Studies on a Transplantable Embryoma of the Mouse

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A general characteristic of most tumors is their ability to propagate a single cell type to an extent limited chiefly by the survival of the host. In the case of transplanted tumors, it generally appears that the cell type is a homogeneous and self-perpetuating entity which increases in number by division as rapidly as the requisite nutrition can be secured. When tumors show two or more cell types, one type (the stroma cell) may be contributed by the host in response to the growth of the other, malignant type, thus keeping pace in the same way as in hypertrophy of normal tissues. The type of cell may change during the course of time, as when sarcoma arises from stroma cells. Under certain conditions, two tumor cell types may coexist together for a long period (1). When one cell type grows considerably more rapidly, however, it soon outgrows the other (2).

In the case of normal growth from embryonal through adult life a contrary condition exists. Here growth rate is subject to progressively increased retardation, while the growth and development of the various component tissues falls into line according to an intimate regulation. The evidence is accumulating that the normal regulation of development and growth depends upon certain chemical factors.

Malignant tumors of an embryonal or teratoid nature are of great interest since their behavior is like that of other neoplasms, although they are composed of many cell types. The coexistence of numerous cell types suggests that they are subject to some sort of organizing factor which prevents one tissue from outgrowing another.

EXPERIMENTAL OBSERVATIONS

Such an embryonal tumor appeared in a female mouse of the C3H strain which had been inbred for three generations in our laboratory, and previously for at least twenty generations. The mouse bearing the tumor had been pregnant at 3 months of age and delivered 3 young which died shortly after birth. Three months later an intra-abdominal tumor was noted; it was subsequently removed and transplanted into other mice of the same strain.

At the time of removal the tumor measured 2.5 cm. in its longest diameter and was heterogeneous in appearance with many small black areas and with no visible necrosis. Microscopically it contained many types of tissue, both embryonal and mature. It appeared to have arisen in the left ovary; the right ovary was normal in appearance.

The tumor was readily transplantable by subcutaneous, intrasplenic, and intrahepatic inoculation of small fragments, and has taken subcutaneously in over 75 per cent of all C3H mice inoculated, both males and females. Transplants have grown to 2 cm. in size in from 6 weeks to 6 months. It has now been transplanted through 13 generations and over 250 tumors have been examined.

In the course of this continued transplantation the neoplasm showed no essential change in type. All individual tumors are composed of some undifferentiated "embryonal" tissue and some mature tissue of diverse types.

The embryonal tissue is more or less readily distinguishable and contains virtually all of the mitotic

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Concentration of mitotic activity in embryonal areas of tumor. Tumor from a mouse treated 9 hours before by subcutaneous injection of 0.03 mgm. colchicine. Mitoses are extremely numerous in the embryonal (darkly stained) areas, and virtually absent elsewhere. Ninth generation transplant from original tumor. Mag. X 80.

Fig. 2.—Islands of embryonal cells with some resemblance to early embryonic epithelium. Second generation transplant. Mag. X 150.

Fig. 3.—Secreting epithelia. Second generation transplant. Mag. X 150.

Fig. 4.—Epithelial pearl formation. Fourth generation transplant. Mag. X 150.

Fig. 5.—Cyst formation. Seventh generation transplant. Mag. X 150.

Fig. 6.—Bone and cartilage. Fifth generation transplant. Mag. X 150.

All sections stained with hematoxylin-eosin.
activity, which is intense. This is brought out with particular clarity in tumors from colchicine-treated mice (Fig. 1). Most of the embryonal tissue is composed of small cells with little cytoplasm, growing without architectural detail (Fig. 2). Occasionally cells of this type are arranged in structures with some resemblance to neural tubes and these are usually surrounded by mature nerve tissue. Structures resembling trophoblasts, as described in human embryomas by Peyron (3), have not been positively identified.

The mature tissues have included squamous epithelium with pearl formation, pigmented and ciliated epithelia, epithelia containing goblet cells, and various glandular structures, often secreting and sometimes forming cysts containing watery or mucinous material (Figs. 3-5). Smooth muscle, cartilage, bone, and nerve tissue are common. Mitotic activity in all these tissues is very rare, although occasional figures are seen in structures resembling skin or secreting glands. Notably absent are any tissues resembling liver, kidney, or gonads. Throughout all of the mature tissues runs a stroma of more or less differentiated fibroblasts in which mitotic activity is likewise rare. The various tissues showed very little evidence of organization aside from a fairly good stroma-epithelium relationship. Bone has been seen in juxtaposition to connective tissue, and also apparently developing from cartilage (Fig. 6).

Although the general makeup and behavior of the tumor remains constant, great changes in the predominant cell type may occur. In one series of five transplant generations, both nerve and bone have appeared and then disappeared.

In making random selection of tissue for transplantation, an effort was made to secure fragments from remotely separated areas. In some cases obviously differentiated tissues (bone, large heavily pigmented areas, and walls of cysts) have been used. These tissues generally have not taken, or have given rise to extremely slowly growing transplants. Otherwise, random selection of tissue has not resulted in sorting out of tissue types.

Tumors resulting from a series of relatively slowly growing tumors (6 generations in 18 months) show no obvious histological differences from those growing more rapidly (10 generations in the same time). It has been observed in several instances that transplantation of a rapidly growing tumor gives rise to a slowly growing one, from which certain transplants in turn grow rapidly. It is thought that this may be due to varying proportions of embryonal tissue in the fragments used.

A few tissue cultures of the tumor have been made for the special purpose of studying cell division. Good growth of a very pleomorphic type has been obtained with both epithelial and fibroblastic growth. A study of 50 mitoses has failed to show any constant anomaly of division.

In a few preliminary experiments material containing organizers in high concentration 2 was inoculated into our tumors without effecting any apparent change in their architecture.

Metabolic studies by Warburg's method were made on 2 tumors in the series, both of which were composed about equally of embryonal tissue and mature tissue. Most of the mature tissue resembled brain. One of these (No. 209) 3 was an 8th generation tumor; the other (No. 256) 3 was in the 12th transplant generation. The results as shown in Table I and Fig. 7 indicate high aerobic glycolysis and low respiration. It was also observed that succinate had only a slight effect upon oxygen consumption (increasing it about 30 per cent). The respiratory quotients of the 2 tumors were 0.80 and 0.81. Thus the metabolism of this tumor resembles that of most malignant tumors and differs from that usually seen in embryo or brain (4).

<table>
<thead>
<tr>
<th>Table I: Metabolism of Embryoma and Other Tissues</th>
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<tbody>
<tr>
<td>Tissue</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Rat liver</td>
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<tr>
<td>Rat brain</td>
</tr>
<tr>
<td>Chick embryo, 6 day</td>
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<tr>
<td>Mouse yolk sac</td>
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<tr>
<td>Mouse embryoma, transplant</td>
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<td>Mouse embryoma, transplant</td>
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<tr>
<td>Mouse embryoma, transplant</td>
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<tr>
<td>Walker rat tumor 256</td>
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</tbody>
</table>

* Numerous additional values for various tissues will be found in the summaries by Burk (4).
† We are indebted for these data to Dr. Francis N. Craig.

DISCUSSION

The occurrence of this ovarian tumor in one mouse following several generations of inbreeding makes it seem probable that the tumor originated as the result of a mutation or a rare embryological accident rather than as a consequence of specific inherited susceptibility. Among 3,000 descendants of one male mouse in our stock, no other ovarian tumors have been seen.

In the light of the fact that relatively uniform tumors often undergo essential changes in the course of transplantation, it might have been supposed that such a complex tumor as this would do likewise.

2 Obtained through the courtesy of Professor Leigh Hoadley.
3 Numbers refer to transplanted tumors descended from the original embryo.
Such a change might take the form of dedifferentiation as the result of cultivation, as mentioned by Lewis (11) in connection with the metamorphosis of myoblastoma to undifferentiated sarcoma; it might occur as the result of the eventual supremacy of a slightly more rapidly growing component; or it might be expected that the random selection of small bits of tissue for implantation would yield a number of different tumor types. The results have shown, however, that this protean tumor reproduces itself with great consistency.

The lack of organization in this tumor is especially marked. Falin (13) has seen considerable organization in zinc-induced fowl teratomas in which cartilage and ciliated epithelium created structures strikingly like bronchi. Despite the failure of organization, some sort of balancing process obviously keeps cell division and maturation going on at such relative rates that both embryonal and mature tissue have persisted throughout a total period equal to the normal life span of a mouse. In contrast, on the one hand, to the usual situation in transplanted tumors where constancy or dedifferentiation is the rule, and on the other hand, to the maturation of tissues in unison in normal embryogenesis, we see an organization of divided responsibility in which some cells elect to mature while others carry on growth.

This situation might be explained if each cell divi-

![Respiratory metabolism of an embryoma transplant (No. 209) and of other tissues. This shows the metabolic similarity of embryoma to other malignant tumors. For data, see Table 1.](image)
sion were uniformly unequal leading to one mature and one immature daughter cell; this would bear an analogy to polar body formation in the ovum. This is an attractive hypothesis, but in the absence of positive evidence from the morphology of mitoses, it would be unwise to carry the analogy too far.

If it were to be supposed that this is an embryo (either parthenogenetically derived or dating back to the previous pregnancy) which has been modified by the loss of some organizing factor, it must be recalled that in the process it has assumed biochemical characteristics (e.g., high glycolysis and low respiratory quotient) common to most true tumors, rather than those of the apparent constituent tissues.

Summary
An embryoma arising in the ovary of a C3H mouse has been transplanted through 11 serial transplants and maintains its pleomorphic nature in vivo and in tissue culture. On biochemical grounds, our studies suggest that it is a true tumor, and that it grows from foci of embryonal cells which divide rapidly throwing off some cells which mature into diverse specialized tissues, and others which retain the ability to grow indefinitely. An analogy is seen between this divided responsibility of daughter cells and polar body formation in ova.

References
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