Heterologous Transplantation of Human Tumors

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The cancer literature contains numerous conflicting reports on the transplantability of tumors not only from one animal species to another but from strain to strain of a single species. The anterior chamber of the eye was first suggested as a site for transplantation of tissue by Van Dooremala in 1879, but it was not until 11 years later that the suggestion was put to test (10). In 1884 Zahn transplanted a human enchondroma into the anterior chamber of the eye of a rabbit but failed to evince its growth (10). In 1912 Ruben succeeded in the homologous transplantation of a sarcoma of the rat into the anterior chamber of the eye but failed in its heterologous transplantation into the eye of the rabbit (10). In 1914, Murphy (11) grew the Jensen rat sarcoma serially in chick embryos but did not succeed in propagating the tumor in chickens. Two years later, by simultaneously implanting the tumor and adult chicken tissue into chick embryos, he determined that lymphocytes (spleen and bone marrow) provide a defensive mechanism that prevents foreign tissues from growing (12). Later in 1914, he reported the successful growth of Ehrlich mouse sarcoma in rats depleted of lymphocytes by having been exposed to roentgen-ray treatment (13).

While sporadic reports have appeared from time to time regarding the heterologous transplantability of human tumors, it was not until the work of Greene (4) in 1938 that the anterior chamber of the eye, of first the guinea pig and then the rabbit and the mouse (5), was firmly established as a favorable site for transplantation. Despite general acceptance of this fact, however, there is considerable disagreement regarding the suitability of the method for experimental purposes because there is wide variation in the per cent of positive takes, the rate of growth is slow, and serial transplantability is poor (1, 10, 19, 24). As unsuitable as the anterior chamber of the eye may be considered by some workers, other sites, such as the brain, cornea, testis, spinal cord, pregnant uterus, and muscle, are even less favorable (2).

In 1951 a new approach to the problem was presented by Toolan (19). On the basis of Murphy's finding of 1914 (13), she inoculated irradiated rats and mice subcutaneously with minced cell suspensions of human tumors and obtained active growth for two generations or more in 83 out of 100 tumors transplanted. In 1958 she recorded 90 per cent of 101 human tumors surviving and proliferating for 12–20 days in irradiated and/or cortisone-treated rats inoculated subcutaneously and in hamsters inoculated in the cheek pouch (20). By 1955 (21, 22), out of a group of over 1,000 human tumors transplanted, she reported that three epidermoid carcinomas, a soft tissue sarcoma, and an embryonal rhabdomyosarcoma were being propagated with regularity and, in one instance, in such quantity as to produce as much as 3 pounds of tumor a week.

Employing similar or slightly modified procedures, Sommers et al. (18) in 1952 reported 40 out of 75 human tumors surviving in irradiated rats and 30 out of 65 human tumors surviving in treated or nontreated hamsters; Hoch-Ligeti and Hsü (6) in 1958 recorded successful transplantation of eight out of nine tumors in cortisone-treated rats; Patterson et al. (15) in 1954 reported survival and/or growth in 23 out of 33 tumors in cortisone-treated hamsters with one case of muco-epidermoid carcinoma of the parotid gland surviving 9 months and, at the time, being maintained in the sixteenth generation; and Patterson (14) in 1955 stated that out of 80 malignant human tumors eight were being maintained by serial passage.

Because of the enthusiastic reports quoted above, we undertook the heterologous transplantation of human tumors with the thought of ultimately using successfully propagated growths in experimental chemotherapy. Since our results differ quantitatively somewhat from those of other investigators we consider our findings worthy of publication.

MATERIALS AND METHODS

The materials and methods used in our experiment were similar to those described by Toolan (20) with but a few minor deviations. For the major experiment, weaning female Wistar rats weighing approximately 45 gm. were used. One
half of the animals were purchased from the Car- 
worth Farms, while the other half were obtained 
from the Bark-Bridge Farms. In minor experi-
mants, AKM mice, Swiss mice, and newborn Wis-
tar and Sprague-Dawley rats were used. Approxi-
mately one half of the weanling animals were ex-
posed to 150 r total-body irradiation (in the man-
ner proposed by Toolan [20]) on 2 successive days, 
while the remaining one half were given a single 
exposure of 150 r. Tumors were implanted into the 
animals from 1 to 7 days following completion 
of the irradiation. Animals used in minor 
experiments were not irradiated.

Each tumor used was obtained directly from the 
operating room, was handled aseptically, and was 
usually implanted within 20 minutes after removal. 
A portion of the tumor was finely minced with 
scalpels, with the use of Toolan's solution (the 
details regarding the preparation of which were 
kindly supplied by Doctor Toolan) as a suspending 
medium, and up to 0.5 cc. of the suspension were 
then injected subcutaneously into the left flank. 
Simultaneously, one or more solid pieces of tumor 
were inserted by the trocar method subcutaneous-
ly into the right flank. Newborn rats received only 
the minced suspension. Each rat, in both experi-
ments, was given 5 mg. of cortisone acetate in the 
subcutaneous tissues of the back at the time of 
implantation of the tumor and three subsequent 
times on alternate days. Some received an additional 
single booster injection 1 week after the 
fourth injection. Mice were treated similarly, ex-
cept that each dose of cortisone was reduced to 
0.75 mg.

Each animal was carefully examined from the 
6th to the 14th day after the tumor was implanted, 
and, if it was thought that the tumor was growing, 
the animal was sacrificed and the tumor was trans-
planted to animals of the next generation by the 
trocar method. Some animals were allowed to live 
as long as 86 days after implantation. The original 
tumor and all subsequently implanted tumors and 
tumor sites were examined microscopically when 
the animals were sacrificed or when they died. The 
initial number of animals used for each tumor 
varied from two to seven, depending upon the 
amount and appearance of the tumor and the 
number of animals available.

RESULTS

The total number of operative specimens trans-
planted was 220. Of these, fourteen ultimately 
proved to be benign and 206 malignant. Of the 
malignant tumors, 182 were carcinomas, five were 
melanoblastomas, and nineteen were sarcomas (Table 1).

In the minor experiments, in which AKM mice, 
Swiss mice, newborn Wistar rats, and Sprague-
Dawley rats were used, out of a total of 84 human 
tumors implanted there was survival of tumor tis-
uie in nine, but in no case did the survival go be-
yond the second generation. In each instance, the 
survival appeared to be a simple persistence of 
neoplastic cells that were carried over, and in none 
was there any evidence of active proliferation or 
growth.

In the major experiment, in which weanling 
rats alone were used, out of a total of 206 human 
tumors transplanted there was survival of tumor 

<table>
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<th>Types and Numbers of Human Tumors Transplanted</th>
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<td>Carcinomas</td>
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<td>Colon</td>
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1 The irradiation was given by Mr. Henry Boudreau, first 
under the supervision of Doctors Paul C. Swenson and Theo-
dore P. Eberhard and later under the supervision of Doctor 
R. L. Nichols.
as growing. The lesion was removed surgically from a 77-year-old woman on December 29, 1954, and was originally transplanted into four weanling Wistar rats in the usual manner. Initial growth occurred in three of the rats. The tumor has now been propagated for 10 months and, at the time of writing, is being maintained in sixteen animals in the 24th generation. After several attempts, the tumor has also been successfully transplanted serially into animals receiving irradiation alone and cortisone alone. At present it is maintained in nineteen animals receiving 150 r in the fifth generation, in eight animals receiving 300 r in the second generation, and in fifteen animals receiving 6-mg. doses of cortisone in the eighth generation. It has not been successfully transplanted into animals receiving 8-mg. doses of cortisone alone or into animals receiving no treatment whatsoever.

Pathologically, the original tumor was located in the ascending colon just distal to the ileo-cecal valve (Fig. 1). It was a large, rather soft, fungating growth that penetrated from the mucosa to the serosa and encircled the entire circumference of the bowel. Tumor tissue for implantation was obtained aseptically from the serosal portion. Histologically, the growth consisted of diffuse sheets of ill-defined polygonal cells with moderate to scanty cytoplasm and round or irregular hyperchromatic nuclei (Fig. 2). There was virtually no evidence of glandular formation. The stroma was of a loose connective tissue variety. It was well vascularized and scanty. The characteristics of the transplanted tumor have remained unchanged from the first to the present 24th generation. Definite increase in size of the transplanted fragment is determinable about 4 days after implantation. Thereafter, the tumor steadily grows larger until it reaches a diameter of from 1.5 to 2 cm. in from 12 to 15 days. Beyond this, it grows slowly until about the 20th day, after which it gradually decreases in size and ultimately regresses. At the 2-week stage, the tumor is well encapsulated, moderately firm, and homogeneously pinkish gray (Fig. 3). Necrosis appears centrally at about the 18th day and thereafter replaces more and more tumor. Histologically, the growth presents the same pattern and cellular appearance as it did originally (Fig. 4).

**DISCUSSION**

While we have been able to substantiate the findings of Toolan and others that malignant human tumors can be serially transplanted to irradiated weanling rats treated with cortisone, our percentage of positive takes is far below that reported by other investigators. We can say with assurance that only one tumor out of 206 transplanted actually grew in the heterologous host. Furthermore, although the characteristics of the tumor have remained unchanged, its slow growth, its small bulk, and the artificial prompting necessary for its propagation render the probability of its ultimate use in experimental chemotherapy quite unlikely.

Aside from the drawbacks mentioned, however, the fact that human tumors can be grown at all in heterologous hosts is a most fascinating phenomenon which, in our present state of knowledge, readily lends itself to much speculation. That normal animals possess a defensive mechanism against heterologously and, in some instances, homologously transplanted tissues is well known and accepted. The precise explanation of this mechanism, however, has not yet been determined. Cognizant of the antagonism, Murphy (12, 13) in 1914 (as already mentioned) discovered that lymphocytes were in some way concerned with maintaining the defenses of the host against foreign tissue. Thus, he was able to break down the defenses by depleting the lymphocytes through the action of irradiation or benzol. More recently, Toolan and Kidd (28), working with CSH mammary carcinoma and lymphosarcoma 6C3HED in A strain mice, actually demonstrated the invasion of transplanted tumors with lymphocytes and subsequent necrobioses and disappearance of the neoplastic cells. The role of lymphocytes in tumor immunity was again demonstrated in 1951 by Howes (7), who was able to grow adenocarcinomas E 0771 and 775 in resistant C57BL mice, and by Foley and

![Fig. 1.](image1.png) The gross appearance of the carcinoma of the ascending colon that grew in irradiated and cortisone-treated rats.

![Fig. 2.](image2.png) The microscopic appearance of the tumor depicted in Figure 1. ×200.

![Fig. 3.](image3.png) The gross appearance of a 15-day-old tumor (originally transplanted from the specimen shown in Fig. 1) in the seventeenth generation of propagation.

![Fig. 4.](image4.png) The microscopic appearance of the same tumor illustrated in Figure 3. ×200.
Silverstein (8), who were able to grow lymphosarcoma 6C3HED in resistant CF1 mice by first treating the host animals with cortisone.

Thus, the role of lymphocytes in protecting an animal from the growth of foreign tissue has been well established; but how is this phenomenon explained? Howes (7) stated that the failure of transplantation "may be caused by an immune reaction that is partially responsible for the absorption of the transferred tissue," while Toolan (21) spoke simply of "natural" and "acquired" immunity. Recently, two important discoveries have been made which may help to elucidate the problem further. In 1954 Pillemer et al. (17) demonstrated and isolated a new serum protein—a euglobulin which they called properdin. This substance (a) destroys bacteria, (b) neutralizes viruses, (c) lyases erythrocytes in the presence of complement and Mg++, and (d) in rats is severely reduced by 500 r total-body irradiation, making the animals highly susceptible to bacterial infection (16, 17). Further evidence of its role in immunity lies in the fact that irradiated rats and mice given properdin are protected against infection. The second pertinent discovery was that of Kidd (8, 9). He conclusively demonstrated that there is some substance in normal guinea pig serum which causes regression of subcutaneously implanted Gardner lymphosarcoma 6C3HED and lymphoma II in mice. Thus, purely from a theoretical point of view, because (a) total-body irradiation to rats causes a marked depletion of lymphocytes and properdin, (b) total-body irradiation to rats is necessary to destroy "something" in order that human tumors may grow, and (c) a "protein" in normal guinea pig serum can destroy lymphosarcoma in mice, is it then not possible (a) that lymphocytes have something to do with the manufacture of properdin, (b) that properdin and the "protein" in guinea pig serum are the same, and (e) that properdin may be the natural tumor inhibitor in the animal body? Of course, whether any of this speculation is true remains to be proved. At present, experiments are being planned to investigate some of the questions raised.

SUMMARY

Two hundred and six malignant human tumors were transplanted subcutaneously in irradiated (150 r) weanling Wistar rats treated with cortisone (6-mg doses). Growth occurred in only one instance—that of an anaplastic carcinoma from the ascending colon of a 77-year-old woman. At the present time the tumor has been cultivated for 10 months and is being maintained in sixteen animals in the 24th generation. It has also been successfully transplanted serially into animals receiving irradiation alone (150 r or 500 r) and cortisone (6-mg doses) alone. It has not, however, been successfully transplanted into untreated, normal animals.

In considering the protective mechanism of animals against heterologously transplanted tissue, it is suggested that properdin may play the leading role and that this euglobulin may be the natural tumor inhibitor in the animal body.

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