The Response of Explanted Human Tumors to Radiation*

SHIELDS WARREN AND OLIVE GATES

(Laboratory of Pathology, Harvard Cancer Commission and Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.)

The culture of human tumors in the cheek pouch of the golden hamster (1-4) provides human cancer tissue for study of the effects of therapeutic radiation with minimal complications introduced by radiation of the host. Eversion of the pouch bearing the tumor permits its exposure, while the rest of the hamster is shielded. The tumor grows in the loose submucosal areolar tissue of the pouch and hence is readily accessible for repeated and nontraumatic observation.

GROWTH IN HAMSTER POUCH

CHART 1.—Sample scatter graph of growth rate of Deac 1 when transplanted under optimal conditions.

The tumors vary in the amount of stroma that they carry and in the relationship of that stroma to the tumor cells. Thus, Deac 3 has but little and very vascular stroma, but it is very closely related to the tumor cells. Deac 7, on the other hand, grows virtually without incorporated stroma.

The dense fibrosis associated with poorly growing tumors should be distinguished from the much less dense supportive stroma that accompanies satisfactory tumor growth. Moreover, since normal adult human tissues do not proliferate in the hamster, it may be guessed that the supporting tissues are those of the hamster. Further, it is probable that stroma carried from one animal to another by transplantation of the tumor does not proliferate but that stroma is derived from each new host.

Four of the human cancers being propagated in hamsters in this laboratory have been used in the present study. Deac 1, an epidermoid carcinoma arising in the parotid gland, was in its 38th generation in the hamster at the beginning of the radiation experiments. Its growth rate is fairly constant if viable portions are chosen for transplantation and if infection is avoided. Chart 1 illustrates its growth pattern in experienced hands, Chart 2 in inexperienced hands. Under favorable circumstances it reaches a diameter of 0.5 cm. in 12 days and 1.0 cm. in 23 days. Since this tumor infiltrated readily, ulceration occurs earlier than in other forms. The tumor keratinizes heavily in its central portion (Fig. 1), and this liquefies or rarely calcifies after about 15 days. At its margin, few of the cells are keratinized. The stroma is moderate in amount and tends to be mucoid. In some foci, the tumor simulates alveoli. This cancer may be considered as representative of those tumors which are

* Read in summary at American Association for Cancer Research, Atlantic City, N.J., April 18, 1956.
† This work was aided by U.S. Atomic Energy Commission Contract AT (30-1)-901 with the N. E. Deaconess Hospital, and by the American Cancer Society, Massachusetts Division, Inc.

Received for publication May 31, 1956.

163
clinically radio-responsive but not often cured by radiation.

Deac 3, an embryonal carcinoma transplanted from the retroperitoneal metastasis of a testicular primary, had been carried for 35 generations. It grows more slowly than does Deac 1, reaching a diameter of 0.5 cm. in about 18 days and 1.0 cm. in 31 days. In some portions Deac 3 grows in papillary form (Fig. 2). It carries very little fibrous stroma. However, it is very vascular and is susceptible to early hemorrhage and degeneration. It falls into the group of clinically radiosensitive cancers.

Deac 7, a malignant melanoma transplanted from a lymph node metastasis of a cutaneous lesion, had been carried for seventeen generations. It reaches a diameter of 0.5 cm. in 17 days and 1.0 cm. in 25 days. In the patient the melanoma was pigmented, but all the tumors explanted to the hamster have been nonpigmented, though the cytology has remained characteristic (Fig. 3). The tumor shows minute foci of necrosis early, but this does not appear to retard its growth rate or hamper its transplantability. It represents the group of clinically radioresistant tumors.

TC 41, an undifferentiated tumor derived from a metastatic nodule (?) thyroid origin) had been carried in our laboratory for 34 generations. It was first isolated by Dr. Mary Green, at the University of Pittsburgh. It is a rapidly growing tumor, reaching a diameter of 1.0 cm. in 14 days. While it shows some focal necrosis, this is rarely extensive until the tumors are large. Because of its firmness and homogeneity, it is an easy tumor to transplant (Fig. 4). TC 41 is also representative of the clinically radioresistant tumors.

In Chart 3 the growth rates of the four tumors are compared.

While generalizations are difficult, the size of the tumors is usually limited by the size of the cheek pouch. Tumors that become so large as to fill the pouch (2 cm. or more in diameter) ulcerate, become infected, and degenerate rapidly, whether irradiated or not. From time to time the growth rate of the transplanted tumors may vary somewhat, usually because of bacterial infection or minor errors in technic.

MATERIALS AND METHODS

A fragment of tumor from 0.1 to 0.3 cm. in diameter was transplanted by trocar between the layers of the everted cheek pouch (4) of nembutal-anesthetized golden hamsters about 60 gm. in weight. Immediately after inoculation, and twice weekly thereafter, the animals received 2.5 mg. of cortisone acetate subcutaneously. Deac 7 and TC 41 have grown well and have been successfully transplanted without cortisone; but, to keep the conditions comparable, host animals for these tumors were treated as the others. In general, 90 per cent of unirradiated tumor transplants are satisfactory for use. When the tumor reached a size of 0.5 cm. or more without infection, hemorrhage, gross necrosis, or ulceration, all of which are uncommon in our hands, the pouch and tumor were everted after the animal was anesthetized, and the pouch was pinned to a cork (Fig. 5). Four animals may be so arranged on a board as to permit four tumors to be simultaneously irradiated. The animals were shielded by ½-inch lead. Control tumors in the opposite pouch and the heads of the animals received as scattered radiation less than 1 per cent of the experimental dose, and the bodies virtually none. No radiation signs or symptoms appeared in the animals, and only the irradiated pouch and tumor have shown change. No epilation has occurred, again emphasizing the small amount of scattered radiation. By transplantation of the tumor from the irradiated bed to fresh hosts, effects due to radiation changes of the supporting stroma and overlying pouch epithelium may be avoided. Hence, such radiation effects as were noted are direct effects on the tumor cells.

The radiation was from a 250-kv Maximar and given at a rate of 240 r.p.m., with filtration of 0.25 mm. Cu and 1 mm. Al. Doses (measured in air) range from 1,200 to 8,000 r for each treatment. The cork and board used to immobilize the ani-
mals for treatment minimized scatter. The tumors were treated only once in any one animal. The pouches bearing irradiated tumors were replaced after treatment. At intervals of from 1 hour to several days, the pouches were everted under anesthesia and the tumors examined. Transplants have been made immediately after irradiation and up to several days. The radiation effects were estimated by changes in size, gross and microscopic appearance, and transplantability of the tumor to other hamsters, and sometimes to tissue culture.

RESULTS

The mucosa of the pouch showed marked radiation changes of the normal epithelium and connective tissue at about 3,000 r or above (Fig. 6). Some reaction was shown after doses of 1,200 r. At 6,000 r, or above, ulceration and fibrosis may partly obliterate the pouch after about 2 weeks. Pouches so damaged can no longer be everted because of adhesions, although tumors may survive in them.

We have concentrated on appraisal of relatively short-time effects, since the unirradiated tumors usually run their course of satisfactory growth in a given animal in less than a month. All dose levels used have produced some deleterious effects on the tumors. Measurement alone of the tumor is not a satisfactory criterion of effect, since "tumor" masses may persist or even increase which, on microscopic study, are found to be completely necrotic or purulent. The appearance of minute focal necroses in practically all older tumors, whether treated or not, tends further to obscure solely gross observation of the radiation effects. Epidermal inclusion cysts of traumatic origin may also be formed rarely and further confuse the findings.

Histologic study is essential to determine the nature of persisting or growing masses but appears to give less information as to radiation effect than does ability to grow in successive transplants.

The cytologic changes in tumor cells secondary to radiation are rarely sufficiently characteristic to permit clear assessment of the extent of their response. Perhaps pertinent to the occurrence of degenerative changes is the observation of Wyman (5) that cortisone (in heavier doses, to be sure) may cause vasoconstriction in the cheek pouch (Figs. 7-10). Even mitotic activity may not be obviously altered at the time of examination. Viability and rate of growth after explanation give the best indications of radiation-induced damage to the tumors.

In general, the clinically more radiosensitive tumors grew more slowly following radiation, regressed more frequently, and gave a lower proportion of successful transplants.

Radiated cells of tumors of all four types varied in their ability to grow in new hosts, indicating that radiation damage to a given tumor was not uniform. Thus, one transplant from a given tumor might not take, while another from the same nodule might start to grow and then regress, another might grow well and follow the life span of an untreated tumor. Even at 1,200 r there was evidence of impairment of vitality. The response, survival, and growth of the radiosensitive Deac 3 after treatment was much poorer than that of other tumors.

The melanoma, Deac 7, showed the best survival and growth after irradiation, although Deac 1 and TC 41 did well.

After a dose of 6,000 r, a Deac 1 tumor and a Deac 7 tumor each survived 53 days, when the animals were killed.

Following initial radiation, tumors have been transplanted to fresh hosts, and subsequent generations of the tumor have been again irradiated, so that some tumor cell lines have received as high a total dose as 19,200 r and have remained viable and have provided vigorous explants equaling the growth rate of unirradiated explants.

For example, a transplant of Deac 1 received 1,200 r in the 42d generation. Transplants of this irradiated tumor then received 6,000 r at one time in the 44th generation, 6,000 r in the 45th generation, 6,000 r in the 47th generation, and grew at its usual rate in the 48th generation, reaching a diameter of 1.0 cm. in 29 days. There was no significant alteration in histologic appearance of biopsies of the tumor or in growth rate of the explants. However, different transplants vary remarkably, and uniform resistance or susceptibility will not be found.

SUMMARY

1. These experiments have not been quantitative thus far but indicate sufficient variation in radiation response of cells from the same human cancer as to warrant further work on problems of radiosensitivity and clinical recurrence following therapy.

2. Some hamster-transplanted human cancer cells survived heavy doses of radiation and were able to proliferate as long as fresh supporting tissues were provided for them.

3. Different portions of an irradiated tumor may respond differently to a given dose of radiation.
4. In general, human tumors growing in the hamster varied in their radiosensitivity or resistance as their general type appears to do in man.

REFERENCES
FIG. 3.—Usual growth pattern of Dsc 7, malignant melanoma. Note frequent mitoses. ×300.

FIG. 4.—TC 41. Undifferentiated tumor of thyroid origin. ×300.
FIG. 5.—Hamster bearing Deac 7 with pouch everted and prepared for irradiation. A lead shield, not shown, protects the animal.

FIG. 6.—Abnormal epithelial cells and connective tissue of the hamster cheek pouch 13 days after 6,000 r. X300.
Fig. 7.—Deac 1. Eleven days after 6,000 r. Note viable tumor cells. ×300.

Fig. 8.—Deac 3. Thirteen days after 6,000 r. Note viable tumor cells and mitotic activity. ×300.
FIG. 9.—Deac 7. Seven days after 6,000 r. Note viable tumor cells. ×300.

FIG. 10.—TC 41. Fifteen days after 6,000 r. Note similarity to Figure 4. ×300.
The Response of Explanted Human Tumors to Radiation

Shields Warren and Olive Gates

Cancer Res 1957;17:163-166.