Some Cytologic Effects of Therapeutic Radiation

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In a series of experiments an attempt has been made to determine the effect of different types and dosages of radiation on cytoplasmic components at different intervals after irradiation. This is a report of a quantitative study of the centrioles in interkinetic cells of the Walker rat carcinoma 256 following radiation (single dose 2,400 r and 4,800 r) and of the mouse sarcoma CR 180 (single dose 2,400 r). Also quantitative observations were made on the grossly abnormal mitoses in the radiated tissue.

REVIEW OF LITERATURE

It has been shown that with appropriate technic centrioles can be demonstrated in Walker rat carcinoma 256. Also there appears a variation in the frequency of multiple centrioles (more than the normal two) following a single dose of 2,400 r. In addition, irregularly shaped nuclei, giant cells, multipolar mitoses, and other evidences of grossly abnormal cell divisions were observed by Fogg and Warren (6).

Many workers have reported on the effect of radiation on nuclei, mitoses, and the formation of abnormal cells. Recently, Dederer (2) reported the production of giant spermatocytes in Philosamia cynthia after x-ray dosages of 2,000 r to 8,000 r. She notes that the giant cells may be of different types, single, binucleate, or multinucleate and concludes these variations must have been produced by the fusion of cells rather than by nuclear division. Whitman (12) radiated normal fibroblasts in tissue culture and Walker rat carcinoma 338 and noted abnormalities, especially in the nucleus. Kemp and Juul (7) confirmed the earlier work of Donaldson and Canti (3) and of Strangeways and Hopwood (11) which showed that abnormalities could be produced by radiation but that these did not differ essentially from cells growing in an unfavorable medium. Crowther (1), in a theoretical review on the biological action of x-rays, states as his belief that the destructive nonspecific action of radiation is due to chemical changes in the cytoplasm. Packard (9) has reviewed the literature and recently a general discussion of the subject with bibliographies has been presented in Duggar's Biological Effects of Radiation (4).

OBSERVATIONS

The method of killing the animals, the nature of the rat carcinoma 256, and the preparation of the tissue were the same as described in previous papers (5, 6). By using identical technic it was possible to show bodies usually paired and probably centrioles in the mouse sarcoma CR 180. At intervals of 18, 24, 48, 72, 96, and 120 hours after radiation animals were killed and the tumor tissue prepared for study. Tumors from a nonradiated rat and a nonradiated mouse were also fixed and stained to serve as controls. The paired bodies embedded in a surrounding mass of acidophilic centrosomal substance which are identified as centrioles can be seen in Fig. 1.

Fig. 1.—Nonradiated interkinetic cell of the Walker rat carcinoma 256 showing 2 centrioles in relatively clear acidophilic area near nucleus. Photograph of Bouin preparation, phosphotungstic acid hematoxylin stain. Mag. X 3,500.
By 24 hours 15 per cent of the counted cells show multiple centrioles. There are a few which have more than 4 centrioles. Table III shows that approximately 94 per cent of the mitoses at this interval present some apparent gross abnormality, usually fragmented pieces of chromosomes or doubling of chromosomal substance in one cell.

We have reported (6) that with this tumor and dose the increase in the number of cells showing higher numbers of centrioles continues up to 72 hours. After 72 hours the percentage decreases although cells with a high number of centrioles persist. Note in Table I that there are 37 cells out of a thousand tissue is recorded in Tables II and IV. An examination of Table II shows the same pattern of response as after 2,400 r; namely, an increase in the percentage of cells showing multiple centrioles up to about 72 hours and the appearance of cells having 6 or more centrioles. Then follows a gradual decrease. It is noteworthy that with the higher dose the percentage of cells showing multiple centrioles is not as high as with the 2,400 r. However, Table IV shows that while high percentages of abnormal mitoses continue longer than after 2,400 r the percentage remains about the same to 96 hours after which there is a lag of 24 hours before the drop toward the normal condition is apparent.

### Table I: Effect of 2,400 r on Centrioles in Walker Rat Carcinoma 256

<table>
<thead>
<tr>
<th>Hours after radiation</th>
<th>Number of cells counted</th>
<th>Number of centrioles per interkinetic cell</th>
<th>Per cent of multiple centrioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 1,000</td>
<td>900 81 17 1 0 0 0 0 0 0 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 1,000</td>
<td>848 40 100 2 10 0 0 0 0 0 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 1,000</td>
<td>605 97 274 5 15 2 2 0 0 0 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 1,000</td>
<td>401 62 355 14 113 7 41 1 6 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 1,000</td>
<td>538 77 236 11 94 7 30 0 7 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 1,000</td>
<td>666 26 6 2 0 0 0 0 0 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1,000</td>
<td>958 39 2 1 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II: Effect of 4,800 r on Centrioles in Walker Rat Carcinoma 256

<table>
<thead>
<tr>
<th>Hours after radiation</th>
<th>Number of cells counted</th>
<th>Number of centrioles per interkinetic cell</th>
<th>Per cent of multiple centrioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 500</td>
<td>480 9 11 0 0 0 0 0 0 0 0 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 500</td>
<td>454 3 9 0 4 0 0 0 0 0 3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 500</td>
<td>442 14 40 0 3 0 0 0 0 0 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 500</td>
<td>437 7 43 0 11 0 0 1 0 0 12.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 500</td>
<td>443 5 50 0 3 0 1 0 0 0 11.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 500</td>
<td>451 1 27 0 2 0 0 5 2 2 7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 500</td>
<td>958 39 2 1 0 0 0 0 0 0 4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sand that contain 8 or more centrioles. It is worthy of note (Table III) that up to 72 hours there was also a high percentage of cells exhibiting abnormal mitoses. The abnormal mitoses at 48 hours to 72 hours and later showed multipolar mitoses, widely scattered chromosomes, and the occurrence of giant cells in profusion. Subsequent to 72 hours the number of abnormal mitoses decreases and by 120 hours following radiation it is approaching the control condition.

The effect of a single dose of 4,800 r on the same tissue was recorded in Tables V and VI. An examination of these tables shows the same pattern of response as after 2,400 r; namely, an increase in the percentage of cells showing multiple centrioles up to about 72 hours and the appearance of cells having 6 or more centrioles. Then follows a gradual decrease. It is noteworthy that with the higher dose the percentage of cells showing multiple centrioles is not as high as with the 2,400 r. However, Table IV shows that while high percentages of abnormal mitoses continue longer than after 2,400 r the percentage remains about the same to 96 hours after which there is a lag of 24 hours before the drop toward the normal condition is apparent.

### Table III: Effect of 2,400 r on Mitoses in Walker Rat Carcinoma 256

<table>
<thead>
<tr>
<th>Hours after radiation</th>
<th>Number of cells counted</th>
<th>Normal mitoses</th>
<th>Gross aberrations</th>
<th>Per cent of aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-18</td>
<td>No significant number of mitoses, normal or abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 500</td>
<td>28 472 94.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 500</td>
<td>42 458 91.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 500</td>
<td>45 455 91.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 500</td>
<td>227 273 54.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 500</td>
<td>450 50 10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 500</td>
<td>489 11 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table IV: Effect of 4,800 r on Mitoses in Walker Rat Carcinoma 256

<table>
<thead>
<tr>
<th>Hours after radiation</th>
<th>Number of cells counted</th>
<th>Normal mitoses</th>
<th>Gross aberrations</th>
<th>Per cent of aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-18</td>
<td>No significant number of mitoses, normal or abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 500</td>
<td>56 68 432 86.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 500</td>
<td>50 50 450 90.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 500</td>
<td>42 458 91.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 500</td>
<td>51 449 89.8</td>
<td></td>
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<tr>
<td>120 500</td>
<td>53 447 89.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 500</td>
<td>489 11 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
However, at the earlier intervals the consistency of the response to the radiation is apparent although the percentage of cells showing multiple centrioles was less for each interval than was noted in Walker carcinoma 256 after similar treatment. On the other hand both tissues consistently showed a very high percentage of abnormal mitotic divisions.

It will be further noted (Table VII) that the percentage of abnormal mitoses in all three tissues studied is approximately the same through the 96-hour interval. In contrast, the percentage of multiple centrioles in Walker carcinoma 256 following a single dose of 2,400 r is consistently higher than in the same tissue following a single 4,800 r dose or in the mouse sarcoma CR 180 following a single 2,400 r dose.

**DISCUSSION**

Every slide of radiated tissue studied showed necrotic cells, varying in number, and it is suggested that as a result of radiation most of the cells suffer injury, lethal in some, transient in others. The hypothesis has previously been advanced that the temporary increase in the number of centrioles is due to the fact that cytoplasmic division of certain cells is inhibited although nuclear division and the division of the centriole continue within limits. The preponderance of centrioles in multiples of two further supports this suggestion. Also the number of centrioles per cell does not reach its maximum until a lapse of time adequate for the production of several cell generations. The possibility of the formation of centromeres or fragmentation of the centriole bears further investigation.

The data suggest that a single dose of 4,800 r approaches the maximal single dose for the rat as 2,400 r does for the mouse. One may deduce from this that the vitality of the cells is so reduced by the radiation that few of the cells which initiate division are capable of performing subsequent abortive divisions which might give rise to multiple centrioles. Whereas, in Walker carcinoma 256 treated with 2,400 r, the lesser injury permits a greater number of cells to continue through later abortive divisions thus producing a greater number of multicentriolar cells. The data suggest further that if a cell initiates division before 24 hours the mitosis will usually be abnormal. If a radiated cell delays division until after 120 hours the effect of the radiation has been reduced to such a point that the process will probably be normal.

**SUMMARY**

Centrioles can be demonstrated in the Walker rat carcinoma 256 and the mouse sarcoma CR 180 after suitable fixation and staining.

Radiation causes an increase in the percentage of cells in both tissues showing multiple centrioles for a period of 24 to 96 hours. In Walker carcinoma 256 with doses of 2,400 r and 4,800 r it was the higher dose which gave a lower percentage of multiple centrioles for any given period after radiation.

A quantitative study of multiple centrioles shows that sarcoma CR 180 with a dose of 2,400 r is more sensitive to radiation than Walker carcinoma 256 after a similar dose.
From the time mitosis is resumed after irradiation the percentage of cells showing abnormal mitoses is consistently high for a period up to 96 hours for both tumors regardless of dose.

REFERENCES

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