Effects of 14 MeV Neutrons and X-Rays, Singly or Combined on the Reproductive Capacity of L Cells

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ABSTRACT
In order to clarify whether the effects of neutrons on mammalian cells are qualitatively different from those of X- or gamma-rays, L cells were irradiated with D-T neutrons and 180 kvp X-rays, singly or combined. From analysis of dose response curves, it is concluded that cell killing mechanism of neutrons is not the same as that of X-ray, but there is something common in part between them. RBE values of neutrons with respect to X-rays calculated from dose response curves was 2.5-1.5 for 50%-1% survival.

INTRODUCTION
In the previous study\(^1\), the effect of neutrons on the reproductive capacity of mammalian cells was compared with that of 180 kVp X-rays, and the following points were ascertained; 1) Temperature and oxygen tension during irradiation and dose rate can modify the dose response curves of HeLa cells exposed to neutrons and X-rays. 2) Using two dose irradiation technique, it was found that the recovery of HeLa cells from damage to reproductive capacity was comparable following neutron and X-irradiation. 3) A quantitative difference was observed between the effects of neutrons and X-rays, but it could not be determined whether this was due to the qualitative difference in the cell killing mechanism.

The purpose of this to clarify whether the effects of neutrons on mammalian cells are qualitatively different from those of X- or gamma-rays. L cells were ex.
posed to neutrons (or X-rays) immediately after X-irradiation (or neutron irradiation) and the dose response curves were compared with those of cells exposed to neutrons or X-rays alone.

**MATERIALS AND METHODS**

The line used in this experiment was L 5, a subclone of mouse L cells obtained from Dr. T. Terasima (National Institute of Radiological Sciences, Chiba) and was adapted for this experiment to grow in Eagle MEM supplemented with 20% calf serum, 0.04 mg/ml of CaCl₂·2H₂O and antibiotics. The doubling time of cells during the phase of logarithmic growth was 24 hours. Cell suspension was prepared by trypsinization from stock cultures (2-3 days old). After counting with a hemocytometer under a phase contrast microscope, cell suspension was diluted with growth media. For 60-70 minutes before irradiation and during irradiation, the cell suspension was bubbled through with a mixture of 5% CO₂-95% O₂. The temperature was maintained at 37°C in a water bath. Cells were irradiated in suspension with X-rays and neutrons, singly or combined. In the experiment in which the combined effects of X-rays and neutrons on cell reproductive capacity were studied, cells were irradiated with neutrons (or X-rays) 5 to 10 minutes after a conditioning dose of X-rays (or neutrons). Thereafter, the cell suspension was kept in situ for 10 minutes and a sufficient number of cells was transferred to each vessel containing 5 ml of media so as to give about the same number of surviving cells for each dose level studied. All plates were then incubated at 37°C in a humidified atmosphere of air containing CO₂, with a constant pH of 7.3. The medium was not replaced during the incubation period of 12-14 days. At the end of this incubation period, colonies containing more than 50 non-giant cells were scored as reproductively intact.

Neutrons were produced by ³H (d, n) ⁴He reaction. A absorbed dose was calculated from the number of alpha particles counted with an alpha detector placed inside the beam duct. As factor for converting flux density to dose, 6.7x10⁻⁹ rad/n/cm² was used. Gamma contamination to biological materials, being less than 5% of the neutron dose, was ignored. Dose rate was 30 rads/minute. X-rays were produced at 180 kVp and 20 mA and filtered to yield a HVL of 1.03 mm Cu. Dose rate was 93 rads/minute.

**RESULTS**

Fig. 1 shows the dose response curves of L 5 cells irradiated with X-rays and 14 MeV neutrons and the influence of previous X-rays irradiation (or neutron) on the neutrons (or X-rays) survival.

The RBE values of neutrons with respect to 180 kVp X-rays calculated from dose response curves were summarized in Table 1, 2.5-1.5 for 50%-1% survival. The parameter Do and extrapolation number n of the dose response curves of neutron irradiated cells were 88 rads and 1.4, respectively, and Do and n of the survival curve of cells irradiated with neutrons after a conditioning dose of 416
rads of X-rays (9.0% of the cells survived) were 50 rads and 3.5, respectively.

The dose response curves of X-irradiated cells and those of cells irradiated with X-rays after a conditioning dose of neutrons were obviously of non-constant parameter, having a downward curvature. The surviving fraction of X-irradiated cells after neutron irradiation and that of neutron irradiated cells after X-ray irradiation were lower than those of cells irradiated with X-rays or D-T neutrons alone.

Fig. 2 shows the survival curve of L cells irradiated with 416 rads of X-rays and various doses of neutrons, combined. Survival curve of L cells irradiated with 147 rads of neutrons and various doses of X-rays, combined, is shown in Fig. 3.

### Table 1. RBE values of 14 MeV neutrons on the reproductive capacity of L cells

<table>
<thead>
<tr>
<th>% Survival</th>
<th>Dose (rads)</th>
<th>RBE</th>
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<tbody>
<tr>
<td>50</td>
<td>190 X-ray</td>
<td>75</td>
</tr>
<tr>
<td>50</td>
<td>310 Neutron</td>
<td>1.82</td>
</tr>
<tr>
<td>30</td>
<td>260 X-ray</td>
<td>125</td>
</tr>
<tr>
<td>30</td>
<td>310 Neutron</td>
<td>1.72</td>
</tr>
<tr>
<td>20</td>
<td>395 X-ray</td>
<td>230</td>
</tr>
<tr>
<td>20</td>
<td>480 Neutron</td>
<td>1.68</td>
</tr>
<tr>
<td>10</td>
<td>540 X-ray</td>
<td>330</td>
</tr>
<tr>
<td>10</td>
<td>480 Neutron</td>
<td>1.63</td>
</tr>
<tr>
<td>5</td>
<td>635 X-ray</td>
<td>430</td>
</tr>
<tr>
<td>5</td>
<td>540 Neutron</td>
<td>1.47</td>
</tr>
<tr>
<td>3</td>
<td>540 X-ray</td>
<td>330</td>
</tr>
<tr>
<td>3</td>
<td>480 Neutron</td>
<td>1.63</td>
</tr>
<tr>
<td>1</td>
<td>635 X-ray</td>
<td>430</td>
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<tr>
<td>1</td>
<td>540 Neutron</td>
<td>1.47</td>
</tr>
</tbody>
</table>

### DISCUSSION

An attempt was made to determine whether or not the effects of 14 MeV neutron on the reproductive capacity of L5 cells were qualitatively different from those of X-rays. Dose response curves of L cells irradiated with X-rays were of non-constant parameter type and quite different from those of HeLa cells reported elsewhere. Berry reported that replacement of culture media during incubation affected the survival rate of HeLa cells, but for L cells it was not possible to straighten out the downward trend in the survival curves when cultured with medium F.

The RBE values of 14 MeV neutrons on L cells with respect to X-rays were somewhat smaller than those of 14 MeV neutrons on HeLa cells. Comparison with the reported data indicates the values are not so small. The values of RBE depended on the surviving fraction and decreased progressively from 2.5 to...
1.5 for survival from 50% to 1%. The mechanism by which cells are killed may be different according to type and dose of radiation. The answer to this question must await future research.

In order to compare the effects of 14 MeV neutrons on living cells with those of X-rays, dose response curves of L cells irradiated with neutrons and X-rays, singly or combined, were analysed. As shown in Figure 1, the surviving fraction of cells irradiated with X-rays after neutron irradiation of 147 rads was lower than that of cells irradiated with X-rays alone. And the survival rate of L cells exposed to neutrons after X-ray irradiation was lower than that of L cells irradiated with neutrons alone. From this results, it may be concluded that there is an interaction between effects of X-rays and of 14 MeV neutron on cell reproductive capacity, when cells were first irradiated with X-rays or with neutrons. This clearly demonstrates that there must be something common in the cell killing mechanism of X-rays and that of neutrons. There may be critical sites common to X-rays and neutrons and previous irradiation may either decrease the threshold dose level for effects, or it may be related to inactivation of the repair mechanism for the damage from the subsequent irradiation on cell reproductive capacity as shown in the interaction of X-rays and UV-rays. Rossi proposed that for absorbed dose of cells, dose per assumed critical site is more appropriate than average absorbed dose per gram in considering the cell killing mechanism. If critical site is 0.5 micron in diameter, electrons produced by Co-60 gamma rays can occasionally provide energy increment comparable to the energy typically lost by the protons liberated by 1 MeV neutrons. Its frequency becomes larger as the size of the critical site becomes smaller and conversely smaller the larger the size; even at diameter of a few microns, it is still quite detectable. Something in cell killing mechanisms common between X-rays and neutrons reported in this paper may be illustrated with this relation.

As dose was expressed in absorbed dose (rad) and biological and physical conditions for irradiation were the same in all the experiments, the effects of radiation can be discussed under the concept of RBE. Thus the effects of different types of radiation can be considered in relation to rem, and the surviving fraction of cells irradiated with one type of radiation depends on only the dose (rem) irrespective of type of radiation. On the assumption that the surviving fraction with A rads of X-rays is I and that with 2A rads is II, the surviving fraction of cells irradiated at the same time with A rem of X-rays and A rem of neutrons should be I² if the cell killing mechanism of neutrons is completely different from that of X-rays. On the contrary, if the cell killing mechanism of neutrons is identical to that of X-rays, the surviving fraction should be II. In this experiments X-rays and neutrons were not administered at the same time and neutrons (or X-rays) were given 5 to 10 minutes after X-ray (or neutrons) irradiation. As recovery in colony forming capability after irradiation of cells in suspension was apparently absent in the interval of 10 minutes, the recovery rate of sublethal damages of cells irradiated with X-rays and neutrons in interval of 5 to 10 minutes may be
Fig. 2. Survival curve of L cells irradiated with 416 rads of X-rays and various doses of neutrons, combined.

Fig. 3. Survival curve of L cells irradiated with 147 rads of neutrons and various doses of X-rays, combined.

From the dose response curve of X-irradiated cells, the surviving fraction of cells irradiated with 416 rads and twofold dose of 832 rads of X-rays is 0.090 and 0.00034, respectively. The neutron dose corresponding to 416 rads of X-rays is 240 rads. If the cell killing mechanism of neutrons is identical to that of X-rays, the surviving fraction of cells irradiated with 240 rads of neutrons after preirradiation of X-rays of 416 rads should be 0.00034 and on the contrary if the cell killing mechanism of neutrons is completely different from that of X-rays, it should be \((0.090)^2 = 0.0081\). From Figure 2 it is possible to read the value for the surviving fraction of cells irradiated with 240 rads of neutrons combined with 416 rads of X-rays. It is 0.0033 and is between 0.00034 and 0.0081. The surviving fraction of cells irradiated with 300 rads and a twofold dose of 600 rads of X-rays is 0.24 and 0.016, respectively. The dose of neutrons corresponding to 300 rads of X-rays is 147 rads. Figure 3 represents that the surviving fraction of cells irradiated with 300 rads of X-rays after preirradiation of 147 rads of neutrons is 0.023 and is between 0.016 and \((0.24)^2 = 0.057\). From these observations it may be concluded that cell killing mechanism of neutrons is common in some points but not all the same as that of X-rays.

Studies on damage of cells irradiated with neutrons and X-rays are in progress in order to establish what are the common or the different points in cell killing mechanism by difference in the quality of radiation.
REFERENCE