Ovarian teratomas are rare tumors in mice, and few cases have been mentioned in the literature. Transplantation studies with only one embryoma were reported by Jackson and Brues (3). This embryoma occurred spontaneously in the ovary of a C3H mouse and was successfully transplanted into mice of the same strain through eleven serial transplants. The pleomorphic nature of the embryoma was maintained in vivo and in tissue culture.

More recently, Fawcett (2) described bilateral ovarian teratomas found in a Swiss albino mouse and remarked about the infrequency of this type of tumor.

We are presenting observations on an ovarian teratoma that occurred in our mouse colony and has been maintained by serial transplantation. The tumor occurred in a mouse of the C3HJ3 substrain. This line was developed by transferring ova of a mouse of the C3H inbred strain, 8 days after fertilization, into the uterus of a C57BL/6 female. The descendants of the young born to the C57BL/6 mother, and nursed by her, were continued to be inbred by brother-sister matings, and constitute the C3HeB substrain.

The animal had a palpable abdominal tumor and was killed at the age of 16 days. The left ovary was greatly enlarged and measured approximately 10 X 15 X 30 mm. The right ovary was normal. Parts of the enlarged left ovary were fixed and prepared for sectioning, and parts of it were used for transplantation. Microscopic examination showed that the ovarian tumor was composed of undifferentiated "embryonal" cells and many types of differentiated tissue. Remains of the ovary were present and contained a few atretic follicles and at least one normal follicle with ovum. Some of the undifferentiated cells were round or oval-shaped and were arranged in small nests (Fig. 1); others showed more varied forms and were scattered irregularly. Both types revealed mitotic figures. The differentiated tissues included nervous tissue (Fig. 2), hyaline cartilage, bone, striated muscle fibers (Fig. 3), and the following kinds of epithelia: stratified squamous, cuboidal ciliated, simple columnar, and goblet cells. These epithelial cells formed the lining of cysts of various sizes. Small groups of alveoli formed by cuboidal epithelium and occasionally surrounded by smooth muscle fibers were seen. At some areas abundant amounts of pigment granules were present in epithelial cells and in stellate cells. Mitotic figures were rare. The various tissues intermingled without evidence of organization. The tumor was designated as E 6496 and was diagnosed a teratoma.

For transplantation, several pieces of the original tumor were selected, cut into very small particles, and well mixed. The teratoma was transplanted subcutaneously with a trocar into five weaning age hybrids of C57BL/6 × C3H. In about 4 weeks all five mice had palpable tumors at the site of transplantation. One of these animals was killed, and the tumor was retransplanted. Thereafter, subcutaneous transplantations were made about every 40 days. Samples from each tumor that was used for transplantation were sectioned for microscopic studies. At each successive transplantation care was taken to include areas of the tumor that differed in color and density. Therefore, several parts were cut up and minced well. An attempt was also made to separate the different components of the tumor: for example, small pieces of black pigmented parts, soft white parts, or hard bony parts, etc., were transplanted into identified mice. Many of the tumors resulting from these transplants were also sectioned.

To date, the tumor is in its ninth transplant generation. It grows in almost all the males and females of the C3H, B strain in which it originated, in the C3H strain, and in F1 hybrids from C57BL/6 × C3H. Occasionally the tumor invades the abdominal wall, but so far metastasis has not occurred. The sizes attained by the tumors vary greatly. Three of the largest dissected tumors weighed 34, 30, and 29 gm. about 70 days after transplantation. The tumors kill the hosts in an average of 80 days.

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At the second and third transfer generations, the tumor was transplanted subcutaneously into mice of the following unrelated strains: BALB/c, A/He, RIII/JFe, AKR, DBA/1 and C57BL/6. These animals were kept under observation for 5 months. In one of the RIII/JFe animals, a hard nodule of pin-head size was palpable at the site of the transplantation. Sectioned and examined microscopically, this proved to be a small piece of bone, which was probably present in the transplanted tumor and persisted in the new host. In another RIII/JFe mouse, a small area containing pigment granules was found at the site of the transplant. None of the animals showed any tumor growth.

At the seventh and eighth serial transfers, subcutaneous transplantsations were done into the following foreign strains: C57L, DBA/1, C57BL/6, RIII/JFe, AKR, A/He and BALB/c. These animals have been under observation for 2–5 months and are still alive. None of them has any palpable tumors. Table 1 summarizes the results of transplantations in the related and unrelated strains.

Microscopic examination of sections of about 90 tumors showed that the tumors resulting from the transplantations, like the original teratoma, were mixed, containing undifferentiated "embryonal" and a variety of differentiated tissues. In general, the proportion of differentiated adult tissues was greater in larger tumors which grew in the hosts for a longer period, than in the small tumors which were allowed to grow for a shorter time. The attempt to isolate different components of the tumor by transplanting small pieces of black pigmented, or soft white parts, etc., was not successful. In some cases these components were somewhat more conspicuous in the resulting tumors, but in all cases many other tissues were also present, and uniform growth of the isolated components did not result.

Figure 4 shows parts of the tumor in the first transplant generation. Nervous tissue, stratified squamous epithelium, striated muscle fibers, bone surrounded by fibrous connective tissue, and a cyst lined by ciliated columnar epithelium represent the differentiated tissues. The undifferentiated cells are irregularly scattered and show many mitotic figures. Figure 5 shows parts of a tumor of the second transfer generation in which cartilage, bone with marrow spaces, stratified squamous epithelium, and small alveoli are visible.

The fifth transplant generation is represented by Figures 6, 7, and 8. In Figure 6 a small cyst lined by stratified squamous epithelium and a large cyst lined partly by pigmented epithelium and partly by goblet and columnar cells can be seen. The goblet cells were functioning, and the cyst contained mucus, the product of their secretion. Figure 7 shows a few striated muscle fibers and loose connective tissue. In Figure 8 a tube is present, lined in parts by pigmented columnar and stratified squamous epithelium and by goblet cells intermingled with columnar epithelium. Finger-like structures resembling intestinal villi project into the tube. Transplantation of parts of the fifth generation tumor shown in Figure 6 resulted in the sixth generation tumor shown in Figure 9. The animal bearing this tumor was killed 82 days after transplantation. Cartilage, bone, dense fibrous

### Table 1

<table>
<thead>
<tr>
<th>Transplant generation</th>
<th>No.</th>
<th>Unrelated strain</th>
<th>Host strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>5/5</td>
<td>BALB/c</td>
<td>R11L/JFe</td>
</tr>
<tr>
<td>Second</td>
<td>14/14</td>
<td>A/He</td>
<td>0/4</td>
</tr>
<tr>
<td>Third</td>
<td>24/24</td>
<td>AKR</td>
<td>0/8</td>
</tr>
<tr>
<td>Fourth</td>
<td>29/31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fifth</td>
<td>29/29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sixth</td>
<td>27/27</td>
<td>C57BL/6</td>
<td>0/4</td>
</tr>
<tr>
<td>Seventh</td>
<td>15/15</td>
<td>DBA/1</td>
<td>0/4</td>
</tr>
<tr>
<td>Eighth</td>
<td>7/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ninth</td>
<td>6/6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The related strains include C57BL, C5H, and F1 hybrids from C57BL/6 X C5H6.

† Numerators are the numbers growing the tumors; numbers in the denominators are the total number of animals receiving tumor transplants.

This tumor shows more differentiated tissues than the one in Figure 6 which grew in its host for only 48 days.

In all the sections examined, regardless of the degree of differentiation, the distribution of the various tissues was confused, and organization was almost completely absent.

It was evident in all the tumors that the differentiated adult tissues contained few mitotic figures, while the undifferentiated embryonal cells were proliferating rapidly. Mitotic figures in these cells were carefully studied to ascertain whether they contained the haploid or the diploid number of chromosomes. Chromosome counts of metaphase plates showed the chromosomes clearly to be diploid. On the basis of a quantitative study of mitotic figures, the growth rates of the original ovarian teratoma and tumors of the first and fourth transplant generations were compared.
Each of the transplanted tumors had been growing in its host for 41 days. The sections selected from all three of these tumors were approximately equal in size and contained about the same amounts of undifferentiated embryonal and differentiated adult tissues. The counts were made at a magnification of $\times 900$ (oil immersion), and all the mitotic figures present in the sections were counted. There were 56 mitotic figures found in the section of the original ovarian teratoma, 477 in the section of the first transplant generation, and 369 in the section of the fourth transplant generation. The rates of growth in the first and fourth transplant generations were not significantly different from one another but were greatly increased compared to the original tumor. Perhaps it can be surmised that in the ovary the growth rate of the tumor was held in check in some way, and after transplantation into new hosts grew without such restriction.

**DISCUSSION**

There are many hypotheses regarding the origin of teratomas. According to Ewing, the origin of the ovarian teratoma is now referred by the great majority of observers to the sex cell or ovum. As was mentioned previously, chromosome counts of metaphase plates of teratoma E 6496 showed them to be diploid. Although this does not enable us to determine the origin, it rules out the possibility of haploid parthenogenetic development of this tumor from an ovum or polar body.

It is generally accepted that the undifferentiated embryonic tissues of teratomas are pluripotent and are able to give rise to cells that differentiate into the diverse components of these complex tumors. The fact that teratoma E 6496, transplanted into new hosts through nine serial transplants, maintained its pleomorphic structure is a proof of this pluripotency. Indeed, it is highly improbable that the amount of tissue introduced subcutaneously at each successive transplantation could contain all the diverse components found in the tumors of the new hosts. The varied elements could be produced only by the continuous growth and differentiation of transferred pluripotent cells. Attempts to separate and grow the differentiated components failed because complete separations were not accomplished, and, while the differentiated components may have persisted, actual growth depended on the undifferentiated pluripotent cells.

Studies of all the tumors show that the undifferentiated cells are growing most rapidly. These, therefore, are the most essential elements. In giving rise to the various tissues the undifferentiated cells show their potentialities and give proof of their pluripotency.

It is generally considered that teratomas containing embryonic components are usually malignant. Teratoma E 6496 contains such components. The results with the transplantation of this tumor were similar to those of Jackson and Bruins, who also observed that their embryoma maintained its pleomorphic nature through eleven serial transplants.

Subcutaneous transplantations of the teratoma into foreign strains did not result in progressive growth. In its specificity to grow only in the strain in which it originated, and in the F1 hybrids of this strain, the teratoma behaved like most other mouse tumors. The tumor will be maintained by transplantation, and further studies are planned.

**SUMMARY**

A spontaneously occurring ovarian teratoma containing undifferentiated "embryonic" and many types of differentiated tissue is described. The tumor was transplanted subcutaneously and grew in males and females of the strain in which it originated (CSH.B), in the C3H strain, and in F1 hybrids of C57BL/6 × C3H.

The transplanted tumors maintained the pleomorphic character of the original tumor through nine serial transplants. It is considered that the essential elements of this tumor are the undifferentiated cells which are pluripotent. In giving rise to the differentiated tissues, the undifferentiated cells showed their potentialities and gave proof of their pluripotency.

**ACKNOWLEDGMENTS**

We are indebted to Dr. Allen B. Griffen for the determination of diploidy of the chromosomes and for the mitotic counts.

**REFERENCES**

All the sections were stained with hematoxylin and eosin except Figure 2.

**Fig. 1.**—The original ovarian teratoma showing undifferentiated round or oval-shaped cells arranged in a small nest. ×400.

**Fig. 2.**—Nervous tissue in the ovarian teratoma. Phosphotungstic acid stain. ×400.

**Fig. 3.**—Striated muscle fibers in the ovarian teratoma. ×400.

**Fig. 4.**—Tumor produced by the first transplant generation, showing bone, a cyst lined by ciliated epithelium, striated muscle, nervous tissue, and a cyst lined by pigmented stratified squamous epithelium. ×200.

**Fig. 5.**—Tumor produced by the second transplant generation showing bone with marrow spaces, stratified squamous epithelium and a few small alveoli. ×200.

**Fig. 6.**—Tumor produced by the fifth transplant generation showing a small cyst lined by stratified squamous epithelium and a large cyst lined by pigmented epithelial cells and goblet cells intermingled with columnar cells. ×200.

**Fig. 7.**—Striated muscle fibers in a tumor of the fifth transplant generation. ×400.

**Fig. 8.**—Tumor produced by the fifth transplant generation, showing a tube lined by pigmented columnar and stratified squamous epithelium and columnar epithelium intermingled with goblet cells. Finger-like structures resembling intestinal villi project into the tube. ×200.

**Fig. 9.**—Tumor produced by the sixth transplant generation. Cartilage, bone, cysts lined by cuboidal epithelium and other cysts lined by stratified squamous epithelium can be distinguished. ×200.
Studies on a Transplantable Teratoma of the Mouse

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