# Antilocalization of Cancer Cells Injected in Irradiated Area of Rats[1] (34711)

REUBEN L. KAHN, GERARD VERLY, NORMAN S. MILLER, RAYMOND PRESSOIR, AND CHARLES W. BUGGS

*Departments of Microbiology and Pathology, College of Medicine, Howard University, Washington, D. C. 20001*

Recent investigations showed (1, 2) that a focally irradiated area of the skin of rabbits loses much of its capacity to localize injected substances. This antilocalizing characteristic of irradiated tissue was observed following the injection of protein or bacteria. These substances escaped when injected in an irradiated area and reached the blood stream, but became localized when injected in a corresponding nonirradiated area. Using another experimental approach, it was shown more recently (3) that, while nonirradiated inflammatory tissue tends to localize injected substances, irradiated tissue has an antilocalizing characteristic.

The question arose whether this antilocalizing characteristic of irradiation would be manifested if cancer cells were used instead of protein or bacteria. More particularly whether cancer cells injected in a focally irradiated area of rats would lead to more extensive metastasis than the injection in the corresponding nonirradiated area of controls.

*Materials and Methods.* Rats used were of the Sprague-Dawley variety obtained from the Charles River Breeding Laboratories, Wilmington, Mass. When received, the rats were immature, ranging from 60 to 80 g in weight. The rats were allowed to mature to a weight of 150–200 g before use.

The Walker 256 carcinoma-sarcoma tumor was used for transplantation, obtained from the Hazleton Laboratories, Falls Church, Virginia. The tumor was first transplanted to randomly selected Sprague-Dawley rats to serve as donors for the experiment in which the remaining rats from the same group would be used.

Transplantation of the tumor from donors was carried out at 7 to 9 days growth. After removal of the tumor from the donor, cancer tissue, free from fat and necrosis, was reduced to very minute particles by mincing the tissue with scissors. The minced tissue was suspended in normal saline and the suspension was then ready to be injected.

In all experiments, a uniform volume consisting of 0.3 ml of the tumor suspension was injected in the subcutaneous region of the right hip of both irradiated and nonirradiated rats. For injections, a 0.5-ml syringe fitted with a 13-gauge needle, was used. This size needle assured passage of tumor particles from syringe to injected area.

*Irradiation.* All rats were first anesthetized by an intraperitoneal injection of sodium pentobarbital. X-Irradiation of the right hip of the experimental rats then followed.

A lead shield, suitable to the size of the rat, was used to cover the animal. The lead shield had a circular opening, 6 cm in diameter, which was the exposed area of the hip to be irradiated.

To limit penetration of the X-rays mainly to the subcutaneous tissue, 85 kVp were employed; also no added filtration. The FSD was 20 cm; The HVL was 1.5 mm Al. The Roentgens used were 1000 at a rate of 263.16 R/min. The tumor suspension was injected in the irradiated area 24 hr after exposure.

*Results. Macroscopic observations.* Based on 11 rats in which the right hips were irradiated, using 1000 R, and 11 which served as nonirradiated controls, the tumors in the controls were massive with correspondingly large central areas of necrosis. The tumors in the irradiated rats were about 7-fold less in

[1] Supported by the American Cancer Society.

weight with smaller central areas of necrosis. Figure 1 illustrates the difference in size of the tumors in 5 irradiated rats and in 5 controls, picked at random. Table I gives the measurements ($cm^2$) of these tumors.

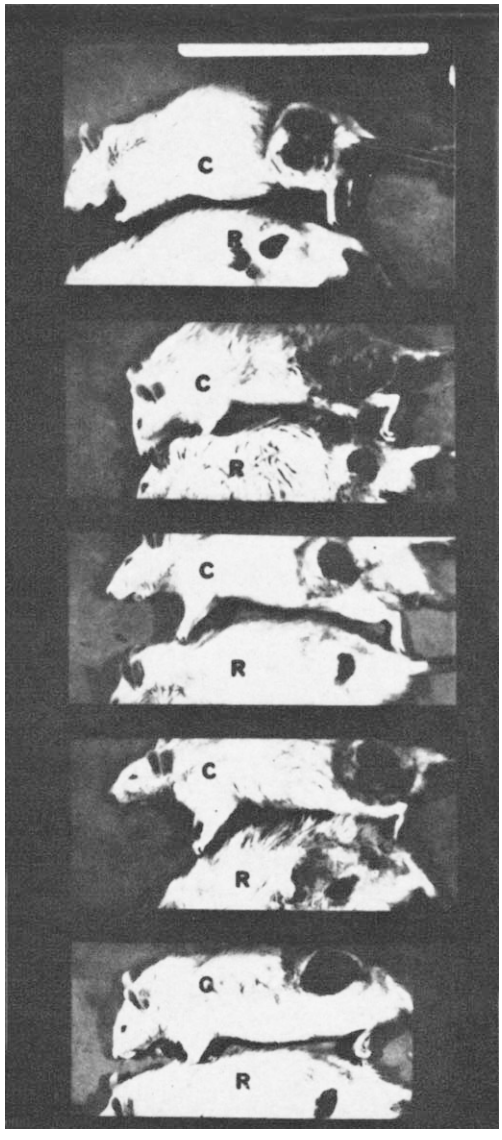


FIG. 1. Subcutaneous injection of 0.3 ml of a suspension of Walker 256 tumor cells in irradiated right hip (R) of Sprague-Dawley rats and in nonirradiated hip of controls (C). The smaller tumors on the skin surface in the irradiated areas are believed to be due to the antilocalizing effect of the irradiation, resulting in greater spreading and invasiveness of the tumor cells below the skin.

TABLE I. Differences in Size of Tumors in Irradiated and Nonirradiated Areas of Rats ($cm^2$).[a]

| Rat no. | X-Irradiated | Rat no. | Non-irradiated |
|---|---|---|---|
| 1 | 1 × 1 | 1 | 4 × 2 |
| 2 | 2 × 1 | 2 | 4 × 3 |
| 3 | 2 × 1 | 3 | 4 × 3 |
| 4 | 2 × 1 | 4 | 4 × 3.5 |
| 5 | 2 × 1.5 | 5 | 4 × 5 |
| Totals ($cm^2$) | 9.5 | | 66.5 |

[a] Exposure = 1000 R; tumor suspension injected 24 hr after irradiation; measurements taken 19 days after injection of tumor.

The tumors in the nonirradiated control rats were completely encapsulated by a thick fibrous wall, designated by the rating of ++++ in Table II. This type of encapsulation is characteristic of the Walker 256 tumor employed in this study. The tumors in the irradiated rats were flattened and extended toward the hip bone. They showed either partial capsule formation with relatively thin walls, designated by + or ++, or no capsule formation.

No invasiveness of tumor tissue outside the fibrous capsule was noted in the nonirradiated controls, but spreading of tumor tissue was noted in the irradiated rats. A greater degree of necrosis was noted in the tumors of the nonirradiated than in the irradiated rats.

*Microscopic observation.* Sections of tissue studied from irradiated and control animals showed the following morphological differences:

1. The masses of neoplastic cells tended to be larger and better preserved in the control animals. In irradiated animals, sections taken from the site of the tumor implant showed few neoplastic cells; some of these cells were found to have spread from the site of implant to the hip bone.

2. The reactive fibrosis around the neoplastic mass was minimal in irradiated animals and no distinct separation between neoplastic mass and normal tissue was evident. The fibrosis was made up of loose connective tissue often associated with fibrinous exudate. In control animals, the tumor was separated

TABLE II. Morphological Differences in Walker 256 Tumors Between X-Irradiated and Nonirradiated Rats.

| Rat no. | Capsule formation | Local invasiveness | Viable cells | Necrosis | Inflammation |
|---|---|---|---|---|---|
| | | | X-Irradiated | | |
| 1 | +++ | +++ | ++ | ++++ | ++++ |
| 2 | ++ | ++ | ++ | + | ++ |
| 3 | ++ | ++ | + | + | ++ |
| 4 | + | + | + | + | + |
| 5 | 0 | + | + | + | + |
| | | | Nonirradiated | | |
| 1 | ++++ | ++ | ++++ | ++++ | ++++ |
| 2 | ++++ | + | ++++ | ++++ | ++++ |
| 3 | ++++ | 0 | ++++ | ++++ | ++ |
| 4 | ++++ | 0 | ++++ | ++++ | ++ |
| 5 | ++++ | 0 | +++ | +++ | + |

from the adjacent tissue by fibrous tissue and no exudate was present.

3. In order to determine the occurrence of metastasis in different tissues, it is planned to carry out extensive experiments under various conditions of irradiation. Lack of collaboration with a pathologist did not enable us to carry out such metastasis studies at this time because microscopic studies were required of some 20 tissues of each animal. On that basis, it was possible to study for metastases in 10 of the animals, 5 irradiated and 5 controls, randomly selected from each group. It was found that 2 of the 5 irradiated animals showed tumor cells in the liver sinusoids and in glomeruli and capillaries of the kidneys. No metastasis was found in the 5 control animals similarly studied.

*Discussion.* The basic difference in Walker 256 tumor when injected in focally irradiated and nonirradiated rats is probably largely due to the marked difference in the capsule formation of the tumor. The heavy encapsulation in the nonirradiated controls tended to circumscribe the tumor and prevent it from spreading, thus reducing invasiveness of the tumor cells. The light encapsulation of the tumors in the irradiated rats tended to increase invasiveness of the tumor cells. This invasiveness, apparently due to the antilocalizing effect of the irradiation, very likely had a double effect. It reduced the size of the tumor in the injected area and it led to metastases in the liver sinusoids and in the glomeruli and capillaries of the kidneys. Of interest is the fact that more viable cells were noted in the nonirradiated controls at the site of the implant, as well as more necrosis than in the irradiated rats.

*Summary.* Walker 256 tumor cells were injected subcutaneously in an X-irradiated area of the right hip of Sprague-Dawley rats and in a corresponding nonirradiated area of controls. The tumors which developed in the injected areas were much larger in the controls than in the irradiated animals. The tumors were heavily encapsulated by fibrous tissue in the controls, but with little or no encapsulation in the irradiated animals. More viable cells and necrosis were present in the tumors of the controls. Special characteristics of the irradiated animals and not of the controls were invasiveness of the tumor and the presence of metastases.

The authors thank Alimam Neal for technical assistance.

1. Kahn, R. L., Kim, S. H., Curtis, A. C., and Simons, C. S., J. Infec. Dis. **107**, 325 (1960).

2. Kahn, R. L., J. Infec. Dis. **110**, 107 (1962).

3. Kahn, R. L., Botzler, R. G., and Miller, N. S., Proc. Soc. Exp. Biol. Med. **124**, 153 (1967).

Received Nov. 5, 1969. P.S.E.B.M., 1970, Vol. 133.