An Ovarian Teratocarcinoma as an Ascitic Tumor*

G. BARRY PIERCE, JR.,† FRANK J. DIXON, JR., AND ETHEL L. VERNEY

(Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh 13, Pa.)

SUMMARY

A teratocarcinoma of the ovary of a mouse, when converted to an ascitic form, lost its differentiated structures and became monocellular in type. The monocellular tumor was shown to be a parietal yolk sac carcinoma. It had little or no morphogenetic propensity. The evidence presented supports the contention that embryonal carcinoma is a stage in the development of teratocarcinomas.

We have been investigating the pathogenesis of teratocarcinomas, particularly the development of the adult, apparently benign, differentiated structures common to them. Although nothing is known concerning the mechanism of the changes involved, we have demonstrated that the differentiated tissues of a teratocarcinoma are derived from embryonic cell types found within the tumor (12). These embryonic cell types include visceral entoderm, mesoderm, and embryonal carcinoma. We were principally concerned with the latter in this study and wished to determine whether embryonal carcinoma was truly a multipotential stem cell (8) of germinal origin (2, 3, 7) capable of giving rise by itself to differentiated structures. These postulates have never been proved.

The rationale of the experiment to be described was based upon the fact that only fast-growing tumors are convertible to an ascitic form. Ascitic conversion of a teratocarcinoma might result in the isolation of the fast-growing embryonal carcinoma or stem cells of the tumor, which could then be studied in a pure population. Although a pure population of embryonal carcinoma was not isolated, support was found for the contention that it is multipotential and probably of germinal cell origin.

MATERIALS AND METHODS

The teratocarcinoma, E6496, used in these experiments was originally described and transplanted by Dr. E. Fekete of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine (6). It arose in the ovary of a C3H mouse and contained a multiplicity of differentiated tissues. The transplants of this tumor usually did not metastasize but destroyed their hosts by cachexia.

Our colony was stocked through the courtesy of Dr. L. C. Stevens, of the Roscoe B. Jackson Memorial Laboratory, and the tumors have been maintained in the subcutaneous space of C3H mice and have been transplanted at approximately 42-day intervals. Tissues to be transplanted were removed aseptically from the host and were minced in a few drops of Hanks's balanced salt solution. Recipients were given injections intraperitoneally of ½ ml. of the tumor mince. The hosts were sacrificed upon the development of distended bellies; their peritoneal cavities were opened aseptically, and any fluid present was aspirated. Recipients in subsequent generations received ½ ml. of hemorrhagic ascitic fluid intraperitoneally. Ascitic fluid from each generation was also injected subcutaneously in another series of animals, and the subcutaneous tumors that developed were carried as a subline in the subcutaneous space. They served as a further control on the ability of free-floating cells of the ascites to differentiate.

RESULTS

Eleven tumors maintained in the subcutaneous space served as controls for this study. Grossly, the tumors were brainlike, and some contained black pigment and contractile muscle. A variety of differentiated tissue types were characteristically found upon microscopic examination. The best differentiated were often located peripherally, while the large central portion of each tumor was composed of undifferentiated embryonal carcinoma, often arranged in small ball-like aggregates that

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impacted an organoid appearance to the tissue (Fig. 1). The embryonal carcinoma cells had poorly staining cell borders giving them a symplasmic appearance. The cytoplasm was amphophilic in color and deeply staining, whereas the nuclei were pleomorphic and each contained several large nucleoli. Mitotic figures were numerous. Frank embryonal carcinoma could often be traced, with gradual transitions, into primitive neuroectodermal tissue, the latter containing many mitoses (Fig. 6). Transitional areas between frank embryonal carcinoma and undifferentiated mesenchyme (Fig. 2) were often noted, with the latter containing significantly fewer mitotic figures than embryonal carcinoma. These transitional areas suggested but did not prove that the embryonal carcinoma gave rise to primitive neuroectodermal and mesenchyme.

The control tumors always contained, in addition to embryonal carcinoma, islands of keratin-producing squamous cells, glands (no attempt was made to list the various types of acinar structures present), and brain (including primitive neuroectoderm, ependyma, and astrocytes) (Figs. 3, 4). Mesenchymal derivatives, muscle, connective tissue, and primitive mesenchyme were present in 82 per cent of the control tumors, as were densely pigmented cells. The pigment granules were often found in either columnar or cuboidal cells that lined small slits and that were interpreted as representing abortive attempts on the part of the tumor to form uveal tracts (Fig. 4). Cartilage was found in 45 per cent of tumors and bone in only 9 per cent. The bone was usually located on the margin of cartilaginous masses, although evidence of endochondral ossification was difficult to find. The hyaline cartilage was always embedded in a tissue composed of spindle-shaped mesenchymal cells that resembled by their arrangement procartilage or perichondrium (Fig. 4). Neither pigmented, cartilagenous, nor osseous cells were found in mitotic division. In none of the murine teratocarcinomas studied have we found areas of transition between embryonal carcinoma and cartilage, muscle, or bone, which observation makes one doubt whether these elements ever develop directly from embryonal carcinoma. In 9 per cent of tumors a peculiar cell type was found embedded in a hyaline matrix. The hyalin was eosinophilic and homogeneous in appearance, while the enclosed cells had small hyperchromatic nuclei and relatively lightly staining cytoplasm. These were considered to be parietal yolk sac cells embedded in a hyalin that corresponded to Reichert’s membrane of the mouse embryo (Fig. 5).

When teratocarcinomas, composed of the tissue types described, were minced and injected intraperitoneally in strain C3H mice, hemorrhagic ascitic fluid and solid serosal implants of tumor were found. The histologic components of the peritoneal implants were tabulated and averaged for each generation (Table 1). The microscopic structure of peritoneal implants of solid tumor in generations 1 and 2 differed only slightly from that of the control tumors maintained in the subcutaneous space (Table 1). The chief departure was the loss of the pattern of the embryonal carcinoma; instead of being in ball-like aggregates, as was found in the controls, this tissue grew in large aggregates broken only by the formation of ependyma and neuroectoderm.

The peritoneal implants found in ascitic generation 3 were markedly altered in their composition (Table 1). Neither bone nor cartilage was found in the sections examined, while the incidence of glands, pigment, squamous cells, connective tissue, and muscle was markedly reduced. By the 5th generation, pigmented cells had disappeared, and by the 7th generation, squamous cells, mesenchyme, and muscle had disappeared. No glands were found in the implants in generation 8. The tumors were composed of embryonal carcinoma, brain, and carcinoma with hyalin; in generation 9 they were composed of only embryonal carcinoma and carcinoma with hyalin. The cells of the carcinoma with hyalin, although similar to embryonal carcinoma, were sufficiently different to warrant separation as an entity (Figs. 7–9). The cytoplasm of these cells was less amphophilic, the cell membranes stained more sharply, and the nuclei and nucleoli were smaller than those of embryonal carcinoma. One could find an eosinophilic refractile hyaline material sometimes with the configuration of basement membrane lying between the cells. At other times the hyaline material was so abundant that the tumor cells seemed to form only small islands in it. Beginning with the solid implants in the third generation there was a gradual increase in the incidence and amount of carcinoma with hyalin. Furthermore, this cell type became more pleomorphic in succeeding generations (Figs. 5, 7), and correlated with such a marked increase in growth rate that it was necessary to reduce the size of the inocula from ½ to 1 ml. of ascitic fluid to prevent destruction of the hosts within 4 or 5 days of inoculation. With succeeding generations the incidence of metastases of carcinoma with hyalin to mediastinal lymph nodes and lungs increased.

Other changes were noted in the carcinoma with hyalin. In areas of florid growth, the cells were remarkably similar to embryonal carcinoma, and the
hyalin was scant and resembled basement membranes. Ball-like masses of hyalin, several hundred \( \mu \) in diameter, were often found in this tumor, and the enclosed cells appeared darker and had fewer mitoses than the surrounding ones (Fig. 7a). In generation 12, clumps of cells were found in the florid areas of the carcinoma with hyalin that bore a strong resemblance to visceral yolk sac (Fig. 8). Such cells, with their abundant eosinophilic vacuolated cytoplasm that contained brown pigment, were often separated from the cells of the carcinoma with hyalin, but many transitional forms could be seen between them, suggesting a common entodermal origin for them. In implants from the fourteenth generation, trophoblastic giant cells with typical huge nuclei and abundant amphophilic cytoplasm were found in the carcinoma with hyalin. However, no transitional forms were found between the cells of trophoblast and carcinoma with hyalin (Fig. 9).

The animals grafted in the subcutaneous space with ascitic fluid from each generation developed tumors that were maintained as separate sublines (Table 2). A comparison of Tables 1 and 2 indicates that the subcutaneous sublines mirrored the findings in the peritoneal implants. Although they did not lose their differentiated tissues at exactly the same time that their counterparts in the intra-

### TABLE 1

**INCIDENCE OF MICROSCOPIC STRUCTURES* IN INTRAPERITONEAL TUMORS RESULTING FROM SERIAL PASSAGE OF INTRAPERITONEAL FLUID**

<table>
<thead>
<tr>
<th>TISSUES PRESENT</th>
<th>CONTROLS</th>
<th>SUBCUTANEOUS SPACE</th>
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<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15</td>
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<tr>
<td>Embryonal carcinoma</td>
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<td></td>
</tr>
<tr>
<td>Brain</td>
<td>100 100 100 100 100 50 50 50 50 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Glands</td>
<td>100 100 100 100 100 50 50 50 50 0 0 0 0 0 0</td>
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<tr>
<td>Squamous cells</td>
<td>82 91 93 90 70 25 25 25 25 0 0 0 0 0 0 0</td>
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<tr>
<td>Connective tissue and muscle</td>
<td>9 9 9 7 0 0 0 0 0 0 0 0 0 0 0 0</td>
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<tr>
<td>Pigment</td>
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<tr>
<td>Cartilage</td>
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<td>82 91 93 90 70 25 25 25 25 0 0 0 0 0 0 0</td>
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<tr>
<td>Bone</td>
<td>45 55 43 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Hyaline carcinoma</td>
<td>9 9 9 0 0 0 25 50 50 100 100 100 100 100 100 100</td>
<td></td>
</tr>
<tr>
<td>Trophoblast</td>
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<td></td>
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<tr>
<td>Visc. yolk sac</td>
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<td></td>
</tr>
<tr>
<td>No. tumors:</td>
<td>11 22 14 7 7 8 8 8 10 12 10 5 6 13 8 4</td>
<td></td>
</tr>
</tbody>
</table>

* All values expressed as percentages.

### TABLE 2

**INCIDENCE OF MICROSCOPIC STRUCTURES* OF SUBCUTANEOUS TUMOR SUBLINES DEVELOPED FROM HEMORRHAGIC ASCITIC FLUID**

<table>
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<tr>
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<tr>
<td>Brain</td>
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<tr>
<td>Glands</td>
<td>100 100 100 100 100 65 60 100 56 0 0 0 0 0 0</td>
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<tr>
<td>Squamous cells</td>
<td>100 100 100 100 100 65 60 100 56 0 0 0 0 0 0</td>
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<tr>
<td>Connective tissue and muscle</td>
<td>9 9 9 0 0 0 25 50 50 100 100 100 100 100 100</td>
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<tr>
<td>Pigment cells</td>
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<tr>
<td>Cartilage</td>
<td>82 91 64 57 65 0 0 0 0 0 0 0 0 0 0 0</td>
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<tr>
<td>Bone</td>
<td>82 91 93 90 70 25 25 25 25 0 0 0 0 0 0 0</td>
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<tr>
<td>Bone</td>
<td>45 55 43 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
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</tr>
<tr>
<td>Bone</td>
<td>9 9 9 0 0 0 25 50 50 100 100 100 100 100 100</td>
<td></td>
</tr>
<tr>
<td>No. tumors:</td>
<td>11 22 14 7 7 8 8 8 10 12 10 5 6 13 8 4</td>
<td></td>
</tr>
</tbody>
</table>

* All values expressed as percentages.

A developed from 1st generation fluid; B developed from 2d generation, etc.
peritoneum lost theirs, the order of their loss was almost identical. Only a few samples of ascitic fluid from each generation were grafted into the subcutaneous space, so one might anticipate the variations noted in the tables on that basis. Long-term residence in the subcutaneous space, after one intraperitoneal passage, did not markedly influence the morphogenetic capability of the cells, although the tumors had a slightly greater tendency to simplify their patterns than did stock tumors that had always been maintained in the subcutaneous space.

The cells of the ascitic fluid changed with succeeding generations. In the early generations the fluid contained, in addition to fragments of teratocarcinoma and single tumor cells, many red blood cells and inflammatory cells. In latter generations almost all of the tumor cells were found in small clumps, usually arranged as vesicles. Although some were empty, most were filled with a smooth homogeneous eosinophilic hyaline material.

DISCUSSION

These experiments were undertaken in an effort to secure embryonal carcinoma cells from a teratocarcinoma and to test their totipotentiality. The reasons for using this method were based on the observations of Leighton (10), who for reasons different from ours converted this tumor to an ascitic form and developed a monocellular tumor from it. He did not identify the cell type derived. Klein and Klein (9) proved that by ascitic conversion a population of tumor cells was selected for its fastest growing members. We have shown,\(^1\) by comparing the numbers of colchicine-induced mitoses in the various tissues of a teratocarcinoma, that the embryonal carcinoma cells multiply faster than those of any of the other tissues present in the tumor. However, rather than isolating a population of rapidly growing multipotent embryonal carcinoma cells by the ascitic conversion of this teratocarcinoma as we had anticipated, we secured a carcinoma with hyaline stroma that was sufficiently different from embryonal carcinoma to warrant its separation as an entity. This carcinoma with hyalin was associated with only two other cell types, visceral yolk sac and trophoblast. Transient forms between visceral yolk sac cells and those of carcinoma with hyalin were observed and were interpreted as representing either intermediate stages in the differentiation of visceral yolk sac from carcinoma with hyalin or a common cell of origin for both cell types. In any case an entodermal origin for the carcinoma with hyalin was indicated.

In another publication we discussed the histogenesis of a carcinoma with hyalin stroma that was developed from a teratocarcinoma of testicular origin (18). We concluded that the carcinoma derived from the testicular tumor was a parietal yolk sac carcinoma, that the hyalin was analogous to Reichert's membrane of the developing mouse embryo, and that the cells embedded in it produced the hyalin. In vitro experiments performed subsequently upon the carcinoma with hyalin from the testicular teratocarcinoma have proved that the cancer cells secrete the hyalin.\(^2\) Further work upon embryoid bodies in vivo have clearly shown an entodermal origin for the cells that secrete the hyalin (14). The majority of embryologists feel that parietal yolk sac cells produce the hyalin of Reichert's membrane in the mouse embryo (1), but there is not complete agreement (15). In view of our data we feel that the carcinoma with hyalin is a parietal yolk-sac carcinoma and that the hyalin of Reichert's membrane is of entodermal origin.

The pattern by which the teratocarcinoma lost its differentiated tissues to become the parietal yolk-sac carcinoma is of interest. Control tumors from the subcutaneous space injected intraperitoneally in generation I were composed of as many as seven differentiated tissues. A reduction in the number of tissue types found in the peritoneal implants occurred with the serial passage of ascitic fluid intraperitoneally. Tissues derived in the normal mouse embryo by secondary or tertiary induction were lost first: pigment cells, cartilage, bone, muscle, and glands, leaving tumors composed of embryonal carcinoma, neuroectoderm, and carcinoma with hyalin. After further passages of ascitic fluid, neuroectoderm (derived directly from embryonal carcinoma) was lost, resulting in peritoneal implants composed of embryonal carcinoma and parietal yolk-sac carcinoma. Finally, only parietal yolk-sac carcinoma was found, and aside from trophoblastic giant cells and from areas of differentiated visceral yolk sac no other tissues were found in these tumors.

A counterpart, in experimental embryology, for the end-stage tumors that were composed of parietal yolk-sac carcinoma, trophoblast, and visceral yolk sac was found in the work of Fawcett (5). He transplanted fertilized mouse ova to the anterior chamber of the eye and found that only yolk sac and trophoblast were developed in the grafts. The parietal yolk-sac cells were embedded in hyalin and formed ball-like aggregates that bore a striking resemblance to those in our Figure 7a. He con-

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1. R. Midgely, G. B. Pierce, and F. J. Dixon, unpublished data.

considered the hyalin to be an overproduction of Reichert's hyalin. Visceral yolk-sac cells and trophoblastic giant cells were also included among tissues developed from transplanted fertilized mouse ova (5).

There are two pathways by which the end-stage tumor, the parietal yolk-sac carcinoma, could have been developed in this experiment. Although not likely, it is conceivable that the very benign-appearing parietal yolk-sac cells found in 9 per cent of control tumors could so adapt to intraperitoneal conditions that they would outgrow all other components of the teratocarcinoma, become malignant, and give rise to the parietal yolk-sac carcinoma. This theory would presuppose that benign, slowly growing cells could undergo malignant change as a result of intraperitoneal passage. Another, and to us more likely, explanation for the development of the parietal yolk-sac carcinoma would be a change in form of embryonal carcinoma to malignant distal entoderm. The latter, if more adaptable to intraperitoneal conditions, would outgrow and eventually replace the other tissues present. At the stage when the tumor was composed of parietal yolk-sac carcinoma and visceral yolk sac on the one hand and trophoblast on the other, one might postulate that, although not recognized, embryonal carcinoma must have been present in the tumors to serve as an origin for the trophoblast. There is no theoretic support for an entodermal origin for trophoblast or a trophoblastic origin for entoderm, so the only explanation available would be that they had a common cell of origin. That cell of necessity would be the neoplastic equivalent of the germ cell or embryonal carcinoma.

Our demonstration, previously, of the morphogenesis of the differentiated tissues of a teratocarcinoma (12), the fact that embryoid bodies in mouse teratocarcinomas resemble mouse embryos (12) while those found in human teratocarcinomas resemble human embryos (9), and the data presented here would indicate that the steps in the development of teratocarcinomas may be closely related to the morphogenesis of normal embryos.

So little is known concerning the mechanism resulting in the simplification of the pattern of the teratocarcinoma as a result of repeated intraperitoneal passages of ascitic fluid that it is difficult to evaluate the possibilities. There was an increment in growth rate of the tumors with serial passage of ascitic fluid, and the peritoneal implants contained progressively more parietal yolk sac carcinoma and less differentiated tissues. Moscona (11) has shown that a critical concentration of cells of like propensity is necessary in a population for those cells to form a tissue. We can only speculate on how much the dilution of embryonal carcinoma by the parietal yolk sac carcinoma affected its capacity to form differentiated tissues.

The inverse correlation between growth rate and differentiation observed for the tumor has been noted in several embryonic systems (4). Although it is tempting to assume that the mechanism is a sorting out of populations of embryonal carcinoma, with various abilities to form tissues, on the basis of their growth rates we have no way of knowing the relative importance of this mechanism in relation to other possibilities.

REFERENCES

Fig. 1.—Ball-like aggregates of embryonal carcinoma separated from each other by primitive mesenchymal-like cells. The latter often appear to arise from the embryonal carcinoma by delamination. ×420.

Fig. 2.—Clumps of embryonal carcinoma with transitional cell types and poorly differentiated mesenchyme. ×240.

Fig. 3.—Section from a well differentiated area of a control tumor from the subcutaneous space illustrating keratinizing squamous epithelium, mesenchyme, brain, and ciliated columnar epithelium. ×120.

Fig. 4.—Control tumor from the subcutaneous space illustrating areas of squamous cells, pigment cells, neuroectodermal tubes, cartilage, and a few gland-like spaces. ×120.
FIG. 5.—Section illustrating the small dark cells embedded in hyalin found in only 9 per cent of control tumors. ×240.

FIG. 6.—Embryonal carcinoma, left lower, neuroectoderm, right, with intervening areas of transitional cell forms. ×240.

FIG. 7a.—A carcinoma with hyalin. Ball-like masses of hyalin containing only a few cells were often found in florid areas of growth. The hyalin in the latter areas was scanty. ×120.

FIG. 7b.—A less florid area with more hyalin. ×120.

FIG. 8.—An area of visceral yolk sac embedded in the carcinoma with hyalin. Transitional areas between the cell types can be seen. Hyalin at arrows. ×240.

FIG. 9.—Trophoblastic giant cells found in the carcinoma with hyalin. Hyalin may be seen at the arrows. ×240.
13. ———. Testicular Teratomas II. Teratocarcinoma as an Ascitic Tumor. Ibid., pp. 584-89.
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