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Effect of X-rays on a Tumor of Known Genetic Constitution.*

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In a recent preliminary report[1] attention was called to an apparent genetic change produced experimentally, in a transplantable mouse cancer. As outlined in the previous paper, tumor dbrB of known

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1 Reinhard, M. C., and Warner, S. G., to be published in *Radiology*.

genetic constitution, when transplanted into mice of the dbr strain, would grow in practically 100% of the animals. On the other hand, when transplanted into any one of several other pure strains of mice, namely, Little's C-57 Blacks, Strong's CBA and a strain of albinos known as the A stock, there was complete failure of tumor growth. This tumor, however, when treated with X-rays responded in an entirely different manner, in that comparatively small doses of X-rays produced some change in the tumor which caused it to grow when transplanted in approximately 40% of the animals of the various strains just mentioned, yet did not affect the percentage of takes in the dbr strain. These results lead us to draw the tentative conclusion that the X-rays produced a change in the genetic constitution of the tumor. However, this conclusion was based on a total of only 46 animals of the 3 resistant strains and results from a greater number of animals are necessary to make the data significant.

The present paper brings the status of these experiments to date. In addition to the use of a greater number of animals, the experiment has been amplified to include the following 3 considerations:

1. The continuance of the radiated dbrB tumor into successive transplant generations.
2. The effect of variation of dosage on the dbrB tumor.
3. The effect of radiation on another transplantable tumor (New Buffalo tumor).

The history of the dbrB tumor and the experimental procedure followed were given in detail in the preliminary report. However, a brief summary is included here. Tumor dbrB is a transplantable tumor of known genetic constitution. It originated as a spontaneous adenocarcinoma in 1920, in the dilute brown stock of mice. This tumor has been maintained successfully by repeated transplantations for approximately 19 years. During this period the tumor has been tested repeatedly and its genetic constitution determined from time to time. The results of these tests show that the tumor dbrB has a genetic factor ratio of 3:1.

Tumor fragments implanted into the dbr strain grew to a size of approximately one cubic centimeter over a period of 7 to 10 days. At this time the tumors were radiated, the body of the mouse being covered with lead in which a window was cut so as to permit the X-rays to strike the tumors. The distance, rate and quality of the radiation was the same as reported previously, 30 cm, 63.3 r/minute and λ effective 0.16 Å respectively.

Doses of the order of 1500 r caused regression in the original host. The results of radiating dbrB tumors with 100 r and subsequently

TABLE I.
Results of Transplanting dbrB Tumors.*

Stock	Total	No. positive	No. negative	% positive
		Dose 100r.		
dbr	111	110	1	99.09
C-57 Black	105	48	57	45.71
CBA	60	23	37	38.33
A Stock	81	31	50	38.27
		Dose 50r.		
C-57 Black	57	23	34	40.33

* In order to prove that tumor fragments of dbrB will grow in the dbr strain only, 104 CBA's, 97 C-57 Blacks and 93 of the A stock were inoculated with normal dbrB tumor. There were no takes in any of these mice.

transplanting them into 111 mice of the susceptible strain (dbr) and into 246 mice of the 3 resistant strains are shown in Table I.

In addition to the 100 r dose several dbrB tumors were subjected to a dose of 50 r given also *in vivo*. As shown in Table I there was approximately the same percentage of takes in 57 animals of one of the 3 resistant strains (C-57 Black). This lends proof that small doses of the order of 50 to 100 r are capable of producing genetic changes in this particular tumor.

If this change in the tumor produced by these small doses of fairly hard X-rays is constitutional, we would expect additional transplantation into succeeding generations to yield approximately the same number of takes. Successive transplants of the radiated tumor through the fourth transplant generation to date (Table II) have yielded approximately 40% takes, adding further proof to the contention that a genetic change has been produced.

When the radiated tumor dbrB was implanted into mice of the dbr strain, there were practically 100% takes. This was true also of the control experiment, in which non-radiated tumor fragments grew in approximately 100% of the mice of the dbr strain. Simultaneous transplantation of the non-radiated tumor into the 3 resistant strains (C-57 Black, 41; CBA, 37; A, 43) resulted in no takes, indicating that no spontaneous change had occurred in the tumor during the course of the experiment.

Since we have presumably produced genetic changes with X-rays

TABLE II.
Results of Successive Transplantations of Radiated dbrB Tumor into C-57 Black.

Generation	Total	No. positive	No. negative	% positive
First	56	21	35	37.50
Second	39	16	23	41.02
Third	63	27	31	42.85
Fourth	32	12	20	37.50

in one group of tumors, (dbrB tumor) the question may well be raised, would similar doses produce a comparable change in another group of tumors of different origin. Therefore a second group of tumors (New Buffalo tumor) was selected for additional experimental work following the same procedure as that used for the dbrB group of tumors. The New Buffalo tumor also originated as a spontaneous adenocarcinoma and has been successfully propagated for several years by transplantation in a pure strain of albino mice known as the New Buffalo Strain, where practically 100% takes are obtained. This tumor will not grow in other pure strains, C-57 Blacks, CBA's.

To date we have exposed these tumors to a 100 r dose of X-rays and have transplanted them into only one of the resistant strains, C-57 Blacks. As shown in Table III there were takes in 37% of 51 animals, approximately the same percentage of takes in this particular host strain as was obtained with tumor dbrB. Additional transplantation into the other resistant strains is underway.

TABLE III.
Results of Transplanting New Buffalo Tumor.
Dose 100r.

Stock	Total	No. positive	No. negative	% positive
New Buffalo	51	19	32	37.25

Conclusions. The data presented in this paper involving, (1) the use of approximately 300 animals, (2) 2 different transplantable tumors known as dbrB and New Buffalo Tumor, (3) the maintenance of the radiated tumor through the fourth transplant generation, are indicative of the fact that small doses of X-radiation are capable of producing a change in the genetic constitution of a tumor.

It is interesting to note in this connection that while X-rays have been used to produce mutative changes in certain forms of life, such as plants, insects and animals, the dosage required for these changes is far in excess of that which we used to produce changes in the constitution of the tumors.

As shown by Muller and others, thousands of roentgens are needed to produce mutations and the higher the dosage the greater is the mutational effect. In our experience we have been unable to distinguish any quantitative difference between doses of 50 r and 100 r. Doses of 1500 r cause regression.