Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture

I. The Effect of X-Rays on Cell Outgrowth in Cultures of Normal Rat Fibroblasts and Rat Benzpyrene-Induced Sarcoma

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Exact knowledge of the direct effect of radium and x-rays on malignant cells is a primary condition for an understanding of the mechanism of the therapeutic action of radiation on neoplastic growth.

The investigations of the direct effect of radium and x-rays on living cells and tissues is greatly facilitated by the method of tissue culture. Normal and malignant cells of various types isolated from the body and kept in pure culture in vitro can be directly exposed to radiation and their response can be observed exactly and determined quantitatively. Moreover, by modifying the composition of the medium and other environmental conditions, the physiological state of the cell colonies may be varied, and the effect of these variations on the radiosensitivity of the cells can be investigated. In this way exact study of the direct effect of radiation on normal and malignant cells in different physiological conditions is made easy and comparison of the respective responses of normal and malignant cells to radiation is rendered possible.

The literature contains but few comparative studies of the radiosensitivities of malignant and normal cells in vitro.

Canti (1, 2) irradiated cultures of chick fibroblasts derived from normal periosteum and cultures of Jensen rat sarcoma with tubes containing 70 to 150 milli-hours of radium emanation and found some differences in sensitivity to radium rays between normal fibroblasts and malignant spindle cells. Fibroblasts of normal periosteum showed very little change as a result of exposure to the emanation. The majority maintained their shape, their sharp outline, and the appearance of the nucleus was not altered. In the case of all the wandering cells, however, a marked effect was noticeable after 20 minutes' exposure; cell movement ceased and the cells became spherical with blurred, irregular outlines. When the cells of Jensen rat sarcoma were subjected to irradiation, apparently all of them, whether of the mobile or fibroblastic type, were affected in about the same time as the wandering cells of the normal tissue.

Laser and Halberstaedter (4) compared the growth-inhibiting action of radium rays on cultures of normal and malignant cells, and found that radium, in doses of 35 to 50 mgm. hr. which strongly reduced the growth of colonies of normal chicken osteoblasts, was practically without effect on cultures of Ehrlich mouse carcinoma and Jensen rat sarcoma. Doses of 12.5 mgm. hr. strongly inhibited the outgrowth of rat fibroblasts but had no effect on rat sarcoma cells (Crocker rat sarcoma 16).

Whitman (8) used cultures of Walker rat sarcoma for his experiments and compared the relative effect of 5, 16, and 50 mc. hr. doses of radium on the mitotic activity of malignant cells and normal macrophages always present in his tumor culture. The results supported the conclusion that normal cells are more sensitive to irradiation than are malignant cells. "The number of mitoses of the normal cells (macrophages) was proportionately more reduced by irradiation than that of the malignant cells. The percentage initial fall in the mitotic count of the normal cells was greater for all three doses than for the malignant cells."

Goldfeder (3), Vollmar (5), and Vollmar and Rajewsky (6, 7) irradiated cultures of various tissues and tumors with x-rays of different dosage and intensity and described the effect obtained.

In view of the sparsity of these investigations and their conflicting results it seemed advisable to undertake a systematic study on the relative radiosensitivity of normal and malignant cells in vitro.

These investigations will be carried out on different experimental tumors, both mesenchymal and epithelial,
FIGS. 1 TO 6
and will comprise systematic studies of the radiosensitivity of the various vital functions of normal and malignant cells. The plan will be to compare the effect of radiation on the tumor cells with that on normal cells of the same species and type of tissue.

This first report is concerned with the comparative effect of x-rays on the outgrowth of normal rat fibroblasts and cells of rat benzpyrene-induced sarcoma in cultures.

**Material and Methods**

**Cell cultures.**—The experiments were performed on cultures of rat fibroblasts and on benzpyrene-induced rat sarcoma. The cultures of rat fibroblasts were obtained from explants of the extremities of approximately 10-day-old rat embryos. Cultures used in the irradiation experiments were 2 or 3 passages old. The strain of sarcoma cells used in these experiments derives from a benzpyrene-induced rat sarcoma and has now been kept in this laboratory without loss of malignancy for one year. The cultures were made according to the standard cover slip method of Carrel. As culture medium we used normal chicken plasma and diluted chicken embryo extract in the proportion 1:1.

**Irradiations.**—Irradiations were carried out by means of a demountable x-ray tube operated at a tension of 35 kv. on a current of 20 ma. using copper anticathode. The window consisted of aluminium foil 3 μ in thickness. Absorption analysis showed that the rays which penetrated through the window foil and through the 0.03 mm. thick mica cover glass of the culture were mainly copper-k-rays. The x-ray intensity at the distance of the irradiated object was about 90,000 r/min.

For irradiation experiments fragments of the cell cultures were transferred into fresh medium, and irradiated immediately afterwards. After irradiation the cultures were put into the incubator at 370° C. The growth of the cell colonies was examined after 24 and 48 hours.

**Experimental**

The results of the experiments on rat sarcoma cultures have been summarized in Table I.

At a dose of 10,000 r all irradiated cultures developed continuous growth areas; their extension was always less than that of the outgrowth of nonirradiated cultures. At a dose level of 25,000 r, about one-half of irradiated cultures showed a continuous growth area. The remaining colonies mostly showed only isolated cells and a few failed to show any cell migration whatever. At 50,000 r the number of irradiated cultures with continuous growth area was negligible; about one-half of the cultures showed only single cells; in the remainder no cell migration whatsoever was observed. An irradiation dose of 75,000 to 100,000 r caused complete suppression of growth in the large majority of irradiated cultures. At 150,000 r only a few cultures which still showed some solitary cells remained. At a dose level of 200,000 r all outgrowth ceased.

Table II summarizes the results of experiments on rat fibroblasts.

At a dose level of 10,000 r all irradiated fibroblast cultures show continuous growth zones. At a dose level of 25,000 r continuous growth zones were found in only 60 per cent of the irradiated cultures, the remainder showing only isolated cells. At 50,000 r none of the irradiated cultures showed continuous outgrowth, about 60 per cent solitary cells, and the remainder no migration whatsoever. At 75,000 r the number of cultures with single cells only was further sharply reduced. At 200,000 r all cell migration was completely suppressed.

**Table I: Results of Irradiation of Cultures of Rat Sarcoma**

<table>
<thead>
<tr>
<th>Doses in r</th>
<th>Number of cultures irradiated</th>
<th>Effect of irradiation *</th>
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<tbody>
<tr>
<td></td>
<td>Continuous growth</td>
<td>Slight growth</td>
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<tr>
<td>10,000</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>25,000</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>50,000</td>
<td>27</td>
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<td>75,000</td>
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<td>48</td>
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</tr>
<tr>
<td>150,000</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>200,000</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

* The column marked + indicates the number of irradiated cultures which showed continuous growth areas; column marked ± shows the number of cultures which after irradiation showed only solitary cells; column marked – gives the number of irradiated cultures in which not a single cell had migrated. Figs. 2, 4, and 6 give typical pictures of the irradiation effects classed respectively as +, ±, and –.

**Table II: Results of Irradiations of Cultures of Normal Rat Fibroblast**

<table>
<thead>
<tr>
<th>Doses in r</th>
<th>Number of cultures irradiated</th>
<th>Effect of irradiation *</th>
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<td>150,000</td>
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<td>0</td>
</tr>
<tr>
<td>200,000</td>
<td>28</td>
<td>0</td>
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</table>

* Notations same as Table I.
The results of the experiments have been arranged graphically in Fig. 7.

The courses of the curves for normal and malignant cells are seen to be practically identical. The minimum x-ray dose which causes complete suppression of cell migration under our experimental conditions is the same for both normal rat fibroblasts and cells of rat sarcoma. This dose is 200,000 r.

**SUMMARY AND CONCLUSIONS**

The minimum x-ray dose which totally suppresses cell outgrowth in tissue cultures is the same for both normal rat fibroblasts and cells of rat sarcoma. This dose is 200,000 r.

**REFERENCES**

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