

Induction of Mammary Neoplasia After *in Vitro* Exposure to X-Rays¹ (35438)

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The initial events of radiation carcinogenesis are not well understood. A technique which would confine the initial events of *in vitro* conditions might prove useful in the analysis of events leading to neoplasia following the application of a carcinogen. This report provides data to show that mammary neoplasia of the rat can be induced by the *in vitro* exposure of mammary tissue to X-radiation when the irradiated mammary tissue is grafted back to the rat from whence it came. The most likely conclusion from these data is that X-radiation acts directly on mammary tissue to induce mammary neoplasia rather than acting by way of a systemic radiation effect which, in turn, acts on mammary tissue to produce neoplasia.

Materials and Methods. A method for transplanting autologously mammary tissue patterned after the method of Dao *et al.* (1) was used. Under ether anesthesia, a portion of abdominal-inguinal mammary tissue approximately 3.5 × 1.5 cm was removed bilaterally from female Sprague-Dawley rats 49–53 days old. Each block of mammary tissue was weighed individually to the nearest 0.5 mg, and placed in a plastic petri dish (3.5 cm in diam) containing 5 ml of saline. One piece was irradiated, while the other was sham irradiated. Each rat was numbered and the block of mammary tissue from the right side was irradiated in even-numbered animals and the left in odd-numbered animals. Both pieces of tissue were grafted separately on to the back of the rat from whence the tissues came so that each rat carried both an irradiated and a sham-irradiated piece of mammary tissue. The transplantation procedure included the development of a skin flap in an area free of mammary tissue on the dorsum.

A tunnel slightly larger than the mammary tissue to be transplanted was made in the fascia. The tissue was then placed in this tunnel and fixed to the underlying muscle by a silk suture at each end. In all cases, both pieces of tissue from each animal were outside the animal for about the same length of time and this ranged between 5 and 10 min. All skin incisions were closed with wound clips. All exposures were done with a conventional therapy X-ray machine operated at 250 kVp and 30 mA with 0.5 mm Cu and 1.0 mm Al filtration under conditions of maximum backscatter. The dose was measured with a 100 R Victoreen chamber at a TSD of 30 cm placed at the same position as the tissue in the petri dish. 800 R was given in 1.9 min.

One hundred and ten rats were prepared and followed for the development of mammary neoplasia both in the grafts and *in situ* by means of twice weekly examination by palpation. Eleven rats were killed, after selection by random number tables, at either 7, 8, or 9 months after transplantation, while the remaining 77 were killed at 10 months after transplantation. When rats were killed, all grafts were recovered and weighed, and all grafts were cleared and bulk stained with hematoxylin and examined at 8–20× for mammary development and pathology. These grafts were rated for degree of mammary development where ratings from 1 to 3 indicate progressive degrees of duct development and ratings of 4–5 indicate lobulo-alveolar growth (2). Any suspected pathological area was removed from the graft and sectioned and stained with eosin. Additional tissues suspected of containing *in situ* neoplasia were sectioned and stained with hematoxylin and eosin for microscopic study. Mammary neoplasia was classified according to criteria

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TABLE I. Number of Grafts, Number of Grafts Recovered, Number of Grafts With and Without Mammary Neoplasia Found in Grafts Exposed to 800 R *in Vitro* or Sham-Irradiated.

Grafts	800 R	Sham irradiated
Transplanted	110	110
Recovered	110	110
Without mammary neoplasia	94	109
With mammary neoplasia	16	1
1 FA ^a	11	1
1 AC ^b	2	0
2 FA	2	0
1 FA and 1 AC	1	0

^a FA indicates mammary fibroadenoma.

^b AC indicates mammary adenocarcinoma.

published previously (3).

Results. Sixteen of the 110 irradiated grafts were found to contain mammary neoplasia while only 1 of the 110 sham-irradiated grafts was found to contain mammary neoplasia (Table I). The difference between 16 of 110 and 1 of 110 is significant beyond the 1% level of confidence by a chi-square test (4). A total of 16 fibroadenomas

and 3 adenocarcinomas were found in the irradiated grafts while only a single fibroadenoma was found in the sham-irradiated grafts. A typical graft containing a neoplasm is shown in Fig. 1 and a photomicrograph of a section from the neoplasm is shown in Fig. 2. The mammary neoplasms found in the grafts were never smaller than 3 mm in the largest dimension and the largest weighed 15 g. All of the neoplasms found in the grafts were found in months 9 or 10 after transplantation except one adenocarcinoma first noted by palpation in month 3 in an irradiated graft. The amount of duct growth and lobulo-alveolar development of the transplanted mammary tissue was not different from *in situ* mammary tissue and no effect of radiation on the graft could be detected. The weight of the irradiated grafts without neoplasia was 77% of the original weight while the sham-irradiated grafts weighed 90% of their original weight. By Student's *t* test this difference was significant at the 5% level of confidence.

In situ neoplasia was found in 8 of the 110 rats. Six of these neoplasms were classified



FIG. 1. Portion of a graft 10 months after 800 R *in vitro* with a mammary neoplasm. Hematoxylin, 10X.

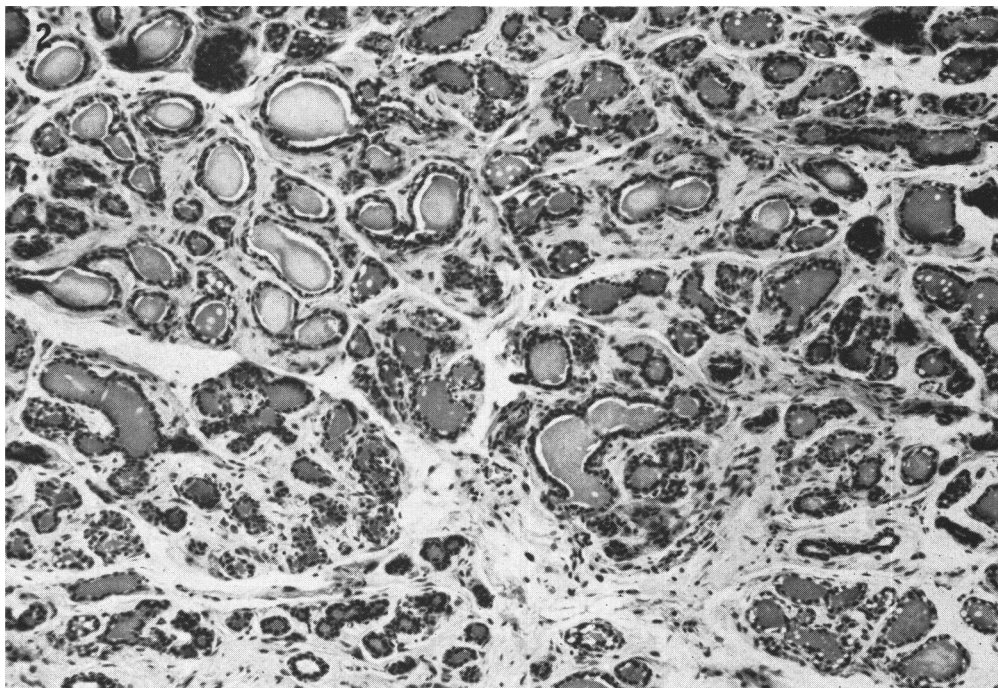


FIG 2. Photomicrograph of neoplasm shown in Fig. 1, H&E $\times 90$.

as mammary fibroadenomas and four of these were on the same side as the irradiated graft and two on the contralateral side. Only one *in situ* mammary neoplasm occurred in a rat with a mammary neoplasm in a graft and in this case, they were in opposite sides of the animal. One skin sarcoma was found on the same side as the irradiated graft and one renal neoplasm was found on the contralateral side.

Discussion. *In vitro* X-ray exposure of mammary tissue was found to produce mammary neoplasia in 16 of 110 irradiated autografts while only 1 of 110 sham-irradiated autografts exhibited mammary neoplasia. These results show clearly that mammary neoplasia can be induced by X-ray exposure of mammary tissue outside of the body of the animal. These results seem much more convincing than those presented previously (5) because the difference in neoplastic response between the irradiated grafts and the sham-irradiated grafts is of a high statistical level of confidence and because the irradiated graft and the sham-irradiated graft were carried in the same animal, thus subjecting both to the same physiological variables. Since mam-

mary neoplasia can be induced by X-ray exposure of mammary tissue outside of the body, the mechanism of action of radiation must be considered to be direct or scopal. Scopal is perhaps the word of choice since scopal has been defined as a radiation effect found within an irradiated volume (6).

A similar experiment has been performed by Dao (7) using 7,12-dimethylbenzanthracene (DMBA) rather than X-radiation as the carcinogen. Dao found mammary neoplasia in grafts after *in vitro* DMBA treatment and he concluded that DMBA must act as a direct carcinogen rather than via the host. It is of some interest that X-radiation (3) and DMBA (8) induce mammary neoplasia following both *in vivo* or *in vitro* application and both agents appear to be scopal in action.

The induction of mammary neoplasia by an *in vitro* exposure technique confines the interaction of X-radiation and mammary tissue to a relatively small amount of tissue and confines the interaction to defined conditions. It is suggested that this technique offers considerable advantages in the future analysis of the initial events which go to

make up radiation carcinogenesis. Such factors as temperature and oxygenation of tissues at the time of radiation exposure, thought to play a role in radiation carcinogenesis, can now be investigated in the tissue of interest in the absence of any systemic influences such manipulations would produce in the intact animal.

Summary. Two blocks of mammary tissue were removed from each of 110 female rats. One was exposed to 800 R of X-rays under *in vitro* conditions while the other was sham irradiated. Both were separately grafted subcutaneously onto the back of the animal from whence the tissues came. All grafts were recovered up to 10 months later. Sixteen of the irradiated grafts developed mammary neoplasia while only one of the sham-irradiated

grafts developed mammary neoplasia.

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