The Heterologous Transplantation of Human Cancers*

Harry S. N. Greene, M.D., and Paul K. Lund, M.D.**

(From the Departments of Pathology and Surgery, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication February 14, 1944)

The capacity of some human tumors to survive and to grow in the anterior chambers of the eyes of animals of alien species was reported in a previous paper (1). The failure of later attempts to transplant morphologically benign tumors in this manner suggested that heterotransplantability might be a characteristic property of cancer. Accordingly, a series of experiments was instituted in an attempt to investigate this suggestion and, although the results to date are confirmatory, the group of benign tumors tested is not yet sufficiently inclusive to allow generalization. However, a fairly comprehensive group of cancers has been tested and their successful transplantation appears of sufficient interest to warrant an independent report.

The transfer of 4 human tumors was described in the paper noted above and the present report will be concerned with the primary transplantation of 10 additional cancers. Several of the tumors have been maintained by serial transfer and more detailed studies of their behavior will be presented in later papers.

MATERIALS AND METHODS

The technic of anterior chamber transfer employed in these experiments has been described in detail elsewhere (2).

In all instances the tumor tissue used was obtained by biopsy, and a portion selected for transfer by means of frozen section examination. Selection was based on the degree of cellularity and the absence of necrosis or desmoplastic reaction. As a rule transfer was performed within an hour of operation, but in a number of cases takes were obtained with tissue kept at icebox temperature for 12 hours.

Previous experience had shown the superiority of the guinea pig over the rabbit as a host for human tissue and this species was used exclusively in the present series of experiments.

RESULTS

The results of transfer are presented in summarized form in Table I. The interval between transfer and the appearance of signs of growth refers in all instances to the earliest take in the group. In the great majority of cases growth became apparent in all the successful transplants within a space of several days, and the recorded figure is representative of the group and is of value as an indication of the growth potential of the tumor concerned. It should be noted, however, that the appearance of growth in individual transplants was occasionally delayed for considerable periods of time. Thus in the case of the mammary sarcoma (tumor 7) 4 of the transplants showed vascularization and increase in size on the 60th day, but such signs were not evident in the fifth animal until the 115th day. Variations of this type were not related to the age or sex of the guinea pig and their cause remains obscure.

Fibrosarcoma.—A fibrosarcoma arose in the chest wall of a 63 year old man, and the tissue used for transfer was obtained from the second and third recurrences of the primary growth. The patient died with widespread metastasis 2½ months after the last

DESCRIPTION OF FIGURES 1 TO 4

All sections were stained with hematoxylin and eosin.

Fig. 1.—Second recurrence of fibrosarcoma of chest wall (tumor 1). Mag. × 475.

Fig. 2.—Anterior chamber transplant of tumor shown in Fig. 1. The guinea pig bearing transplant was killed 108 days after transfer. Note relatively mature fibroblastic proliferation with absence of giant cells. Mag. × 475.

Fig. 3.—Adenocarcinoma of aberrant salivary gland tissue in the hard palate (tumor 2). Mag. × 220.

Fig. 4.—Anterior chamber transplant of tumor shown in Fig. 3. The guinea pig bearing transplant was killed 150 days after transfer. Note abundant mucinous stromal background with cords of epithelial cells forming abortive glandular structures with a decided resemblance to a mixed salivary gland tumor. Mag. × 235.
transfer. Histologically the tumor was made up of fibroblastic elements of sarcomatous character with an unusual amount of giant cell proliferation (Fig. 1).

The first transfer, undertaken on July 14, 1942, gave rise to 2 takes in the 14 animals used. Growth was noted on the 90th day in one instance and on the 194th day in the other and both transplants grew to fill approximately one-quarter of the chamber during a two weeks' period. The first animal was killed for section on the 108th day while the other was held under observation for 150 days. Histologically the transplants were similar to the primary tumor. The fibroblastic proliferation was generally of a more mature type with a reduction in the number of mitotic figures and an absence of giant cell forms. Interacellular substance was more abundant (Fig. 2).

**Salivary gland adenocarcinoma.**—An adenocarcinoma originating in aberrant salivary gland tissue was found in the left posterior portion of the hard palate of a 23 year old man. The patient also presented a number of enlarged, firm lymph nodes in the right submaxillary region. The primary tumor in the palate was removed and transplanted on January 30, 1943, and approximately a month later a right neck dissection was performed, and tissue from the involved nodes also was used for transfer. At the present time, February, 1944, the patient has a mass of large nodes in the left submaxillary region but no further evidence of extension or metastasis is apparent.

Upon microscopic examination the primary tumor

**Table I: The Results of Transfer of 10 Human Cancers**

<table>
<thead>
<tr>
<th>Tumor No.</th>
<th>Microscopic diagnosis</th>
<th>Date of transfer</th>
<th>Material used for transfer</th>
<th>No. of guinea pigs</th>
<th>No. of Takes</th>
<th>From transfer to observable growth, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fibrosarcoma</td>
<td>July 14, ’42</td>
<td>2nd local recurrence</td>
<td>14</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov. 25, ’42</td>
<td>3rd “ “</td>
<td>9</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>2.</td>
<td>Adenocarcinoma of salivary gland</td>
<td>Jan. 30, ’43</td>
<td>Primary tumor</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb. 25, ’43</td>
<td>Lymph node extension</td>
<td>11</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Chondromyxosarcoma of larynx</td>
<td>Feb. 5, ’43</td>
<td>Primary tumor</td>
<td>8</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Malignant melanoma</td>
<td>Mar. 6, ’43</td>
<td>Lymph node extension</td>
<td>10</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>7.</td>
<td>Fibrosarcoma of breast</td>
<td>Aug. 5, ’43</td>
<td>Primary tumor</td>
<td>8</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>8.</td>
<td>Undifferentiated carcinoma of lung</td>
<td>Sept. 28, ’43</td>
<td>Operative implant</td>
<td>7</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>10.</td>
<td>Chordoma</td>
<td>July 23, ’43</td>
<td>Primary tumor</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct. 13, ’43</td>
<td>“ “</td>
<td>6</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

for further observation. In the latter case continued growth was followed by interstitial keratitis with glaucoma, and by the 220th day the corneal opacity so obscured the graft that direct study became impossible. When the animal was killed, on the 275th day, the transplant showed pronounced regressive changes.

The second transfer, 4 months later, resulted in takes in 7 out of 9 animals and growth was evident in all instances on the 21st day. The subsequent course of the transplants was extremely irregular, with phases of apparent reduction in size alternating with periods of renewed growth, and in no instance did the tumor fill the chamber, despite the fact that all the animals were held under observation for 150 days.

Histologically the transplants were similar to the primary tumor. The fibroblastic proliferation was generally of a more mature type with a reduction in the number of mitotic figures and an absence of giant cell forms. Interacellular substance was more abundant (Fig. 2).

**DESCRIPTION OF FIGURES 5 TO 8**

**Fig. 5.**—Anterior chamber transplant of tumor shown in Fig. 3. The guinea pig bearing transplant was killed 150 days following transfer. Fibrous connective tissue, cartilage, and bone. Mag. X 135.

**Fig. 6.**—Extension of salivary gland adenocarcinoma shown in Fig. 3 to submaxillary lymph nodes. Mag. X 220.

**Fig. 7.**—Anterior chamber transplant of tumor shown in Fig. 6. Mag. X 220.

**Fig. 8.**—Chondromyxosarcoma of cricoarytenoid cartilage (tumor 3). Mag. X 230.
was found to be an adenocarcinoma arising in a "mixed tumor" of aberrant salivary gland tissue (Fig. 3). Growth of the transplanted fragments was evident in 3 of the 5 animals on the 60th day after transfer. The transplants grew slowly and no more than doubled their size by the 100th day. The animals were killed on the 150th day, and at this time all the transplants appeared living and occupied approximately one-quarter of the anterior chamber. Microscopic examination disclosed 2 types of tissue. In some areas epithelial cells formed abortive glandular structures in an abundant mucinous stromal background (Fig. 4); in others, mesoblastic tissues predominated with the formation of cartilage and bone (Fig. 5).

The lymph nodes removed on February 5th were found to be almost completely replaced by epithelial cells in adenocarcinomatous arrangement (Fig. 6). Fragments of this tissue were transferred to the eyes of 11 guinea pigs, and growth was apparent in 8 of these between the 20th and 30th days. The transplants rapidly increased in size to fill approximately one-fifth of the chamber on the 50th day. Thereafter, the growth rate decreased, and in several instances no further increase in size occurred despite the fact that the tissues remained alive. The animals were held under observation for varying periods of time up to 102 days.

The transplants in animals killed before the 50th day were microscopically identical with the tissue used for transfer (Fig. 7). However, the transplants in animals killed at later periods showed an entirely different structure, consisting entirely of bone and cartilage similar to that found in transplants of the primary tumor.

Chondromyxosarcoma of larynx.—This unusual tumor arose from the cricoarytenoid cartilage of a 61 year old woman, and the tissue used for transfer was obtained at laryngectomy. The patient is alive at the present time but the tumor has recurred locally.

Histologically the growth was characteristic of tumors of this type and consisted of masses of immature cellular cartilage interspersed with islands of myxomatous tissue (Fig. 8).

Representative tissue was transferred to the anterior chambers of 8 guinea pigs and growth was apparent in 6 of these on the 13th day. The subsequent course of the transplants varied in individual animals. In some, growth was progressive throughout the experiment (122 days) and the fragments grew to occupy one-half of the chamber. In others, a decided reduction in growth rate occurred after the transplants had doubled in diameter (25 days) and only slight increase in size was noted on subsequent examinations.

Histologically the larger, actively growing transplants were identical with the primary tumor (Fig. 9). The smaller, arrested growths, on the other hand, consisted of mature cartilage with extensive areas of bone formation.

Malignant melanoma.—An inguinal lymph node removed from a 44 year old man, 3 years after amputation of a foot bearing a malignant melanoma, was found completely replaced by cells of this tumor. Histologically it consisted of solid masses of melanoblasts with typical nuclear structure and numerous mitoses (Fig. 10).

Representative fragments were transferred to the eyes of 10 guinea pigs, and growth was evident in 6 of the transplants on the 23rd day. Subsequent growth was slow but uniform throughout the group, and in all cases the anterior chamber was one-quarter filled on the 50th day and one-half filled on the 100th day. Histologically the transplants were identical with the primary tumor (Fig. 11).

Epidermoid carcinoma of buccal mucosa.—This tumor originated in the buccal mucosa of a 53 year old woman. The primary growth regressed after irradiation in September, 1942, but in March, 1943, enlarged, firm lymph nodes were found in the neck and on dissection these were found to be diffusely involved by tumor. The patient died with widespread metastases in July of the same year. Microscopically the tumor was a low grade epidermoid carcinoma consisting of well-formed prickle cells with a pronounced tendency to whorl formation and keratin production (Fig. 12).

Fragments of tumor obtained from lymph nodes at the time of the neck dissection were transferred to the eyes of 9 guinea pigs. Takes occurred in 3 of these and the first signs of growth were evident 19 days after transfer. Unfortunately, all the pigs bearing living transplants died during the early course of growth and the period of observation was limited.

**DESCRIPTION OF FIGURES 9 TO 12**

Fig. 9.—Anterior chamber transplant of tumor shown in Fig. 8. The guinea pig was killed 122 days after transfer. Note more mature cartilage and reduction in myxomatous elements. Mag. X 230.

Fig. 10.—Malignant melanoma in inguinal lymph node (tumor 4). Mag. X 575.

Fig. 11.—Anterior chamber transplant of tumor shown in Fig. 10. Mag. X 575.

Fig. 12.—Extension of epidermoid carcinoma of buccal mucosa (tumor 5) to submaxillary lymph node. Mag. X 165.
to 33 days. During this period the transplants remained living and grew to occupy one-third to one-half of the chamber. Histologically they were identical with the primary tumor (Fig. 13).

Adenoacanthoma of urethra.—This growth originated in the posterior urethra of a 42 year old man, and the material used for transfer was derived from its extension in an inguinal lymph node. Microscopically the tumor was of some interest inasmuch as the primary growth in the penis was largely epidermoid in character, whereas the lymphatic extension showed a pronounced adenoid architecture (Fig. 14).

The tumor was transferred to the eyes of 10 guinea pigs and a single take resulted. Growth was evident in this instance on the 27th day, and the animal was killed on the 81st day with the anterior chamber two-thirds occupied by tumor. Histologically the transplant was identical with the tumor tissue used for transfer (Figs. 15, 16).

Fibrosarcoma of breast.—A fibrosarcoma arose in the right breast of a 43 year old woman and pulmonary metastases were present at the time of examination. The breast was removed on August 5, 1943, and the patient died 2 months later. Histologically necrosis was a prominent feature of the tumor and the essential neoplastic elements, consisting of fibroblasts with elongated nuclei, were well preserved only in the immediate vicinity of blood vessels (Fig. 17).

Fragments of the tumor were transferred to the eyes of 8 guinea pigs, and takes occurred in 5 instances. Growth was observed in 4 of the fragments on the 60th day, but the fragment in the fifth animal remained unchanged for 115 days. Despite the long latent period the subsequent course of growth was rapid and in this animal, as well as in the others, the transplants grew to occupy two-thirds of the chamber within 2 weeks of the appearance of vascularization. Histologically the transplants were made up of closely packed, large, sarcomatous fibroblasts, and mitotic figures were numerous (Fig. 18).

Undifferentiated carcinoma of lung.—This carcinoma occurred in the left lower lobe of the lung of a 53 year old man. A total left pneumonectomy was performed on August 19, 1943, and on September 28th an implantation growth was removed from the operative site. The patient died on October 10th. Histologically the tumor consisted of solid, unorganized masses of highly anaplastic epithelial cells (Fig. 19).

Fragments of the implantation growth were transferred to the eyes of 7 guinea pigs and takes occurred in 2 instances. Growth was evident on the 17th day in one case and on the 50th day in the other. The anterior chambers of both animals were filled with tumor 2 weeks after the appearance of vascularization. Histologically the transplants were identical with the tissue used for transfer (Fig. 20).

Epidermoid carcinoma of lung.—This tumor arose in the upper lobe of the lung of a 63 year old woman and had invaded the chest wall at the time of operation. Histologically it was found to be made up of irregular masses of squamous cells with numerous mitotic figures and very little keratin formation (Fig. 21).

Fragments of the tumor from the chest wall were transferred to the eyes of 6 guinea pigs, and growth was evident in 3 of these on the 11th day. Subsequent growth was rapid and in all instances the anterior chambers were filled with tumor at the end of a month. Histologically the transplants were identical with the tumor used for transfer (Fig. 22).

Chordoma.—This tumor arose in the region of the 6th cervical vertebra of a 35 year old man. Biopsies were performed on July 23rd and Oct. 13th, 1943, and transfers to guinea pigs’ eyes were carried out in both instances. The tissue obtained at the first biopsy was made up of highly anaplastic cells and no classification other than sarcoma was made. Following this biopsy, x-ray therapy was instituted and the patient received a total dose of 1,139 r. Tissue from the second biopsy was largely necrotic but in scattered areas masses of cells were well preserved and their physaliphorous character, together with the nature of the stroma, suggested that the tumor might be a chordoma (Fig. 23).

Tissue from the first biopsy was transferred to the anterior chambers of 12 guinea pigs, and although the animals were held under observation for 5 months no growth occurred. On the other hand, takes occurred in 3 of the 6 animals used in the second transfer. Moreover, growth was apparent in these instances in 10 days and the anterior chambers were one-half filled with tumor by the 40th day, when the animals were killed to obtain tissue for serial trans-
plantation. Histologically the transplants were found to consist of typical ballooned cells with characteristic vegetable-like limiting membranes and here and there a tendency to the formation of syncytia (Fig. 24).

DISCUSSION

It has been consistently observed in experiments involving rabbit tumors that heterologous transplantation can be successfully effected only when the growth under study has manifested the ability to invade foreign tissue or to metastasize in the primary host. The transfer of benign tumors, or of potentially malignant tumors during their preinvasive stages, invariably fails and the conclusion appears justified that, in the rabbit, autonomy is attained only after continued development and is not a property of the primary neoplastic focus or of other preinvasive stages.

The present series of investigations was instituted in an attempt to determine the validity of these conclusions as applied to human tumors. The experiments reported in this paper are confirmatory as far as concerns the heterologous transplantability of cancer and demonstrate that, in man as well as in the rabbit, cancer autonomy transcends species barriers.

Numerous attempts have been made to transfer benign human tumors to lower animals, but with the exception of several debatable and poorly understood growths of peripheral nerves no takes have been obtained. In addition, a considerable number of transplantation experiments have been performed utilizing biopsy tissue from precancerous lesions and from anaplastic tumors during preinvasive stages, and without exception transfer has been unsuccessful. Such cases are being followed with additional transfers as more advanced biopsy material becomes available, but the performance of serial biopsies on developing tumors is not tolerated by the majority of human patients and the compilation of adequate data is dependent on fortuitous circumstances. However, in several cases of the series reported here tissue from consecutive biopsies of the same tumor was available for transfer, and the results, together with those obtained from the transfer of benign tumors, indicate that in man, as in the rabbit, autonomy is the outcome of continued development and is not a common attribute of all neoplastic cells.

The chordoma (tumor 10, in Table 1) is a case in point. Here the first attempt at transfer failed whereas the second transfer, undertaken 2½ months later, gave rise to growth in half of the animals used. The fibrosarcoma (tumor 1) and the salivary adenocarcinoma (tumor 2) are also suggestive in this direction and indicate further that autonomy may be of gradual rather than of sudden development. The first transplantation of the fibrosarcoma resulted in takes in only 2 of 14 animals and growth was not evident until the 90th day, while the second transfer, undertaken 4 months later, gave rise to takes in 7 of 9 animals and growth was apparent on the 21st day. In like manner, transfer of the salivary gland adenocarcinoma gave evidence of a gradient in transplantability or autonomy. The first transfer of this tumor, utilizing the primary growth in the hard palate, resulted in 3 takes in the 5 animals used, and growth was evident on the 60th day. In this case the structure of the primary growth was not reproduced on transfer and the histological appearance of the transplant was that of a mixed salivary gland tumor, presumably an earlier morphological form of the primary growth. On the other hand, the second transfer a month later, utilizing an involved lymph node, resulted in takes in 8 of 11 animals and growth was evident in 20 days. Moreover, the microscopic structure of this tumor and of the transplant were identical.

It was also observed in a study of rabbit tumors that transplantability or autonomy was not immediately related to the degree of anaplasia, and a similar condition appears to hold with reference to human tumors. In the case of the chordoma, tissue obtained at the first biopsy was so highly anaplastic that classification was not possible, while the second biopsy specimen contained sufficiently differentiated cells to suggest the correct diagnosis. In contrast, transfer from the first biopsy failed while transfer from the second was successful. Again, tissue obtained from the 2 operations on the fibrosarcoma of the chest wall showed a comparable degree of anaplasia, yet transfer of tissue from the later biopsy gave rise to a much higher percentage of takes with much earlier growth. The transfers of the 2 lung cancers are also of interest from this point of view. The highly anaplastic tumor (No. 8) grew in 2 of 7 test animals and growth was evident on the 17th day. On the other hand, the more differentiated epidermoid tumor (No. 9)

**DESCRIPTION OF FIGURES 17 TO 20**

Fig. 17.—Fibrosarcoma of human, female breast (tumor 7). Mag. X 165.

Fig. 18.—Anterior chamber transplant of tumor shown in Fig. 17. The guinea pig was killed 78 days after transfer. Mag. X 165.

Fig. 19.—Operative implant from primary undifferentiated carcinoma of lung (tumor 8). Mag. X 250.

Fig. 20.—Anterior chamber transplant of tumor shown in Fig. 19. The guinea pig was killed 64 days after transfer. Mag. X 250.
Figs. 17-20
grew in 3 of 6 animals and growth was apparent by the 11th day. A study of the other tumors of this series fails to show the existence of a correlative relationship between autonomy and the degree of differentiation, a finding that is of some significance in view of present attempts in surgical pathology to offer prognoses based on grades of anaplasia.

Transfer to lower animals is not only a diagnostic aid in the case of questionable tissues but also assists in the morphological classification of cancers. Occasionally cancers are associated with a local tissue reaction that obscures the nature of the cell involved in the neoplastic process. Thus the tissue obtained from the first biopsy of the fibrosarcoma of the chest wall contained a large number of giant cell forms, and a question of muscular origin arose. However, only the fibroblastic elements survived guinea pig transfer, and it became clear that the growth was a fibrosarcoma and that the giant cells were reactive rather than neoplastic in nature. Fortunately, also, transplants often show a slightly higher degree of cellular differentiation and organization than is found in the primary host and thus allow a classification of highly anaplastic tumors. For example, the tissue from the vertebral growth (tumor 10) sent to the laboratory for diagnosis was 90 per cent necrotic and tumor cells were scattered and highly anaplastic. A diagnosis of chordoma was made on the basis of a "hunch" rather than from objective morphological considerations—and widely different views were held by other observers. However, the type and arrangement of cells in the guinea pig transplant left no doubt of the true nature of the growth and substantiated the diagnosis of chordoma.

The ability to grow cancer in lower animals affords an approach to many other problems associated with human tumors. After successful primary transplantation the cancer can be carried by serial passage to new generations of animals and subjected to a variety of investigations not permissible during residence in the human host. It should be emphasized in this connection that after preliminary growth in the anterior chamber transfer to other body regions is readily effected.

SUMMARY

A series of 10 human cancers including a fibrosarcoma of the chest wall, an adenocarcinoma of salivary gland tissue, a chondromyxosarcoma of the larynx, a malignant melanoma, an epidermoid carcinoma of buccal mucosa, an adenocanthoma of the urethra, a mammary fibrosarcoma, an undifferentiated carcinoma of the lung, an epidermoid carcinoma of the lung, and a chordoma have been successfully transferred to the anterior chambers of the eyes of guinea pigs. The transplants grow progressively in the alien host and bear a close histological resemblance to the original tumors.

REFERENCES


DESCRIPTION OF FIGURES 21 TO 24

Fig. 21.—Pleural extension from primary undifferentiated carcinoma of lung (tumor 9). Mag. × 165.

Fig. 22.—Anterior chamber transplant of tumor shown in Fig. 21. The guinea pig was killed on the 31st day after transfer. Mag. × 165.

Fig. 23.—Chordoma arising in region of 6th cervical vertebra (tumor 10). Note extreme anaplasia and extensive necrosis. Mag. × 165.

Fig. 24.—Anterior chamber transplant of tumor shown in Fig. 23. The guinea pig was killed 40 days after transfer. Note physaliphorous character of more highly differentiated cells. Mag. × 575.
The Heterologous Transplantation of Human Cancers

Harry S. N. Greene and Paul K. Lund


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/4/6/352.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.