Human Neoplasms in Tissue Culture

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The tissue culture method has been successfully applied to the study of human neoplasms only in recent times. The cultivation of human neoplastic tissue in vitro was first attempted by Carrel and Burrows (2) in 1911 without much success. Maccabruni (8) in 1913 observed proliferating cells around human fetal uterine and ovarian tissues planted in plasma. In 1914, Losee and Ebeling (7) kept human sarcomatous cells alive and growing in human plasma for more than 2 months.

Since these earlier attempts much progress has been made, and it has become apparent that the cultivation of human neoplasms offers no obstacles not also encountered in the cultivation of neoplasms of lower animals. The origination of the roller tube method of tissue culture by Gey (4) and its development by Lewis (6) have made possible the maintenance of more massive cultures over extended periods.

There are certain advantages in a method whereby living neoplastic cells can be directly observed. For instance, opportunity is afforded for the direct observation of cellular movements. The formation of patterns of growth can be studied. By the roller tube method especially, chemical investigations can be carried out upon the fluid medium in which the cells have been living. The effect of substances which may stimulate or inhibit growth of the tumor cells can be observed. The chemical and physical requirements for the continued growth of human neoplastic cells may be investigated. The process of differentiation and organization of cells might well be studied by tissue culture.

The present paper deals with the growth patterns observed in a wide variety of human neoplasms maintained in vitro, and serves as the introduction to a series of studies now under way on human tumors.

MATERIALS AND METHODS

The majority of tumors cultured were obtained from the operating room of the Hospital of the University of Pennsylvania, through the courtesy of Dr. I. S. Ravdin and his staff.

Tissues were collected in sterile dishes and planted as soon as possible. It was found, however, that tissues could be stored overnight in the refrigerator without noticeably affecting their subsequent proliferation in culture. A few of the tumors were obtained at autopsy. Large, solid blocks of tissue were placed in 95 per cent alcohol for a few minutes and then fragments from the deeper parts were removed aseptically for culture. Successful cultures resulted from tissues obtained as long as 27 hours after death.

All tissues were cut into small pieces, up to 5 mm. in diameter, and washed in Gey's solution (5) before planting. Two or 3 drops of heparinized chicken plasma were allowed to run down an ordinary pyrex test tube, forming a streak along the inside of the tube. The tissue fragments were placed in the mouth of the tube with small forceps and then arranged in a row in the chicken plasma with a long pipette having a slightly curved tip. The plasma was clotted by adding a drop of chick embryo extract. A fluid medium was added, consisting of 8 drops of human fetal serum and 5 drops of Gey's solution. The tube was closed with a rubber stopper and placed in a rotating device (3) housed in an incubator at 37° C. This rotator turns about 9 times per hour, causing the fluid medium to wash over the embedded fragments of tissue, thus furnishing a primitive type of circulation. The fluid medium was withdrawn every 4th day, or more frequently if necessary, and replaced.

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Hematoxylin and eosin stained section of an adenoma of the breast for comparison with the other photographs in the series which show the same tumor in vitro. Mag. X 440.

Fig. 2.—Epithelial cells in vitro forming a tongue-like process, characteristic of the early stages of culture. Mag. X 113.

Fig. 3.—Epithelial cells forming a broad sheet. Mag. X 113.

Fig. 4.—Epithelial cells showing beginning acinar arrangement, after a month of culture. Mag. X 113.

Fig. 5.—Epithelial cells showing acinar pattern at a somewhat later stage than seen in Fig. 4. Mag. X 113.

Fig. 6.—An isolated epithelial giant cell in vitro. Such cells are capable of ameboid movement. Mag. X 550.
by fresh medium. Liquefied areas in the plasma were patched with fresh plasma and chick embryo extract. Subcultures to other tubes or to hanging drop cultures were made as desired, or when thought advisable for the health of the culture. The outgrowing cells were observed and photographed directly through the walls of the tubes. Of each tumor, part was placed in formalin, and hematoxylin and eosin stained preparations were made for comparison with the growth pattern developed in vitro. The period of cultivation varied from 1 to 7 months, and many cultures were actively proliferating with no apparent decrease in vigor when discarded.

RESULTS

In Table I are listed the human neoplasms maintained in vitro by the method described above. A discussion of the growth patterns and cell types produced by these tumors follows:

### Table I: Human Neoplasms Grown in Tissue Culture

#### Epithelium

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroadenoma, breast</td>
<td>4 cases</td>
</tr>
<tr>
<td>Cystic mastitis (involutorial hyperplasia)</td>
<td>5 cases</td>
</tr>
<tr>
<td>Acrosome, pancreatic islets</td>
<td>1 case</td>
</tr>
<tr>
<td>Squamous cell carcinoma (branchiogenic)</td>
<td>1 case</td>
</tr>
<tr>
<td>Carcinoma, breast (primary)</td>
<td>9 cases</td>
</tr>
<tr>
<td>Carcinoma, breast (metastatic to lymph node)</td>
<td>3 cases</td>
</tr>
<tr>
<td>Carcinoma, stomach (metastatic to lymph node)</td>
<td>4 cases</td>
</tr>
<tr>
<td>Carcinoma, stomach (metastatic to liver)</td>
<td>2 cases</td>
</tr>
<tr>
<td>Carcinoma, prostate (metastatic to lung)</td>
<td>1 case</td>
</tr>
<tr>
<td>Carcinoma, adrenal (metastatic to lymph node)</td>
<td>1 case</td>
</tr>
<tr>
<td>Carcinoma, liver (primary)</td>
<td>1 case</td>
</tr>
</tbody>
</table>

#### Smooth Muscle and Fibrous Tissue

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leiomyoma, uterus</td>
<td>3 cases</td>
</tr>
</tbody>
</table>

#### Lymphoid Tissue

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphosarcoma</td>
<td>4 cases</td>
</tr>
</tbody>
</table>

#### Endothelium

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiosarcoma</td>
<td>1 case</td>
</tr>
</tbody>
</table>

#### Pigment Cells

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanosarcoma</td>
<td>2 cases</td>
</tr>
</tbody>
</table>

### Mixed Tumor

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td>2 cases</td>
</tr>
</tbody>
</table>

#### Benign Epithelial Tumors (Fig. 1)

Epithelial growth was never noted in cultures less than 72 hours old and at times would start feebly, but usually became more vigorous after the first 2 weeks. The epithelial cells first formed tongue-like projections which later developed into broad sheets (Figs. 2, 3). These membranous sheets usually extended out over the surface of the plasma clot. Liquefaction of the plasma by the epithelial cells occurred particularly during the first few weeks of culture. Thereafter the cells showed little or no liquefying action even if kept without transplantation for several months. Acinar structures (Figs. 4, 5) were formed by the epithelial cells only after a month or so of culture. This acinar arrangement occurred whether or not fibroblasts were present and, as noted by Cameron and Chambers (1), the acini at times seemed actually to form better in the absence of the fibrous tissue. Occasionally, isolated giant cells were produced (Fig. 6). The final growth pattern assumed by the epithelial cells roughly approximated that of the original tumor as seen in the hematoxylin and eosin preparations.

In addition to epithelial cells, the benign tumors in vitro produced macrophages and fibroblasts. The macrophages emigrated during the first 24 to 48 hours, their number varying with different tumors, but always considerable. The number of these cells in all tumors is rarely appreciated in the usual histologic section. One is impressed by their consistent presence when tumors are examined in vitro.

Fibroblasts usually became evident by the 3rd day. These cells grew out in radial arrangement around the explants and in old cultures piled up in layers many cells thick, forming heavy networks of interlacing cells. The general features of the fibroblastic growth were similar in different tumors, although minor variations were noted in the looseness or density of the pattern.

### Malignant Epithelial Tumors

A squamous cell carcinoma of branchiogenic origin (Fig. 7) produced an abundant fibroblastic proliferation (Fig. 8). Epithelial sheets (Fig. 9) were noted as early as the 4th day and continued to form thereafter. Every so often a small cluster of cells in the midst of a sheet, or along its periphery, would become whorled in arrangement, and more dense and hyaline in appearance, suggesting the formation of pearls (Fig. 10). Cells once forming these pearl-like structures were never seen to resume proliferation.

Glandular carcinomas produced, in addition to fibroblasts and macrophages, irregular cords and clusters of epithelial cells, as shown in Fig. 12. The epithelial cells derived from glandular carcinomas never formed acini. They showed an even greater tendency to liquefy the plasma than did the epithelial cells of benign tumors. Of 2 mammary carcinomas having a similar appearance in the hematoxylin-eosin preparations (Figs. 11, 13) one produced epithelial cells in culture and one did not (Fig. 14). Thus tumors which appear similar in the usual histologic section may behave differently in vitro.
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Fig. 7.—Hematoxylin and eosin stained section of a squamous cell carcinoma of branchiogenic origin, for comparison with the other photographs in the series showing the same tumor in vitro. Mag. X 440.

Fig. 8.—Fibroblasts in vitro, having proliferated from the stroma of the squamous cell carcinoma. Mag. X 113.

Fig. 9.—Squamous epithelial cells forming a large sheet in vitro. Mag. X 113.

Fig. 10.—Structures resembling pearls, formed in vitro by epithelial cells from the squamous cell carcinoma. Mag. X 113.
Not infrequently single epithelial cells broke away from the small nests and clusters, and such cells displayed ameboid motion. Proliferation of these isolated cells sometimes formed new colonies. This ability of the isolated neoplastic cell to progress by ameboid movement should be further studied, particularly in its possible relationship to invasion and metastasis. The cohesiveness of the cells should also be investigated to determine, if possible, the means by which a single cell breaks away from the cluster of which it is a unit.

No significant differences were noted in the behavior of primary and secondary tumors.

**Benign Fibrous Connective Tissue Tumors**

Vigorous fibroblastic proliferation occurred in cultures of leiomyofibromas of the uterus (Figs. 15, 16). The fibroblasts soon built up heavy meshworks around the explants. Intermingled with the fibroblasts were less numerous cells of a broader, flatter, though elongated form, which possibly were smooth muscle cells. This type of growth continued for several months of culture with no significant changes in the pattern.

**Lymphosarcoma**

Lymphoblastic cells were produced in abundance during the first 24 hours. This continued in some instances for several weeks. By this time fibroblasts had also grown out, and in 3 of the 4 tumors cultured the fibroblasts eventually dominated the growth while the lymphoid elements gradually disappeared. The remaining case, a retroperitoneal tumor (Fig. 17), showed a quite different sequence of events. From the very first, the emigrating cells were of the monocyte or macrophage type, as shown in Fig. 18. Very few cells were recognized as lymphoblasts. The monocytes continued to proliferate for more than a month, many of the cells wandering out of the plasma clot and attaching themselves to the inner surface of the test tube. Few fibroblasts grew.

Much discussion has taken place over the histogenesis of the lymphoblastomas and the matter has never been satisfactorily clarified. It would seem probable from these preliminary observations that new information might be acquired by investigating the lymphoblastomas in tissue culture.

**Angiosarcoma**

This tumor was obtained at autopsy and planted in tissue culture 27 hours after the patient’s death. In spite of this, excellent growth was obtained. The tissue selected (Fig. 19) was from a metastasis in a rib. The angiomatous character of the tumor was more striking in vitro than in the hematoxylin-eosin section of the original tissue. Tubular structures (Fig. 20) were formed, appearing on the 3rd day and continuing for 6 months of culture. They seemed to grow as solid cords which later became hollowed out into true tubes, increased in size, and frequently formed lateral branches (Fig. 21). These vascular structures were composed of closely approximated endothelial cells which grew vigorously from the start and continued so until the cultures were discarded.

**Melanosarcoma**

Two melanosarcomas were cultured, both from metastases in the liver. Growth was poor. The only cells which emigrated in any number were macrophages and fibroblasts. The macrophages sometimes contained particles of the dark amorphous pigment and were the only emigrating cells which did contain pigment. Both tumors were obtained at autopsy and perhaps growth was poor because of autolysis. One of the cultured tumors developed a latent infection which ultimately destroyed it.

**Description of Figures 11 to 18**

Fig. 11.—Hematoxylin and eosin stained section of a mammary carcinoma. Mag. X 113.

Fig. 12.—Epithelial cells in vitro derived from the mammary carcinoma in Fig. 11. The epithelial cells form irregular clusters. Mag. X 113.

Fig. 13.—Hematoxylin and eosin stained section of a mammary carcinoma, similar in structure to the carcinoma in Fig. 11. Mag. X 113.

Fig. 14.—Tissue culture of the carcinoma in Fig. 13. No epithelial cells are present. The growth consists largely of fibroblasts. Mag. X 113.

Fig. 15.—Hematoxylin and eosin stained section of a leiomyofibroma of the uterus. Mag. X 113.

Fig. 16.—Tissue culture of the tumor shown in Fig. 15. The growth consists largely of fibroblasts. In the right lower corner of the photograph are somewhat broader and flatter cells which may be smooth muscle. Mag. X 113.

Fig. 17.—Hematoxylin and eosin stained section of a lymphoblastoma of retroperitoneal origin. Mag. X 440.

Fig. 18.—Macrophages in vitro. These were the only cells produced in tissue culture by the tumor shown in Fig. 17. Mag. X 113.
Mixed Tumor of the Parotid

Two mixed tumors of the parotid (Figs. 22, 24) were cultured. Only slight epithelial proliferation occurred and was never a prominent feature in the growth pattern. The only other cells produced in the cultures were macrophages and fibroblasts. The fibroblastic outgrowth from these 2 tumors, however, was of considerable interest. In hematoxylin and eosin stained preparations the 2 tumors were very similar, whereas in culture they proliferated in a strikingly different manner. In one case the outgrowing cells were small, delicate, and filamentous with rounded bulges produced by the nuclei (Fig. 23). These cells formed lace-like fragile filaments and were quite unlike any fibroblastic growth encountered in any of the other tumors of the series. The fibroblasts from the other mixed tumor were of the more usual type and differed in no essential way from the stromal outgrowth of other tumors (Fig. 25). The significance of this difference in the appearance of the fibroblastic pattern was not determined.

SUMMARY AND CONCLUSIONS

Various human neoplasms have been cultured by the roller tube method and their patterns of growth have been described and photographed. These tumors included carcinomas, sarcomas, and various benign growths.

Benign glandular epithelial tumors formed acini after a month of culture, whereas malignant epithelial tumors never formed acini.

A squamous cell carcinoma produced structures in vitro resembling epithelial pearls.

Individual neoplastic epithelial cells frequently broke away from the cell clusters and displayed ameboid movement. Such isolated cells sometimes gave rise to new colonies. The possible significance of this in relation to the mechanism of invasion and metastasis is mentioned.

Lymphosarcomas produced lymphoblasts in 2 instances and only macrophages in a third. This suggested a possible heterogeneity in the histogenesis of the lymphoblastomas.

An angiosarcoma formed endothelial tubes in vitro. These structures resembled vessels and produced lateral branches.

Some neoplasms that presented a similar appearance in histologic sections behaved differently in vitro. These differences were noted both in the neoplastic cells themselves and also in the cells of the stroma.

The feasibility of the roller tube method for the study of a wide variety of living human neoplasms is demonstrated and its value as a method in investigative oncology emphasized.

REFERENCES


Description of Figures 19 to 25

Fig. 19.—Hematoxylin and eosin stained section of an angiosarcoma obtained at autopsy from a metastasis in a rib. Mag. X 1050.
Fig. 20.—Tissue culture of tumor shown in Fig. 19. Note tubular structures with branching ends. Mag. X 113.
Fig. 21.—Several large tubules composed of endothelial cells, derived from the angiosarcoma shown in the preceding photographs. Lateral branching is evident. Mag. X 113.
Fig. 22.—Hematoxylin and eosin stained section of a mixed tumor of the parotid gland. Mag. X 113.
Fig. 23.—Tissue culture of the tumor in Fig. 22 illustrating the peculiar appearance of the fibroblasts. Delicate filaments with bulging nuclei are formed. Mag. X 113.
Fig. 24.—Hematoxylin and eosin stained section of a mixed tumor of the parotid. This is not the same tumor as shown in Fig. 22. Mag. X 113.
Fig. 25.—Tissue culture of the tumor shown in Fig. 24. The growth consists of fibroblasts having an appearance and pattern quite different from those shown in Fig. 23. Mag. X 113.
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