The Relative Biologic Effectiveness of 200 KV and 23.5 MEV (Beatron) X-Radiation

I. Production of Leukopenia in Mice

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SINCE PHYSICAL STUDIES of radiation emitted by Betatron machines indicate that this type of radiation possesses possible advantages compared to the conventional 200 KV x-radiation as a therapeutic agent, investigations are needed of the relative biologic effectiveness of these two qualities of radiation. The high sensitivity of the leukocyte value in the peripheral blood to ionizing radiations is well known and its depression is often taken as an indication of radiation injury. In the present investigation, the two types of radiation have been compared using leukopenia as the biologic indicator. It will be shown that in producing a given degree of leukopenia in mice, the relative effectiveness of betatron radiation is 0.65 of that produced by 200 KV radiation.

MATERIALS AND METHODS

1. Biologic Technic

Inbred strains of mice were used in these studies and consisted of CF1, black C57, and a strain, heterogenous genetically, which will be referred to as the Toronto strain. This was obtained from Dr. Milton Bell, Department of Animal Husbandry, University of Saskatchewan. Body weight varied from 15 to 30 Gm. In general, animals of the same strain from several litters born within a week were pooled together to form stocks of males and females. Leukocyte counts were made according to standard laboratory procedures routinely thirty days after birth and again usually one week before the beginning of an experiment. Among other considerations, the variation and increase of this normal leukocyte count served as a guide for the proper random distribution of these animals among the three experimental groups: control group, 200 KV group and betatron group. Blood was taken from a tail vein. As a rule, counts were performed within 24 hours after irradiation on half of the animals and those of the remaining half were done on the third or fourth day. Subsequent countings of each subgroup were followed twice a week for a period of several weeks, making sure that no anemia was produced as a result of excessive bleeding.

2. Radiologic Technic

For immobilization during whole body irradiation, each animal was loaded in a plastic tube with the tail held in a cork plug. The 200 KV x-radiation (filter 1.0 mm. Cu + 0.5 mm. Al; half value layer 1.4 Cu) was generated at a dosage rate of approximately 30 r per minute at a focal skin distance of 50 cm. Twelve mice were irradiated simultaneously on a rotating...
horizontal circular platform with the plastic tubes appearing as radii of the circle. The tubes were inclined at a small angle to the horizontal plane so that points near the center of the platform were at a slightly greater distance from the focal spot of the x-ray machine than were points on the periphery, thus counteracting the increase in dose toward the center of the platform due to scattered radiation and rendering the dose uniform over the length of the mouse. Half way through exposure the tubes were rotated through 180 degrees. The mean dose received by the mice was determined by filling one plastic tube with a block of unit density material and measuring the dose received at the center of this block, using a 25 r Victoreen chamber. Doses recorded are mean doses. The dose received by any part of the mouse did not differ from the mean dose by more than about 4 per cent and all mice received exactly the same dose.

The betatron radiation was generated most of the time at 23.5 MEV and a copper compensating filter was used to obtain a flat distribution of radiation over the area of the field. A cylindrical wooden block 17 cm. in diameter with 10 holes placed parallel to the axis on a circle of diameter 11 cm. was placed with its axis at right angles to the beam at a point 90 cm. from the target. Because of the flat nature of the radiation field the dosage variation over the length of the mouse was very small. By rotating the wheel during the exposure all mice were given exactly the same dose.

The x-ray beam was continually monitored with an integrating type of meter and this integrating meter was calibrated by placing a 100 r Victoreen chamber at the center of one of the plastic tubes filled with unit density material. The average dosage rate was about 100 r per minute. The rim of the wooden wheel extending outside the position of the plastic tube served to build up electronic equilibrium between the gamma rays and the electrons set in motion by the gamma rays.

3. Method of Evaluation

Quantitative estimates of the degree of leukopenia were made by computing the maximum depression of the leukocyte value resulting from radiation. This was the difference between the minimum leukocyte value observed in the irradiated animals and the corresponding leukocyte value of the control animals on the same day and was expressed as a percentage of the latter quantity. It should be noted that due to its increase with age, the leukocyte value of the control animals used in the above measurement was usually higher than the initial value at the beginning of the experiment. The leukocyte values for all animals in a given group were averaged and the percentage standard deviation varied from 10 to 25 per cent.

The relative biologic efficiency of betatron radiation as compared with 200 KV radiation is given by

\[ \eta = \frac{\text{Dose of 200 KV x-radiation which produces a given reaction}}{\text{Dose of betatron radiation which produces the same reaction}}. \]

This was determined by a graphic method. In our experiments, the value of \( \eta \) depended on the level chosen for the comparison. Comparisons were therefore made at 70 per cent leukocyte reduction in each case.

Results

1. Experiments with CF1 Strain

These experiments were carried out with a dosage range varying between 150 and 600 r. Some of the results are shown in figures 1 and 2. Each point in figure 1 was obtained by averaging the leukocyte count for 6 mice. In general, it was found that both the extent of depression in leukocyte value and the rate of recovery depended upon the dose given. The effect was greater and the rate of recovery slower, the higher the dose employed. This statement applied equally well to the response produced by both qualities of radiation. For a given dose,
Fig. 1—Effect of 300 and 500 r on leukocyte count in mice. Leukocyte values are expressed as a per cent of initial count. CF1 mice, 45 days of age. Initial leukocyte count corresponding to 100 per cent was 8,500 ± 1,000 per cu. mm.

Fig. 2—Effect of 600 r on leukocyte count and mortality in mice. Leukocyte values as per cent of initial count. CF1 mice, 37 days of age. Initial leukocyte count corresponding to 100 per cent was 8,150 ± 1,400 per cu. mm. Mortality curve is for 200 KV radiation.
the degree of reaction was higher in animals treated with 200 KV x-radiation than that in those treated with a similar dose of betatron radiation. Recovery of the leukocyte count began earlier and took place more rapidly in the betatron-treated animals. Similar results were obtained with older mice (age 75 days) except that in this case the leukocyte value for the control animals remained more nearly constant.

In figure 2 are reported results obtained with a dose (600 r) approaching the lethal range for these animals. Apart from the quantitative difference in response compared to that observed at lower dosage levels, it was observed that the severe reaction produced by the 200 KV x-radiation was followed by death in a good fraction of the experimental animals within a few days, whereas none of the betatron treated animals succumbed (lower diagram of fig. 2). It was also noted that the leukocyte value of the dying animals continued to decrease without showing any sign of recovery. Only the fraction of animals which possessed relatively high leukocyte values survived to the ninth day. In other experiments with older mice the results of which are not shown, leukocyte reductions greater than those shown in figure 2 were observed without subsequent death. This suggested that there was a narrower margin of tolerance in leukocyte depression among younger animals.

The results of all the experiments with CF1 mice are summarized in figure 3. Here the percentage of leukocyte depression is plotted against the dose for each radiation used. The number of mice used for the determination of each point is indicated on the left-hand side of the figure. It is seen that there exists an ap-

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**Fig. 3**—Comparison of betatron and 200 KV radiation for maximal leukocyte reduction in CF1 mice. Reduction is given in per cent of initial count at time of radiation. Lengths of dashes on left indicate the combined numbers of mice employed in the two series of experiments to obtain the corresponding points on the two curves. Doses for 70 per cent leukocyte reduction shown by the dotted lines. \( \eta = \frac{A}{B} = 0.65 \).
proximately linear relationship between the dose of radiation and the maximum degree of leukocyte depression. For any given degree of leukopenia produced, the dose of betatron radiation required is always higher than that of the 200 KV x-radiation. Since the lines of figure 3 do not pass through the origin, the value for the relative biologic effectiveness will change somewhat for various degrees of leukopenia. At 70 per cent leukocyte reduction, a factor of 0.65 is obtained. The factor varies from 0.54 to 0.66 over the whole dosage range.

![Comparison of betatron and 200 KV radiation for maximal leukocyte reduction in C57 mice. Reduction is given in per cent of initial count at time of radiation. Lengths of dashes on left indicate the combined numbers of mice employed in the two series of experiments to obtain the corresponding points on the two curves. Doses for 70 per cent leukocyte reductions are shown by the dotted lines. \( \eta = \frac{A}{B} = 0.60 \).](image)

2. Experiments with the C57 Strain

The results of similar experiments with C57 mice are shown in figure 4. The general pattern observed with these mice was the same as in figures 1 and 2. Due to the greater uniformity of biologic material and the larger number of animals employed less variation in results was observed. It appears that the C57 strain possesses a higher radiosensitivity to the two qualities of radiation than the CF1 strain. It is also seen that for a given dose the relative degree of depression in leukocyte value produced by the 200 KV radiation is distinctly higher. The relative biologic efficiency for the C57 mice was 0.60.

A high mortality was also observed in the C57 mice treated with 600 r of 200 KV x-radiation. A significant feature of these experiments was the low level of the leukocyte value attained: 70 per cent of the animals died between the twelfth and eighteenth day when the mean leukocyte count was 5 to 7 per cent of normal. Averaging the counts of all mice with leukocyte counts made the day before death gave a value of 2 per cent. This data is of greater significance than that
obtained with CF1 mice because of the substantial number of experimental animals employed (40 controls and 40 irradiated with each type of radiation). Also, compared with the animals used in other experiments, these animals were more mature, having an average age of 112 days and with the controls showing a fairly constant leukocyte value during the experimental period. These results demonstrate that an extremely high degree of leukopenia preceded the radiation death of these animals.

In the case of C57 mice irradiated with 600 r from the betatron, only a small fraction (6 per cent) died after the third week following irradiation. These showed

![Graph showing comparison of betatron and 200 KV radiation for maximal leukocyte reduction in Toronto mice. Reduction is given in per cent of initial count at time of radiation. Lengths of dashes on left indicate the combined numbers of mice employed in the two series of experiments to obtain the corresponding points on the two curves. Doses for 70 per cent leukocyte reduction are shown by the dotted lines. $\eta = \frac{A}{B} = 0.70$.]

only a relatively minor degree of leukopenia and this suggested that other types of radiation injury might be responsible for the death of these animals. Some of these animals showed definitely prolonged bleeding and in many cases hemorrhage was conspicuously present.

3. Experiments with the Toronto Strain

Similar results were obtained with the Toronto strain and the results are shown graphically in figure 5. Considerable differences were observed in the leukocyte value among the various groups of animals used as well as variations in the growth rate of this quantity after birth. Moreover, the age of the animals in any one experiment lacked the degree of uniformity usually found in earlier experiments with other strains. Finally, the experiments were all carried out at the 400 r region with only one exception. In spite of these unfavorable factors, the results of these experiments were similar to those obtained with CF1 and C57 mice.
The general pattern observed with these mice was the same as that shown in figures 1 and 2. With the betatron treated animals, recovery took place earlier and more rapidly. The value of $\eta$ for 70 per cent leukocyte reduction was 0.70.

The Differential Count after Irradiation

In order to obtain some information regarding the relative amount of depression of the different types of leukocytes by the two qualities of radiation, differential counts were performed on a number of C57 mice suffering a high degree of leukopenia following exposure to 600 r of whole body radiation. The results are shown in table 1. It can be seen that the depression of lymphocytes, which constitute about 80 per cent of the normal leukocyte count in mice, largely accounted for the observed degree of leukopenia following irradiation. This was particularly true in animals receiving lethal doses of the 200 KV x-radiation, where in several cases, there was almost complete disappearance of these cells from the circulation prior to the death of the animal. The neutrophils were fewer and as a result contributed less to the observed degree of leukopenia. It was observed that the depression of the neutrophils occurred more slowly and that during recovery juvenile cells of this series were occasionally seen in the circulation. In the case of eosinophils, due to their small numbers (about 1 per cent of the total leukocyte count), their contribution to the leukopenia was relatively insignificant.

While the above findings applied to the general blood picture observed in animals receiving either quality of radiation, nevertheless there was some difference in the relative percentage contribution to leukocyte depression of each cell species depending on which quality of radiation was used. Thus the contribution of lymphocytes was higher in animals receiving the betatron radiation than in the 200 KV group while the contribution of the neutrophils was higher in the latter group. This was the result of several factors, viz., the difference in relative abundance of the different types of leukocytes and the difference in the degree of leukopenia observed. The percentage depression of each cell species was calculated from the pertinent data and is tabulated in the last two columns of the table. It is to be noted that while the depression of lymphocytes increased only slightly from the betatron treated animals to the 200 KV group, that of neutrophils increased by a factor of almost two. The significance of this observation as bearing on the different degree of leukopenia produced by the two qualities of radiation will be discussed later.

**Table 1.—Comparison of the Differential Counts in the Peripheral Blood of Mice (C57) given 600 r of Whole Body 200 KV or 23.5 MEV X-Radiation. Each Group Consisted of 16 Animals**

<table>
<thead>
<tr>
<th>Counts per cu. mm.</th>
<th>$%$ Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Leukocyte .</td>
<td>9600 ± 1500</td>
</tr>
<tr>
<td>Lymphocyte .</td>
<td>7400 ± 1800</td>
</tr>
<tr>
<td>Neutrophils .</td>
<td>2100 ± 400</td>
</tr>
<tr>
<td>Eosinophils .</td>
<td>100 ± 100</td>
</tr>
</tbody>
</table>
Effect of Age on Leukopenia and Recovery

The possible effect of age on the degree of leukopenia and its recovery rate was studied with approximately 400 mice with ages of 40, 50, 60 and 70 days. The animals were divided into three equal groups, one group for control and the other two groups were given doses of 400 r of 200 KV radiation and betatron radiation respectively. The results are shown in Table 2. As can be seen the leukocyte count of the control group increased with age. The minimal leukocyte value reached after irradiation was progressively smaller with increasing age for the 200 KV group but did not change for the betatron group. However, the percentage depression below the control produced by 400 r increased with increasing age for both groups (see second part of Table 2). The time required for 50 per cent recovery is shown in the last part of the table. This increased with age. Recovery of the leukocyte count was more rapid in the betatron treated group.

<table>
<thead>
<tr>
<th>Average age in days</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte value × 1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.7 ± 2.0</td>
<td>13.9 ± 1.7</td>
<td>14.8 ± 2.4</td>
<td>15.8 ± 2.1</td>
</tr>
<tr>
<td>200 KV</td>
<td>4.1 ± 1.7</td>
<td>3.6 ± 1.2</td>
<td>2.9 ± 0.6</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>23.5 MEV</td>
<td>5.7 ± 1.5</td>
<td>5.6 ± 1.1</td>
<td>5.7 ± 0.7</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>% depression from control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 KV</td>
<td>68</td>
<td>74</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>23.5 MEV</td>
<td>56</td>
<td>60</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>Days for 50% recovery of leukocyte count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 KV</td>
<td>36</td>
<td>43</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>23.5 MEV</td>
<td>21</td>
<td>23</td>
<td>25</td>
<td>26</td>
</tr>
</tbody>
</table>

Discussion

The relative biologic effectiveness of the betatron radiation may be expressed as Quastler's conversion factor, but in the present case, the value of this ratio shows some variation with degree of leukopenia produced. Hence, while the graphic method allows us to show the over-all difference in effectiveness of the two types of radiation, for comparison of the ratio for the three varieties of mice, this comparison must be made at one leukocyte level. We selected 70 per cent maximal leukocyte reduction for comparison and at this level, the values were 0.65 for CF1, 0.60 for C57 and 0.70 for the Toronto strain. There is no significant difference between these three values. The average value is 0.65. An explanation to account for the lower biologic effectiveness of the betatron radiation is not apparent. Although the exact factors influencing the biologic effectiveness of a given type of radiation are not generally known, it is commonly agreed that these are both biologic and physical in nature. In this experiment equal doses of radiation by the two types of radiation should correspond to equal

TABLE 2.—Effect of Age on Leukocyte Depression by X-Radiation in Mice.
Mice—Toronto Strain 400 r of 200 KV or 23.5 MEV Radiation.
Approximately 90 Mice in Each Group
amounts of energy absorption so that the difference must be explained in biologic
terms.

The effect of the radiations in causing leukopenia must be due either to the
direct destruction of cells in the circulation or to interference with leukopoiesis.
Since the leukocytes normally survive in the circulation only for a few hours,
the effect of the radiation observed three to ten days later must be the result
of damage to leukopoietic mechanisms. It has been reported above that the lower
degree of leukopenia produced by the betatron radiation is due to a lesser degree
of depression of granulocytes in the circulation compared with that produced by
a similar dose of 200 KV x-radiation. As shown in table 1 the lymphocyte depres-
sion was 80 per cent for the Betatron group and 95 per cent for the 200
KV group, while the corresponding depressions for neutrophils were 52 per cent
and 98 per cent. This raises the question whether or not the precursors of gra-
ulocytes are less sensitive than the lymphocyte series to the Betatron radiation.
It is not possible to answer this question with the present approach. A possible
alternative explanation has been sought on physical grounds, since Spiers has
recently shown that the ionization in a cavity in bone 100 micra in diameter is
increased 30 per cent in a 200 KV beam over that in a high energy beam. The
effect is considerably greater for smaller cavities. This could conceivably be
responsible for a differential effect on the granulocyte series. It is difficult to
judge the significance of this factor, however, without more detailed information.
Cursory examination of rib marrow suggests that the bone marrow cavities in
mice are too large for this to be a significant factor but on the other hand, the
presence of bone spicules and the relatively higher hemopoietic activity of sub-
cortical marrow are factors which would favor this explanation.

The observation that the betatron radiation is less effective in producing leuko-
penia may have practical applications in radiotherapy. It may be possible to
administer doses of betatron radiation beyond the tolerance limit set for the con-
tventional 200 KV x-radiation without inviting the same degree of general radia-
tion injury. In this connection, the fact that it is the granulocytes which are
spared may increase the tolerance in man, where the granulocytes constitute
70 per cent, the lymphocytes 20 per cent, of the leukocytes. This is the reverse
of what is found in the mouse, with 80 per cent lymphocytes and 20 per cent
granulocytes. The betatron will only have a real advantage in the treatment of
deep-seated tumors if the relative biologic effectiveness of betatron radiation
for tumor regression is higher than for leukocyte depression.

SUMMARY AND CONCLUSIONS

1. The leukocyte value in the peripheral blood of three different strains of
mice was followed after a single dose of whole body x-radiation. The maximum
leukocyte depression was used as a basis for comparing the biologic effectiveness
of the betatron radiation and the 200 KV x-radiation.

2. The ratio of effectiveness in producing 70 per cent leukocyte reduction,
for betatron radiation to 200 KV x-radiation, for the three strains was 0.65.

3. The onset of recovery from leukopenia for equal doses of radiation occurred
earlier in animals treated with betatron radiation than in those treated with a
similar dose of 200 KV x-radiation.
4. The degree of leukopenia and delay in recovery was found to increase somewhat with age of animals treated with 200 KV x-radiation and to a less extent in animals treated with betatron radiation.

5. At a dose of 600 r, over 90 per cent of the animals treated with 200 KV died within a few days, while none of the animals exposed to the same dose of betatron radiation died in the same period. The leukocyte count immediately before death was about 2 per cent of control values.

6. For a dose of 600 r, the lymphocytes were equally reduced by 200 KV and betatron radiation, while the granulocytes were much less reduced by betatron than by 200 KV radiation.

REFERENCES


The Relative Biologic Effectiveness of 200 KV and 23.5 MEV (Beatatron)
X-Radiation: I. Production of Leukopenia in Mice

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