STUDIES ON X-RAY EFFECTS.

IV. DIRECT ACTION OF X-RAYS ON TRANSPLANTABLE CANCERS OF MICE.*

BY ELSA HILL, JOHN J. MORTON, M.D., AND WILLIAM D. WITHERBEE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, October 21, 1918.)

It has been shown in this laboratory that x-rays administered in doses sufficient to destroy a large proportion of the lymphoid tissue reduce the resistance of the animal to transplanted cancer. Furthermore, both potential and established induced immunity can be destroyed by a similar process. It has also been shown that small doses of x-rays sufficient to stimulate the lymphocytes increase the resistance to cancer. These observations bring up a number of interesting points with regard to x-rays as a therapeutic agent in the treatment of cancer. The literature on this subject is so extensive and contradictory that no attempt will be made to review the previous work. The question of immediate interest to us in this investigation is whether or not x-rays, even in a dose above that possible for therapeutic purposes, will kill the cancer cell. It is necessary in the light of the experiments mentioned above to rule out the action of this agent on the animal itself.

To determine the cumulative, as well as the immediate effect of the direct action of powerful doses of x-rays upon tumor cells, we undertook to grow a Bashford mouse tumor (Adenocarcinoma No. 63) in successive generations of white mice, subjecting the tumor to like doses of x-rays after excision and before inoculation at each transplantation. An actively growing tumor which had been propagated

* This investigation was carried out by means of funds from the Rutherford Donation.

in the laboratory for some time was used, and an x-ray dose selected which was purposely above the range of therapeutic dosage: Coolidge tube, spark-gap 8 inches, milliamperes 5, distance 6 inches, and time 2 minutes and 35 seconds. The percentage of takes and rate of growth of the tumor were observed in fourteen generations, extending over a period of 17 months with eleven exposures between transplantations.

Later, considering the possibility of greater absorption of less penetrating rays, we undertook a similar experiment with the following dose: Coolidge tube, spark-gap 1 inch, milliamperes 25, distance 8 inches, and time 20 minutes. In this experiment the tumor was observed in four generations.

Method.

In both experiments (Nos. 1 and 2), the technique used was as follows: Wherever possible a healthy, actively growing tumor, approximately 1.5 by 1.5 cm., was chosen for inoculation. The mouse was killed and the tumor removed under aseptic conditions. It was then cut into halves and each half placed in a small Petri dish, covered with a single layer of sterile gauze, and set into a large Petri dish containing sufficient salt solution to keep the gauze slightly moist. One-half remained in the laboratory at room temperature, while the other half was subjected to x-rays. The temperature at the surface of the gauze did not rise above 33°C. in Experiment 1, or 42°C. in Experiment 2. The x-rayed portion of the tumor was then divided into small pieces of uniform size. Care was taken to select for inoculation the outer actively growing portions of the tumor. Asepsis was observed throughout the experiment. Single pieces of the tumor were loaded into hollow needles and ten normal white mice (young adults) were inoculated in the right groin. In loading the needles care was taken to macerate the tissue as little as possible. The control half of the tumor which received no x-rays was then inoculated into ten mice in exactly the same manner. In Experiment 1 the x-rayed half remained out of the body 45 to 50 minutes and the control half 1 hour. In Experiment 2 the x-rayed half remained out of the body 60 to 70 minutes and the control half 1½ hours. The tumors resulting from the inoculations were measured at periods of 1 week
Text-Fig. 1. The generations of tumor transplantations for Series I, extending over a period of 17 months. The final tumor transplant had been exposed to eleven treatments of x-rays during this period.
until the death of the mice. A healthy tumor was selected from the x-rayed series unless because of an epidemic or some other cause this was impossible, when one of the control tumors was chosen for treatment and an inoculation was made into another series of twenty mice in the manner described.

The first series of transplants was begun December 11, 1916, and terminated May 9, 1918 (Text-fig. 1). In Generations 5 and 6 the percentage of takes is inaccurate, probably much too low, on account of an epidemic among the mice which occurred before the usual time of appearance of tumors. For the transplants at this period, on account of the loss from the epidemic, the tumors of the next generation were taken from the control series. If we disregard the sudden drop in those two generations, the percentage of takes among the mice receiving x-rayed portions of the tumor remained between 25 and 45 during the first year, or nine generations. At the same time the percentage of takes among the control mice was between 50 and 70.
From the tenth generation to the fourteenth the percentage of takes does not remain consistently higher among controls and in both x-rayed and normal groups the percentage rises even to 100 (Text-fig. 2).

The rate of growth of the tumors was judged from curves representing the average time of appearance of the tumors and the average size 5 weeks after transplanting. As these two curves were essentially similar, only the former is illustrated (Text-fig. 3). In less than half the generation groups the tumors appeared a week earlier in the control mice than in those which had received the x-rayed tumor. The difference in size between the tumors of the two groups 5 weeks after transplantation is slight and not consistent.

Experiment 2 was begun January 14, 1918, and terminated May 9, 1918. Four successive generations were observed. Without a much longer series of transplants it is impossible to judge the cumulative effect of this dose of x-rays on the general virulence of the tumor. The difference in the percentage of takes is consistently much more marked in this series than at any point in the first series (Text-fig. 4). Initially the rate of growth of the tumors receiving x-rays was also greatly retarded (Text-fig. 5).
TEXT-Fig. 4. A comparison of the percentage of takes for Series 2.

--- Controls. ——— X-rayed cancers.

Percentage of takes

<table>
<thead>
<tr>
<th>Generation 1</th>
<th>Jan.14</th>
<th>Feb.13</th>
<th>Mar.19</th>
<th>Apr.15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

1918
DISCUSSION.

The present tendency of workers on x-ray therapy of cancer is to devise methods of increasing the amount of x-rays delivered at the location of the cancer process. In the light of our observations there is one point which should be taken into consideration; that is, whether or not we are justified in using a procedure which apparently only inhibits the cancer temporarily, while it incidentally lowers the resistance of the individual to the growth. It is well recognized that a proportion of cancers held in check for a time by x-ray treatment will later grow more rapidly. It is not possible to form an idea of what proportion of the total number of patients treated show this result, as few completely and accurately controlled series have been
published. Blood counts on a number of these individuals have been made in this laboratory, and they all showed remarkably low lymphoid counts. Our work, however, has been done mainly on cancer of mice, and we are therefore not warranted in drawing sweeping conclusions until there is more careful confirmation from studies on man. We feel justified, however, in suggesting that powerful doses of x-rays which are only capable of inhibiting cancer growth for a time may bring about eventually a lowered resistance to a return of the disease process.

CONCLUSIONS.

These experiments indicate that the direct action of x-rays in more powerful doses than can be applied therapeutically is somewhat injurious to tumor cells, but by no means destroys them. Experiment 1 also indicates that the cancer cells establish a resistance to the x-rays after repeated doses. This harmonizes with the experience of clinicians who have succeeded in checking cancerous growths for some time but reach a point where no response can be effected by repeated doses. The rays of low penetration used in Experiment 2 are apparently more harmful to tumor cells than the penetrating rays used in Experiment 1.