Further Studies on the Effect of X-Rays on a Tumor of Known Genetic Constitution

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In two previous reports (6, 8) attention was called to an apparent transmissible, genetic change induced in the transplantable mouse tumors (dbrB and Simpson) by comparatively small amounts of x-rays. The change from a specific to a nonspecific tumor following x-radiation manifested itself by the fact that radiated tumors were successfully propagated in several pure strains of mice in which the nonradiated tumor fails to grow. For example, these tumors will not grow in the strains of mice known as the A stock albinos, Little's C57 black and Strong's CBA. On the other hand, when these tumors, either radiated or nonradiated, were transplanted into their host strains there were always approximately 100 per cent takes. However, when exposed *in vivo* in the host strains to doses of 50 to 100 r (λ effective 0.16 A.), and transplanted one week following the radiation into the above mentioned strains, these tumors grew in approximately 40 per cent of the animals. Furthermore the radiated tumors have been successfully propagated through several transplantations in the resistant strains of mice and then successfully transplanted back into the original host strain, indicating that the change induced in the tumors by the radiation was a permanent one for at least 5 transplant generations.

The studies presented in this paper deal with the effect of varying doses of x-radiation on the percentage takes of the Simpson tumor in the C57 black strain. The doses of radiation (λ effective 0.16 A.) varied from 25 to 1,000 r. The same experimental procedure was followed as was described in the previous papers (6, 8). The percentage takes in the so-called resistant strain, C57 black, increased with increased dosage up to a certain point after which there was a decrease. These experimental data are compiled in Table I and have also been plotted on arith log paper as shown in Fig. 1, with the magnitude of the percentage probable errors shown by the vertical lines for each point. As this curve is drawn there is an exponential relation between the dose and the percentage takes. For doses of 0 to 25 r the exponent is 2.26 while for doses of 25 to 400 r the exponent is 1.01.

Muller (5) working with mature male germ cells of *Drosophila* has determined that while the frequency of gene mutations bears a simple linear relationship to the dose administered, the frequency of structural change is an exponential function of the dose, the exponent for doses of 1,000 to 4,000 r being about 1.5 whereas the exponent for lower doses is greater, approaching 2.0, the square of the dose.

Our exponents are comparable to those of Muller in that a large exponent is associated with small doses and a small exponent with larger doses. The explanation for this break in the curve is not at first apparent, but Muller gives a clue. He explains the gene mutations on the theory of individual successful ionizations, and the structural changes on the basis of multiple breaks and translocations of the chromosomes as a result of direct hits. In the latter case, he claims that the recombination is blocked in the spermatozooan stage until fertilization occurs, at which time the fragments are subject to more movement as a result of cell division. This process of cell division favors new combinations of the broken chromosomes.

TABLE I: RELATION BETWEEN PERCENTAGE TAKES AND DOSAGE

Dose r	Total no. animals	No. animals positive	Percentage positive	Probable error (percentage)
25	35	12	34.4	± 5.4
50	24	8	33.4	± 6.5
100	90	41	45.5	± 1.12
200	56	27	48.2	± 4.5
250	26	14	54.0	± 6.6
400	30	21	70.0	± 5.6
600	41	25	61.0	± 5.5
800	17	12	70.6	± 7.5
1000	15	8	53.4	± 8.7

When considering somatic cells of the Simpson tumor from this point of view, there can be no block such as is mentioned by Muller in connection with the germ cells. However, if cell division is more favorable to new combinations of broken chromosomes and the doses of radiation administered to the tumor cells did result in direct hits which split the chromosomes, then it is possible to theorize that low doses, having apparently little or no effect on cell division, afford a maximum opportunity for new combinations of the fragments. To go one step further, it is generally

accepted that increasing doses of radiation have an increasing effect of retardation on cell division. In fact it has been shown by Love (4) and Gordon (3) that doses of the order of 100 to 400 r are capable of markedly reducing mitotic activity. It might be expected, therefore, that if the mitotic activity of the

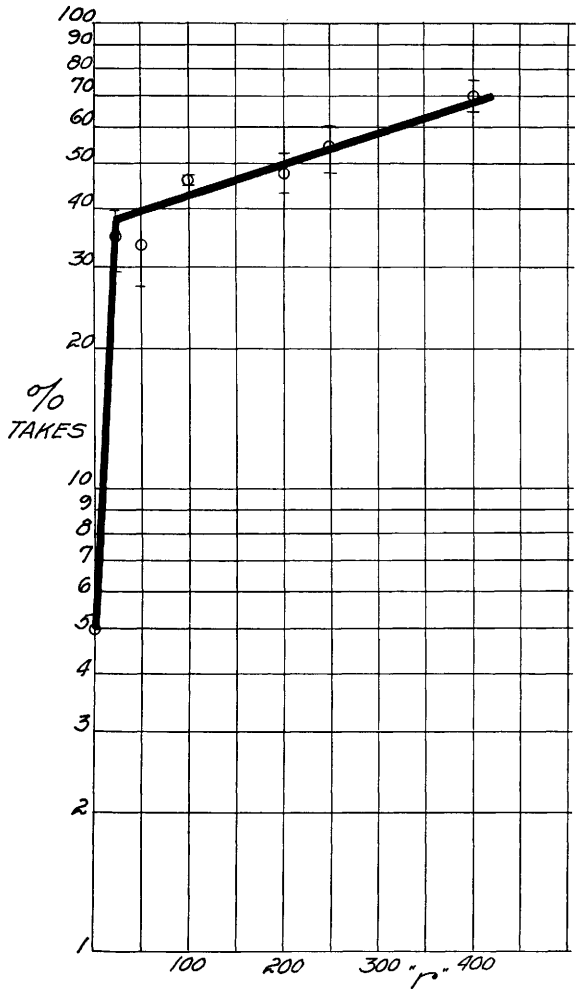

FIG. 1.—Graph of the experimental data of Table I for doses from 0 to 400 r. As drawn this is an exponential relation between the percentage takes and dosage for a dose 0 to 25 r and another exponential relation for doses of 25 to 400 r.

radiated cells is decreased, the possibility of new combinations of the chromosomes is reduced following radiation with doses from 25 to 400 r. Thus the lowered probability of new combinations would cause the exponent to be smaller with increased dosage.

If the theory is correct that progressively increasing doses tend progressively to decrease mitotic activity, in addition to the direct action on the chromosomes, we would have to rule out constant exponents for the two portions of the curve for doses up to 400 r. Instead there should be a progressively decreasing exponent. In other words, our graph should not be represented by straight lines but by a smooth curve. The portion of the curve up to a dose of about 25 r may represent the threshold dose for effect on mitotic activity. Our experimental points could then be connected as shown in Fig. 2. Additional experimental work is in progress to check this curve.

The question whether or not we have produced a genetic change in these tumors by means of radiation is not necessarily answered by the work presented so far, although the fact that these tumors, in addition to growing in the resistant strains, have been carried through several transplant generations, would seem

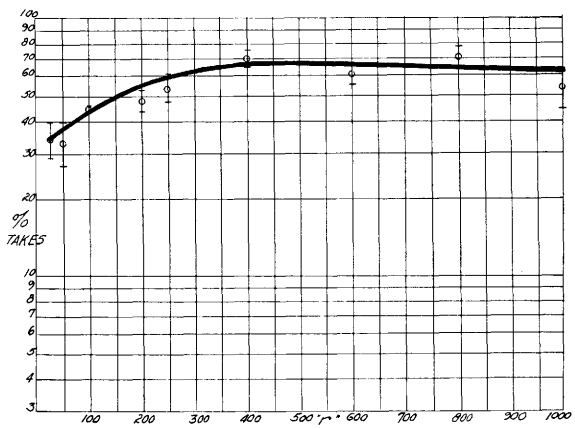

FIG. 2.—Graph of the experimental data from Table I for doses from 25 to 1,000 r, where the experimental points are connected with a continuous curved line which represents a progressively decreasing exponent.

to bear out the contention that we have produced a permanent genetic change.

According to Bittner (1), a genetic change is always in the direction of more malignancy and less tissue specificity. Our radiated tumors are definitely less specific. An indication of a genetic change, according to Schultz (7), is the absence of any time factor and as is further pointed out by Demerec (2), there is no recovery in the case of hereditary effects—such changes, once they have occurred, do not revert.

In order to determine the influence of the time factor and recovery effect, we have carried out the following experiment.

Tumors of the Simpson strain were radiated *in vivo* with 200 r and transplanted into one of the so-called resistant strains of mice (94 animals of C57 black strain), according to the following schedule: immediately after radiation, after 1 day, 3 days, 5 days, and

7 days. The results of this experiment to date show that the percentage takes, approximately 40 per cent, hold fairly constant for all the time intervals mentioned, indicating that the effect is instantaneous and does not depend on any latent period such as is normally associated with radiation experiments. Furthermore, although the number of animals used for this work to date is not sufficient for conclusive proof, there is no evidence of a recovery from the radiation.

Conclusions

Based on the experimental work performed to date on the Simpson tumor, we believe that:

1. An inheritable genetic change has been produced by radiation doses as low as 25 r.

2. The percentage takes with varying doses is intimately connected with the effect of the radiation on cellular activity as well as the effect on the genetic constitution.

References

1. Bittner, J. J. A Genetic Study of the Transplantation of Tumor Arising in Hybrid Mice. Am. J. Cancer, **15**: 2202-2247. 1931.
2. Demerec, M. Heredity and Radiation. Radiology, **27**:217-220. 1936.
3. Gordon, C. J. Immediate Effects of 250 r of X-rays on the Different Stages of Mitosis in Neuroblasts of the Grasshopper, Chortophaga viridifasciata. J. Morph., **66**:11-23. 1940.
4. Love, W. H. Quantitative Effect of X-rays on Mitosis in Mouse Tumor S-37. Med. J. Australia, **1**:814-815. 1936.
5. Muller, H. J. Medical Uses of Radium (Summary of Reports from Experimental Research Centers for 1939). Brit. J. Radiol., **14**:1-10. 1941.
6. Reinhard, M. C., and Warner, S. G. A Preliminary Report on the Effect of X-rays on a Tumor of Known Genetic Constitution. Radiology, **34**:438-439. 1940.
7. Schultz, J. Radiation and Study of Mutations in Animals, in Biological Effects of Radiation. Vol. II, Ed. by B. M. Duggar, McGraw-Hill, 1209-1261. 1936.
8. Warner, S. G., and Reinhard, M. C. Effect of X-rays on a Tumor of Known Genetic Constitution. Proc. Soc. Exper. Biol. & Med., **42**:673-676. 1939.