenic Ovarian Grafting*

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Ovaries grafted into the spleens of gonadectomized hosts are transformed into neoplasms in rats (2, 14), mice (4, 7, 9, 11, 12), and rabbits (15). Although there is a rapidly growing literature on the production of such ovarian neoplasms, very few reports have been concerned with their transplantability. Li and Gardner (11) stated that they made successful primary subpassages of several neoplasms obtained from intrasplenic ovarian grafts in mice but did not elaborate the details. Furth (4) transplanted fragments from eighteen different intrasplenic grafts, and only two were successfully passed. Furth's primary subpassages were set up in a manner designed to test whether these neoplasms were conditioned or autonomous, and his findings indicated that they were conditioned. Peckham and Greene (14) obtained successful subcutaneous primary subpassages of rat granulosa-cell tumors obtained by intrasplenic ovarian grafting when gonadectomized rats were used as hosts, but no successful primary subpassages were obtained when intact rats were used as hosts. Recently, Gardner (7) described the morphology and growth pattern of a number of ovarian neoplasms that arose from intrasplenic ovarian grafts in gonadectomized mice and reported successful subcutaneous primary subpassage of five of seven tumors.

This investigation was made to study the subcutaneous transplantability of neoplasms arising from intrasplenic ovarian grafts, the morphology and secretion of the primary tumors and of subpassages of these tumors, and to look for criteria that might be used to predict successful primary subpassage of ovarian neoplasms.

MATERIALS AND METHODS

Young, adult CHI strain mice were used throughout this investigation. Seventeen females were ovariectomized, and an autologous graft of one ovary was made into the spleen of each mouse. Ten males were castrated, and 2 months later each male received an intrasplenic graft of an ovary from a related female. Throughout the period of the experiment the spleens of the host mice were palpated at monthly intervals, and an arbitrary assessment of the size of the graft was made.

Primary subpassages of these grafts and all subsequent transfers were made from small pieces of tissue (approximately 1 cu. mm.) removed from the periphery of the tumor. These tissue fragments were taken up in a trocar and injected subcutaneously into the region of the right axilla of gonadectomized and intact young adult mice.

All tissues collected at autopsy were fixed in Bouin's solution. After being sectioned, they were stained with hematoxylin and eosin, or an acid fuchsin-Orange G-fast green connective tissue stain.

Histologic studies of a variety of organs furnished a basis for the qualitative assessment of hormone secretion by the tumors. Hooker (8) has shown that the morphology of the endometrium of the mouse provides an index for determining the presence of estrogen and progestin. If progestin is present, the stromal nuclei of the endometrium are vesicular, display a prominent nucleolus, and possess fine, evenly dispersed chromatin. Estrogen is indicated when the luminal epithelium is heightened, the stroma edematous, and the glands developed. Androgen secretion is indicated when the atrophy of the seminal vesicles of castrated hosts is prevented, and in addition the morphology of the submaxillary gland provides an indication of androgen secretion in either sex (10).

RESULTS

Palpable masses were found in the spleens of eight of the ten males and fourteen of the seventeen females between 5 and 7 months after grafting. Nine to 12 months after grafting, the palpation record showed that palpable masses in four...
males and four females had regressed completely, and at autopsy 350–535 days after grafting no trace of the original graft could be found in these mice. In four of these mice (two female and two male) the spleen was adhered to the body wall or kidney, but the other four were completely free of visceral adhesions.

The remainder of the mice were autopsied at 300–535 days after grafting, and neoplastic masses were found in four males and ten females. Autopsies of the mice that did not develop neoplasms produced no significant results. From the fourteen neoplasms ten were chosen at random for this experiment; and of the ten chosen tumors three arose in male hosts and seven in female hosts. The dis-

ised into both gonadectomized and intact hosts at the first subpassage and, therefore, adequately demonstrated their autonomy. Only gonadectomized hosts were used for the other two successful subpassages (ST4 and ST5), and their successful second subpassage into intact hosts suggests the possibility of earlier autonomy.

Five of the six tumors that underwent successful primary subpassage were successfully carried in subsequent serial subpassage. The sixth tumor killed all the second-generation hosts before a third subpassage was made.

**Morphology, general.**—Although the histology of the ten tumors showed considerable variation, they showed definite patterns of organization of three general types: (a) a follicle-like organization, with the cells arranged in large, somewhat rounded groups that were surrounded by capillaries that did not penetrate into the mass of cells; the presence of an antrum in many of these masses made their follicle-like nature more apparent; (b) cells arranged in slender, twisted cords surrounded by capillaries; (c) a type composed of an unorganized mass of cells permeated by sinusoidal capillaries. These organizational patterns correspond to Gardner’s (7) histological types III, V, and IV, respectively. In the four primary tumors that were not transplanted, patterns of organization were seen that corresponded well with the other two histological types reported by Gardner. It should be emphasized that the histological organization of a primary tumor was seldom confined to just one

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>RESULTS OF TRANSPLANTATION</td>
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<table>
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<tr>
<th>TUMOR no.*</th>
<th>SEX OF HOSTS</th>
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<th>AV. LATENT PERIOD (DAYS)</th>
<th>SEX OF HOSTS</th>
<th>TAKES/NO. MICE</th>
<th>AV. LATENT PERIOD (DAYS)</th>
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<td>2/5</td>
<td>30</td>
<td>M</td>
<td>6/7</td>
<td>35</td>
<td>M</td>
<td>10/10</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>3/3</td>
<td>30</td>
<td>MC</td>
<td>7/7</td>
<td>45</td>
<td>F</td>
<td>17/20</td>
<td>50</td>
</tr>
<tr>
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<td>M</td>
<td>1/3</td>
<td>30</td>
<td>M</td>
<td>6/10</td>
<td>60</td>
<td>M</td>
<td>10/25</td>
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<td>5/15</td>
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<td>40</td>
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<tr>
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<td>FO</td>
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<td>60</td>
<td>F</td>
<td>2/5</td>
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<td>F</td>
<td>7/10</td>
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<td>FO</td>
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<td>60</td>
<td>FO</td>
<td>0/10</td>
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<td>FO</td>
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<td>M</td>
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<td>M</td>
<td>8/30</td>
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<td>M</td>
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<td>3/5</td>
<td>50</td>
<td>MC</td>
<td>1/5</td>
<td>50</td>
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* ST 1, 8, 9, 10—when transplanted into five male and five female hosts—failed to grow.
† M = intact male. F = intact female.
MC = castrated male. FO = ovariectomized female.
of the types described, and different sites of the primary tumors often showed different patterns.

The parenchymal cells of the neoplasms were morphologically similar to those found in the parenchyma of the normal mouse ovary. A rigid classification of the neoplasms based on those similarities proved to be quite difficult, because there are almost no clear-cut cytological differences between all types of ovarian parenchymal cells. To classify the tumors, the taxonomic scheme proposed by Li and Gardner (11) was used. A tumor that was composed of vesicular cells that possessed very little cytoplasm was classified as a granulosa-cell tumor. It should be noted that the nuclei of many neoplastic cells in this category had dispersed, fine chromatin in contrast to the coarse, granular chromatin of true granulosa cells. If cells possessing abundant cytoplasm and cells with cells with scanty cytoplasm were present in a neoplasm, it was called a mixed tumor. Such a scheme of classification is merely one of convenience based on superficial morphological features and does not seriously reflect the origin of the neoplastic cells.

In the mouse the term "interstitial cell" refers to a glandular-appearing cell that is responsive to gonadotrophic stimulation, and because these cells may conceivably be involved in the genesis of an ovarian neoplasm it should be emphasized that any classification scheme that neglects these cells cannot be a rigid one until more information is available.

Morphological and endocrinological observations. —Table 2 summarizes the morphological and endocrinological observations of these ten neoplasms. The age of the primary tumors and the sex of their host did not seem to influence the cell type found at autopsy, nor did they seem to influence the pattern of organization.

The primary subpassage of ST2 grew more rapidly than any of the other successful subpasses and has continued to grow rapidly for 37 consecutive subpasses. Although the parent tumor was a mixed tumor, all subsequent transplants from this tumor were composed entirely of homogeneous masses of small parenchymal cells with very little cytoplasm and were classified as granulosa-cell tumors. The large, luteal-like cells seen in the primary tumor were not seen in any sections made of transplants from this tumor. The uterus of the mouse bearing the primary tumor and the uteri of ovariectomized mice bearing transplants of this tumor were greatly enlarged, and sections showed a pseudostratified epithelium, hypertrophied myometrium, and an edematous stroma, all of which are indicative of estrogen secretion. The testes and male accessory glands were atrophic in all the intact male hosts to this tumor. In the second and third transplant generations intact females were used as hosts, and at autopsy their ovaries were found to be atrophic; histologically, these ovaries were comparable to those of a hypophysectomized mouse. The gonadal atrophy found in these host mice suggested that secretion of estrogen by the tumor inhibited the host's pituitary gonadotrophin secretion in the manner described by Deanesly and Warwick for the rat (3) and Nalbandov and Baum (13) for the chicken. There was a marked dilation

TABLE 2

<table>
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<tr>
<th>PRIMARY SPLENOGRAPH</th>
<th>SUBCUTANEOUS SUBPASSAGE</th>
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<tbody>
<tr>
<td>TUMOR NO.</td>
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<tr>
<td>ST1</td>
<td>Male 500</td>
</tr>
<tr>
<td>ST2</td>
<td>Female 400</td>
</tr>
<tr>
<td>ST3</td>
<td>Female 440</td>
</tr>
<tr>
<td>ST4*</td>
<td>Female 480</td>
</tr>
<tr>
<td>ST5*</td>
<td>Male 490</td>
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<td>ST6*</td>
<td>Female 535</td>
</tr>
<tr>
<td>ST7</td>
<td>Male 475</td>
</tr>
<tr>
<td>ST8</td>
<td>Female 497</td>
</tr>
<tr>
<td>ST9*</td>
<td>Female 498</td>
</tr>
<tr>
<td>ST10</td>
<td>Female 497</td>
</tr>
</tbody>
</table>

* Cavernous dilation of the sinusoids of the liver and spleen in the primary and secondary hosts.
of the sinusoids of the liver and the spleen in the host mice carrying this tumor.

Primary tumor ST3 was a mixed tumor that contained all three patterns of organization. The tumors of the first four transplant generations of this tumor were granulosa-cell tumors that contained areas with folliculoid organization and areas without organization. During these first four generations the folliculoid pattern of organization became progressively less prominent and was not seen in any tumors after the fifth generation, so that the tumor after that time was morphologically identical with subpassages of tumor ST2. However, the average latent period throughout fifteen generations has remained longer than that of ST2, and the growth of this tumor after it becomes palpable is a great deal slower than that of ST2. The uteri of ovariectomized hosts bearing this tumor were histologically similar to those described for ST2 and indicated that the tumor secreted estrogen. Intact male and female hosts bearing this tumor showed gonadal atrophy identical to that described for the previous tumor. Hosts bearing this tumor showed the hypervolemic response described by Furth (5).

The primary tumor ST4 had three types of organization present, but transplants from this tumor showed only cords and areas of no organization. The amounts of fibrous stroma in the transplants of this tumor were greater than those seen in any of the other tumors. During the first five transplant generations of this tumor the cord type of organization became progressively less prevalent until the tumor became a mass of homogeneous parenchymal cells of the granulosa type, split into islands by thick strands of fibrous connective tissue. Two different areas of the primary tumor were sectioned for microscopic study, and only the granulosa-type cell was seen in these cross-sections of the tumor. A tumor from one host bearing a second-generation transplant showed an area of luteal-like cells surrounded by granulosa-type cells. This was the only time that luteal-like cells were found in transplants made from any primary tumor except a pure luteoma. Although the diagnosis of the primary tumor was made from two cross-sections of tumor tissue from different sites, it is probable that the primary tumor was actually a mixed tumor. This point emphasizes the hazards of diagnosis from a single section of the primary tumor and makes it appear that an unequivocal diagnosis is possible only after the study of a complete serial section of a primary tumor. Large, stimulated uteri were found in all female hosts bearing this tumor; sections of these uteri contained pseudostratified luminal epithelium and hypertrophic myometria that are indicative of estrogen stimulation. However, the lack of stromal edema and a strong progestational reaction in the stromal nuclei throughout the endometrium suggested that the tumor was producing substantial amounts of progesterone. Intact hosts bearing this tumor showed gonadal atrophy identical to that described in hosts bearing the two tumors previously described. Dilution of the sinusoids of the liver and spleen was present in approximately one-half of the hosts to this tumor.

Primary tumor ST5 possessed all three patterns of organization and both cell types. Subsequent subpassage of this tumor resulted in slowly growing tumors that were an unorganized mass of granulosa-like cells morphologically identical to transplants of ST2. The uteri and vaginas of first- and fourth-generation hosts were comparable in all respects to those of ovariectomized nontumor-bearing controls. The submaxillary glands of the hosts showed well developed male type morphology, indicating the presence of androgen. Furthermore, the seminal vesicles of the castrated male hosts bearing this tumor were stimulated and thus gave stronger evidence that this tumor was secreting androgenic hormone. Cavernous dilation of the sinusoids of the liver and spleen was found in all hosts to this tumor.

The primary tumor ST6 and all subsequent transplants from it were a mass of uniform luteal-like cells. The endometrial stromal nuclei of the host bearing the primary tumor showed a well developed progestational response, while stromal edema and a pronounced epithelial response were lacking. The submaxillary gland morphology of this host was distinctly of the male type. Castrated males used as first-generation hosts had stimulated seminal vesicles and the male type submaxillary gland morphology. These observations are difficult to interpret, because certain androgenic steroids are known to be capable of producing a progestational response. Testosterone is capable of producing an endometrial stromal nucleus response identical to that evoked by progesterone; however, in the mouse the former also causes a stromal edema that is not seen after progesterone (J. A. Green, unpublished data). Other androgenic compounds might be capable of mimicking progesterone in all respects. With that reservation, the tentative interpretation is that the tumor was secreting both progesterin and androgen. Dilution of the sinusoids of the liver and spleen was present in hosts to this tumor.

The tumor ST7 resembled ST6 in all respects, except that there was no sinusoidal dilation in the liver and spleen of hosts to this tumor. This tumor
was lost before a third serial subpassage was made.

The primary tumors ST8 and ST9 resembled ST3 morphologically, but the uterus of the host to the former tumor was castrate-like, and the uterus of the host to ST9 showed all the morphological responses induced by estrogen. The uterus of the host to primary tumor ST10, a luteoma, had fusiform, undifferentiated stromal nuclei identical to those found in an ovariectomized mouse.

**DISCUSSION**

These observations demonstrated that ovarian neoplasms induced by intrasplenic grafting of ovaries in gonadectomized mice will grow after subcutaneous primary subpassage into intact hosts. Furth (4) reported that he obtained no successful subcutaneous primary subpassages from eighteen neoplasms that arose from ovaries grafted into the spleens of gonadectomized mice. His primary subpassages were made 4±9 months after intrasplenic grafting, while all the successful primary subpassages made here were done in excess of 1 year after intrasplenic grafting. Thus, an explanation of this difference in the success of subcutaneous primary subpassage can be made on the basis of Furth’s (6) concept of conditioned neoplasia. Prior to 1 year after intrasplenic grafting, the neoplasms are dependent upon certain conditions that are met in the spleen of a gonadectomized host, but after 1 year the neoplasm has become autonomous. If this is the correct explanation of these conflicting reports, it appears likely that, during the period between 9 months and 1 year following grafting, the neoplasm achieves autonomy. In this connection it is interesting to note that eight palpable intrasplenic masses regressed during this period, in the present investigation. A possible explanation for the regression of these tumors is that they were gonadotrophin-dependent, and their hormone secretion reached a level capable of inhibiting the gonadotrophin secretion of the host’s pituitary gland. Deprived of their stimulus, they regressed completely. This speculation leads to another question. Since a high level of estrogen is capable of inhibiting pituitary gonadotrophin secretion (8, 19) and the liver is not an insurmountable barrier for ovarian hormones produced by intrasplenic ovarian grafts in mice (1), how could an estrogen-secreting tumor achieve autonomy if it is gonadotrophin-dependent? Such tumors achieve autonomy, as shown by three that were transplanted successfully during this experiment, thus raising the possibility that dependency was contingent upon factors other than gonadotrophin.

The morphology of the tumor subpassages was rarely completely identical to that of the primary tumor. Since the three patterns of organization were generally circumscribed and separate from one another in the primary tumor, the failure of one or more patterns to appear in the subpassage may have been due to the chance selection, at the time of primary subpassage, of a site containing the pattern of organization that appeared in the subpassages. The observation that each of the three patterns was seen in subpassages indicates that all three are autonomous and that they do not represent transitional forms. After several consecutive subpassages, the pattern that lacked any structural organization usually emerged. This probably resulted from a more rapid growth rate of this type of tumor parenchyma, and consequently it gradually became the predominant type of tissue found.

The luteal-like cells failed to survive in the subpassages of the three mixed tumors (ST2, ST3, and ST5) that were successfully transplanted. This could mean that luteal-like cells do not achieve autonomy as early as the small granulosa-like cells and were transplanted during their dependent phase. However, the ready transplantability of this type of cell in the case of ST6 and ST7 makes that line of reasoning seem unlikely.

The morphology of the parenchyma of subpassages from ST2 and ST5 were microscopically similar, but their secretory capacities and their growth rates were quite different. The morphology of the other two granulosa-cell tumors that were transplantable (ST3 and ST4) became similar to these two tumors, but their hormone secretion pattern and growth rate remained distinct. Hosts bearing transplants from tumor ST5 did not show any evidence of estrogen secretion. Ovariectomized hosts bearing transplants from tumor ST4 had uteri that contained an endometrium that appeared to show a stronger progesterational response than that found in similar hosts to the other three granulosa-cell tumors. The observations presented in this investigation demonstrate that the morphology of the parenchyma of mouse ovarian neoplasms cannot be used as an absolute index of their hormone secretion.

Neither the morphology nor the secretory ability of the primary tumors furnished criteria for predicting successful primary subpassage in this small group of ovarian neoplasms. Also, it appeared that the gonadal status of the hosts to the primary subpassage had little if any influence on the success of transplantation.
SUMMARY

Intrasplenic grafts of ovaries in young adult, gonadectomized CHI mice produced ovarian neoplasms in four of ten male and ten of seventeen female hosts.

One year or more after the intrasplenic grafts were made, fragments from ten of the neoplasms were transplanted subcutaneously into related hosts. Six of these transplanted neoplasms grew readily, and their morphology and hormone secretion pattern, as indicated by the histology of various target organs, was studied. The morphology of these neoplasms proved to be an inadequate index to the quality of their secretion. Neither the morphology of the primary tumor or its secretory capacity seemed to influence the predictability of successful primary subpassage.

On this basis of this investigation and reports of other investigations, it is postulated that neoplasms arising from ovaries grafted intrasplenically achieved autonomy 9-12 months after grafting.

ACKNOWLEDGMENTS

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