In general, DNA is assumed to be the critical target in mammalian cells exposed to ionizing radiation. However, the spectrum of lesions induced, their subsequent repair, and the interrelationships between these damage and repair mechanisms to cellular endpoints such as cell killing, mutation, and carcinogenesis remain important topics for research. Of the various types of lesions known to be produced by radiation, DNA strand breaks have been the subject of intensive study, primarily owing to the availability of sensitive methods for their detection in cultured cells irradiated in vitro. Although the results from such work have contributed significantly to our understanding of the effects of radiation on mammalian cells, the models developed from these studies may not be strictly applicable to the situation in vivo. The evidence that has accumulated over the last few years suggests that there may be significant differences between the in vitro and in vivo situations that influence both the formation and repair of radiation-induced DNA lesions. Such factors include the degree of oxygenation, intracellular levels of reduced glutathione (GSH), and degree of cellular differentiation.

In order to examine in greater detail how these factors may influence the formation and repair of DNA damage in vivo, we measured the efficiency of production and the kinetics of rejoining of strand breaks in various tissues of mice that had been exposed to whole-body doses of X rays. DNA damage was measured using the alkaline elution technique modified for use with cells from animal tissues in vivo using a microfluorometric DNA assay, which allows these measurements to be made in nonproliferating cells (i.e., cells unable to incorporate radioactive nucleosides).

Methods

The methodology used in these investigations will only be briefly outlined since the details have been described previously. C3H mice from a specific-pathogen-free breeding colony were used at 12-16 weeks of age. They were restrained in a Perspex chamber and whole-body irradiated using a General Electric Maximag 250 KVP X ray machine (dose rate, 3.53 Gy min⁻¹). The dose-response relationships shown in this paper have been adjusted for the difference in dose received by the different tissues. The animals were sacrificed at various times following irradiation, and their tissues were immediately removed and immersed in ice-cold Puck's saline A containing 5 mM EDTA. A total of about 3 min was required for irradiation and dissection.
under conditions where repair of DNA damage could occur. To induce acute
tissue hypoxia, the mice were either killed by cervical dislocation 15 min
prior to irradiation or were asphyxiated with nitrogen gas. Suspensions were
prepared from each tissue, and DNA damage was measured using the alkaline
elution technique modified for use with cells from animal tissues. DNA was
assayed using the fluorescent dye Hoechst 33258. This method generates
elution profiles identical to those obtained with radioactivity assays using
$^{14}$C-dThd-labeled CHO cells. DNA strand breaks were quantitated from such
DNA elution profiles by calculating a relative strand scission factor (SSF),
deфинирован as the absolute log of the ratio of the percentage of DNA retained on
the filter for the irradiated sample to that for an unirradiated, control
sample, in each case after an eluted volume of 17.5 ml. An SSF of 0 indicates
no strand break formation.

Intertissue Variations in Sensitivity to X Ray-induced DNA Strand Breakage In
Vivo and in Vitro

The efficiency of DNA strand-break production in different mouse tissues
irradiated in vivo was compared. Figure 1 shows the dose responses for
spleen and jejunum, which relate the relative degree of DNA strand breakage
to the radiation dose. Similar dose responses were generated for other
tissues. Cell suspensions also were prepared from various tissues from
unirradiated mice, and then irradiated with graded doses of X rays on ice in
vitro; these results for spleen and jejunum are also shown in Fig. 1. The
slopes of the resulting dose-responses, calculated by linear regression
analysis, are included in Table 1, and provide an indication of the relative
radiosensitivity of the tissues based on the efficiency of strand-break
production. Liver, brain, and kidney were analyzed after a single dose of 4
Gy in vitro, and the calculated slopes of the extrapolated SSFs are listed in
Table 1 for comparison.

The efficiency of DNA strand-break formation among tissues irradiated in
vivo varies by a factor of more than two, each tissue having its own char-
acteristic response. This confirms the observations of Ono and Okada ,11,12
who showed that the efficiency of DNA strand breakage was considerably less
in mouse testicular cells than in either liver or thymus cells irradiated in
vivo. They attributed this finding to a hypoxic condition of testicular
cells. In contrast to the effects of irradiation in vivo, cells from normal
tissues irradiated in vitro had similar sensitivities (Table 1). Further-
more, each tissue was 2- to 5-fold more sensitive when irradiated in vitro
than when irradiated in vivo.

Table 1. Slopes of in vivo and in vitro dose-response curves$^a,b$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>In vivo</th>
<th>In vitro</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>0.68±0.05$^c$</td>
<td>1.20±0.11</td>
<td>1.76</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.60±0.04</td>
<td>1.24±0.09</td>
<td>2.07</td>
</tr>
<tr>
<td>Brain</td>
<td>0.53±0.05</td>
<td>1.26$d$</td>
<td>2.38</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.43±0.06</td>
<td>1.28$d$</td>
<td>2.98</td>
</tr>
<tr>
<td>Liver</td>
<td>0.35±0.05</td>
<td>1.35$d$</td>
<td>3.86</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.30±0.03</td>
<td>1.34±0.21</td>
<td>4.47</td>
</tr>
</tbody>
</table>

$a$: Adapted from reference 9;
$b$: Units of slope are SSF per 10 Gy;
$c$: 95% confidence interval;
$d$: values were extrapolated from data obtained using a single dose of 4Gy.

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