The Biology of Testicular Cancer

I. Behavior after Transplantation*†

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This study is concerned with the behavior after transplantation of eleven human testicular cancers heterografted to the hamster cheek pouch. It was undertaken in an effort to establish an adequate supply of uniform and predictable tumor cells upon which investigations could be made into the biology of testicular cancer.

Several reports are available concerning the behavior after heterotransplantation of a broad spectrum of human neoplasms. It is difficult to compare the degree of success achieved by the various investigators, because each used methodology that best suited his own objectives. Sommers, Chute, and Warren (9) heterografted 75 human cancers into x-radiated rats and found that 40 of these grafts survived on the 8th day. In a second experiment they found that 30 of 65 human tumors survived in the hamster cheek pouch, but none could be maintained beyond the third generation (2). Toolan (10, 11) selected only the most rapidly proliferating of her heterograftable human tumors for experiments in the chemotherapy of cancer. Only 3 per cent of over 1,000 tumors heterotransplanted to x-radiated and/or cortisone-treated rats met her requirements (12). Greene (4) successfully heterografted three of seven testicular cancers (two embryonal carcinomas and one choriocarcinoma) to the anterior chamber of the guinea pig's eye. He developed no permanently heterotransplantable tumors but, rather, attempted to show that a cell's ability to survive heterotransplantation was a function of its stage of development. Patterson (8) has obtained permanently heterotransplantable neoplasms with eight of 80 human tumors and has studied their growth characteristics. Herbut (6) modeled his procedures after those of Toolan and developed permanent transplantability in one of 206 human tumors heterografted. In our attempts to ensure adequate supplies of neoplastic tissue we have heterografted eleven human testicular cancers. Of these, three have become permanently transplantable, and available evidence indicates that they will serve as satisfactory laboratory models of their prototypes in human hosts.

MATERIALS AND METHODS

The methods used were modifications of those of Lutz et al. (7), employing cortisone acetate1 to abrogate host resistance (10, 11). Four- to 6-week-old weanling golden hamsters of either sex, weighing 45-55 gm., were used as hosts. If was found necessary to cage them separately to prevent deaths from fighting. They were fed Purina pellets and water and were allowed to live for at least 60 days following grafting before their heterografts were considered negative.

The tumors to be heterografted were obtained as removed from the human host at either surgery or autopsy and were processed immediately, with the use of aseptic technics. The portions to be explanted were carefully chosen from the periphery of the tumor that was free from necrosis. They were soaked in Hanks' balanced saline solution (BSS) (5) to which had been added penicillin (100 units/cc) and streptomycin (85 μg/cc). The tumor tissue was minced with a scissors in a few drops of BSS until the mince was fine enough to be aspirated into a 1-c.c. syringe. It was injected in 0.1-c.c. amounts, via 90-gauge needles, into the apex of each hamster cheek pouch.

The pouches were prepared for heterografting by being swabbed with a mild antiseptic in an effort to remove gross contamination. The operative areas were blotted dry with soft gauze in order to prevent organisms in a fluid phase from being carried by the needle into the graft site. All manipulations of the heterografting procedure and subsequent visual examinations of the explant sites were carried out with the hamsters under a nembutal anesthetic.

The animals were given 2.5 mg. cortisone acetate subcutaneously on the dorsum immediately after heterografting and were given a total of 3.75 mg. weekly thereafter in two doses (8). Their cervical regions were examined by palpation for the presence of developing tumors 3 times per week, but the cheek pouches were everted and the explants visualized only once each week. The methods used were modifications of those of Lutz et al. (7), employing cortisone acetate1 to abrogate host resistance (10, 11). Four- to 6-week-old weanling golden hamsters of either sex, weighing 45-55 gm., were used as hosts. If was found necessary to cage them separately to prevent deaths from fighting. They were fed Purina pellets and water and were allowed to live for at least 60 days following grafting before their heterografts were considered negative.

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* This investigation was supported in part by the Martha O. Freas Grant-in-Aid No. MOP-7A, American Cancer Society.

† This is Reprint No. 90 from the Department of Pathology, University of Pittsburgh School of Medicine.
A few of the recipient animals were given injections of the following hormones in an attempt to influence the growth of seminomas and teratocarcinomas: estrone, 800 I.V. per week; chorionic gonadotrophin, 20 I.U. per week; progesterone, 2.5 mg. per week; follicle-stimulating hormone, 300 I.U. per week; prolactin, 3 I.U. per week.

Experiments with either x-radiation (400 r whole-body x-radiation prior to transplantation of tumor) of recipient animals or the principle of acquired tolerance (1)—in which newborn animals are given injections of living tumor—were undertaken in an effort to find a more convenient means of abrogating the recipients' mechanism of resistance to heterografts than by the use of cortisone acetate. Newborn golden hamsters, for the most part only 1 hour old, were given injections on the dorsum of living human embryonal carcinoma. The appearance and continual growth of tumors would indicate that the hamsters had acquired tolerance to the human testicular cancer.


| Table 1 |
|---|---|---|---|---|
| Tumor no. | Histopathologic diagnosis (9) | No. animals grafted with primary tumor in testis | No. animals grafted with metastatic tumor | Result of heterografting | Fate of human host |
| 69 | Seminoma  | 27 |  |  |  |
| 71 |  | 24 |  |  |  |
| 74 |  | 21 | 5 |  |  |
| 90 |  | 10 |  |  |  |
| 97 |  | 18 |  |  |  |
| 61 | Embryonal carcinoma  | 2 | 10 | PITT 61 | Deceased* |
| 94 |  | 9 |  | PITT 94 | Deceased* |
| 98 |  | 20† |  |  | Deceased (same case as T94) |
| 75 | Teratocarcinoma  | 5 |  |  |  |
| 76 |  | 15 |  |  |  |
| 77 |  | 10 |  |  |  |
| 94 |  | 17 |  |  |  |
| 100 |  | 15† | 3d generation |  |  |
| 89 | Choriocarcinoma  | 4† |  | PITT 89 |  |

* The patients that succumbed did so within 2 years of the onset of symptoms.
† Tissues acquired from autopsy.

RESULTS

In all, fourteen heterotransplantation attempts were made with tumors obtained from eleven patients (Table 1). The series included five seminomas, four teratocarcinomas, two embryonal carcinomas, and a choriocarcinoma.

None of the seminomas became permanently transplantable, although tumors appeared in three of 85 recipients in the first generation. None of these, however, survived transfer to the second generation.

Five attempts were made at heterografting the three teratocarcinomas. The tumors used in four were obtained surgically primary and included three teratocarcinomas primary in the testis and one metastasis in a retroperitoneal lymph node. These four did poorly in the hamsters. Gross and microscopic evidence of proliferation was found in one of them (T 96), but insufficient growth occurred to allow transplantation to the second generation.

The fifth (T 100) was prepared 55 hours post mortem from lung and liver metastases obtained from a 32-year-old white male whose primary testicular teratocarcinoma we had explanted as T 96 3 months previously. Five takes have occurred in the first generation 25 days following the grafting.

It is still too early to know either the type of cell comprising the tumor takes or whether a permanently transplantable tumor will result.

Attempts to condition the hosts harboring either seminomas or teratocarcinomas by using estrogen, progesterone, follicle-stimulating hormone, chorionic gonadotrophin, or prolactin failed to alter the outcome of the experiments. The grafts became sclerotic or, in some cases, calcified, and they contained no living cells when examined on the 60th day.

T 61 and T 94 (embryonal carcinomas) and T 89 (choriocarcinoma) are the permanently heterotransplantable tumors, and, because no standard nomenclature has been adopted for permanently heterotransplantable human tumors, they have been named PITT 61, PITT 89, and PITT 94. They comprise the material upon which our experiments are being conducted and will be considered individually.

PITT 61.—The embryonal carcinoma explanted formed a tangerine-sized, painful mass in the right scrotal testis of a 21-year-old white male. He had developed bilateral gynecomastia, and repeated gonadotrophin tests performed upon his urine were positive. Death occurred 18 months after the onset of symptoms, 1 year after or-
Heterotransplantation to the cheek pouches of two hamsters was performed at the time of orchiectomy. A single small tumor, which was soft in consistency and pink to purple in color, developed in the first generation 37 days after grafting. PITT 61 has since been passed through 25 generations in over 1,400 animals in this laboratory. The generation time, defined as the average time required for tumors in a given generation to attain transplantable size, has gradually decreased from the original 37 days and now averages 22–24 days. The microscopic morphology of the primary testicular tumor in the human host was that of an undifferentiated embryonal carcinoma, the features of which have been duplicated in each heterograft generation. The tumor when sectioned was found to be composed of solid sheets of anaplastic tumor cells. In most cases the nuclei seemed to float in an amorphous syncytial-like mass of amphophylic cytoplasm, but in later generations the cell membranes have stained more prominently. A few dark cells that may be either degenerate embryonal carcinoma cells or attempts at the production of syncytiotrophoblast were scattered throughout.

As the tumors became older, the cells farthest removed from their blood supply often underwent a coagulative type of necrosis with the development of blood-filled spaces. The cells which remained around the supplying blood vessels appeared to float in the pools of blood and imparted a papillary appearance to the tumor. In later generations, glandlike arrangements have occasionally been seen, with tall goblet-like cells lining up around small blood vessels (Figs. 1–4).

If the tumors were allowed to remain indefinitely in the cheek pouch, they became so large that the pouch prolapsed, strangulated, and the tumor became gangrenous. Neither local invasion of the cheek pouch mucosa nor distant metastases have been found; indeed, the tumors were always painless masses, a typical embryonal carcinoma, the features of which have been duplicated in each heterograft generation. Two cell types have predominated. The more numerous resembled those of embryonal carcinoma, with the exception that their cell borders were more prominent and their cytoplasm less darkly stained. These cells formed frond- or pluglike aggregates which were surrounded by either the host’s connective tissue stroma or syncytiotrophoblast. The fronds, when surrounded by syncytiotrophoblast, formed structures reminiscent of placental villi. The syncytiotrophoblastic giant cells contained nuclei that varied in size and number from multiple small ones to a single large nucleus identical to that of the type cell of an embryonal carcinoma. The cytoplasm was deeply amphophylic and contained vacuoles, some of which enclosed red blood cells (Figs. 5–8).

Second-generation times averaged 29 days, while the third through sixth generations averaged approximately 22 days. The histologic characteristics of both primary tumor and transplants are similar to those of PITT 61.

**PITT 89.**—The explants were taken from metastatic choriocarcinoma found in the liver and lungs of a 24-year-old white male, autopsied 1 hour after death. An embryonal carcinoma of the right testis had been removed in 1954 following which he received deep x-ray and nitrogen mustard in both 1954 and 1955. He died with disseminated metastases 3 months after the last course of nitrogen mustard and approximately 2 years after the onset of symptoms.

Four animals received grafts within 2 hours of his death. By the 24th day, three of these had produced tumors that were transplanted to the second generation. The tumors were purple in color, owing to their vascularity, and were soft in consistency. Although invested by a dense capsule, they were locally invasive and, if allowed to attain too great a size, often fungated through the mucosa of the cheek pouch. Metastases did not occur in any of the heterologous hosts, and the usual termination was ulceration of the pouch with suppuration and sloughing of the tumor. In later generations a lessening of this invasiveness occurred, and the tumors reached a larger size with fewer instances of ulceration. Some tumors have even prolapsed the pouches.

As in the case of PITT 61, PITT 89 carefully reduplicated the morphology of the original explants in each heterograft generation. Two cell types have predominated. The more numerous resembled those of embryonal carcinoma, with the exception that their cell borders were more prominent and their cytoplasm less darkly stained. These cells formed frond- or pluglike aggregates which were surrounded by either the host’s connective tissue stroma or syncytiotrophoblast. The fronds, when surrounded by syncytiotrophoblast, formed structures reminiscent of placental villi. The syncytiotrophoblastic giant cells contained nuclei that varied in size and number from multiple small ones to a single large nucleus identical to that of the type cell of an embryonal carcinoma. The cytoplasm was deeply amphophylic and contained vacuoles, some of which enclosed red blood cells (Figs. 5–8).

As the fronds or villi increased in width, the central cells became necrotic, and upon removal of
the debris the resulting space became filled with blood. At that time the morphologic pattern was one of blood-filled spaces surrounded by thin rims of viable tumor, but ultimately all the tumor cells succumbed, leaving only the blood-filled spaces.

In the experiments attempting to induce tolerance in neonatal hamsters, seventeen of 52 newborn (1-4 hours old) hamster hosts acquired tolerance to human embryonal carcinomas when living PITT 61 tumor cells were heterografted at that time, as evidenced by continued tumor growth in these otherwise untreated hosts. Two of these tumors regressed by the 33rd day. After removal of their tumors, after 22 days, the other fifteen animals were regrafted with living PITT 61 or DEAC 3, but in only one instance was there proliferation of the regrafts in the cheek pouches.

Control animals not conditioned with cortisone acetate when heterografted with PITT 61 at 4 weeks of age failed to produce tumors, although one of twenty animals in the group had a nodule of surviving embryonal carcinoma that measured 6 × 4 × 3 mm. on the 36th day following grafting. This surviving tissue was surrounded by a dense cuff of lymphocytes.

A group of 30 animals previously grafted in adult life with the aid of cortisone were chosen at a time when they had developed embryonal carcinomas of approximately 7 × 4 × 3 mm. in size. The biweekly cortisone injections were discontinued, and 400 r total-body irradiation was substituted as the means of conditioning the host. In two instances there was regression of the tumor within 8 days, but the remaining 28 proliferated for 2 weeks following irradiation. Between the 15th and 20th days after x-ray, however, these 28 tumors underwent rapid regression, and most of them appeared to have undergone infarction.

DISCUSSION

The results obtained in this study—three permanently heterotransplantable neoplasms out of eleven testicular cancers heterografted—compare favorably with those obtained by Patterson (8), who developed eight permanently heterotransplantable cancers from 80 human tumors of various kinds heterografted to cortisone-treated hamsters. His DEAC 3, an embryonal carcinoma, has been successfully propagated in our laboratory and has been used in the experiments on acquired tolerance.

The comparatively high incidence of takes in this series may be attributable to two factors: It is possible that testicular cancers are more adaptable to heterologous hosts than most human tumors; or that, through the continued use of cortisone acetate as employed here, tumors in the first generation are afforded protection for a longer period of time, thereby allowing those explants an opportunity to adapt to their new environment and proliferate.

As heterologous tumors are propagated, they are unconsciously subjected to a selection of those cells that grow fastest. The generation times have, in the cases of PITT 61 and PITT 94, shortened from 37 and 29 days, respectively, to an average of 22 days by the fifth generation. It would seem, therefore, that a tumor with a relatively long first-generation time, by this selection of its fastest growing cells, may develop into a more prolific strain than at first indicated.

To our knowledge there are no reports of the growth of primary testicular seminoma in heterologous hosts, although Dr. H. S. N. Greene (4) has successfully transplanted seminomatous tissue found in a mediastinal teratoma to the anterior chamber of the guinea pig's eye. Second-generation grafts were made in three instances in our series of seminomas; but it was impossible to say whether they were truly nodules of proliferating tumor or whether they were chronic granulomas, because there was so little tissue available that none was used for histologic appraisal.

SUMMARY

1. Three of eleven human testicular cancers heterografted to the cheek pouches of cortisone-treated hamsters became permanently transplantable.
2. Attempts at heterografting seminomas and teratocarcinomas of the testis have thus far been unsuccessful.
3. Seventeen of 52 neonatal hamsters acquired tolerance to living human embryonal carcinoma cells heterografted within 1 hour of birth, but the tolerance produced was not permanent.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of Dr. C. A. Kuehn, Director of Urologic Services; Dr. E. R. Fisher, Director of Laboratories, Veterans Administration Hospital, Pittsburgh; Dr. J. J. Lee, Director of Urologic Services; and Drs. T. J. Moran and R. S. Totten, Dept. of Pathology, Presbyterian Hospital of Pittsburgh, in making tissues available for transplantation.

REFERENCES

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Fig. 1.—Embryonal carcinoma, human testis removed at surgery. T 61. Note the solid pattern composed of anaplastic cells with little supporting stroma. X360.

Fig. 2.—PITT 61, permanently heterotransplantable human embryonal carcinoma derived from T 61. Note the papillary fronds surrounded by blood-filled lacunae, a departure from the usual solid pattern. X100. Hamster No. 2928, 23rd generation.

Fig. 3.—PITT 61. Microscopic appearance of a typical solid area. X360. Hamster No. 2796, 23rd generation.

Fig. 4.—PITT 61. Microscopic appearance of cells pali-saded around small vessels. X360. Hamster No. 2797, 23rd generation.
Fig. 5.—Choriocarcinoma, human pulmonary and hepatic metastases removed 1 hour post mortem, composed of cytotrophoblast and syncytiotrophoblast. ×360.

Fig. 6.—PITT 89. Permanently heterotransplantable human choriocarcinoma adapted from T 89. Fronds of tumor cells, some surrounded by syncytiotrophoblast, lie in juxtaposition to areas of necrosis and hamster stroma. ×100. Hamster No. 3623, ninth generation.

Fig. 7.—PITT 89. Note the large vacuolated syncytiotrophoblastic cells. ×360. Hamster No. 3623, ninth generation.

Fig. 8.—PITT 89. Note the two cell types: vacuolated erythrocyte-containing syncytiotrophoblast at center, surrounded by cytotrophoblast. ×360.
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