**Alpha particles: experimental work**

The realization that radon is a significant dose component for the general population led to a greatly increased interest in the radiation biology of alpha particles.

Experimental difficulties:
- Short range
- Changing energy and LET within target.

Limited early studies with alpha particles led to the “conventional wisdom” that even a single particle traversal of the nucleus would kill the cell.

Exponential survival curves interpreted as “single-hit, single target.” (Barendsen, 1960).

Indirect mechanisms were invoked to explain the survival (and mutation) of cells exposed to alpha particles.

Munro (1970) plutonium needle experiment showed nucleus much more sensitive than the cytoplasm.

One of the first studies to show that cells could survive a large number of alpha particle nuclear traversals was [Lloyd, et al., Int. J. Radiat. Biol., 35, 23-31, 1979]

- Alpha particles derived from a Tandem accelerator, 5.6 MeV, 85 keV/µm at cell surface.
- Track detectors used to count particle fluence.
- Cell nuclei measured.

**The Lloyd paper contains one of the first observations that cells flatten out considerably when attached, and that the nuclear cross section changes between spherical and attached cells. Serious implications for radiation effects with limited penetration alpha particles**

- Pelleted and fixed, nuclear cross section ~ 100 µm$^2$

- Attached and flattened, nuclear cross section ~ 300 µm$^2$, thickness ~2 µm.
Principles of Radiation Interactions

$Lloyd, 1979$

$D_0 = 4.4 \times 10^6$ alphas/cm$^2$

Conclusion was that $\sim 14$ alpha particles pass through a 313 µm$^2$ nucleus, at a dose $= D_0$.

Relation to other work:

- $40$ µm$^2$ 1-2 traversals = mean lethal dose (Barendsen, 1960)
- $150$ µm$^2$ $\sim 5$ traversals = mean lethal dose (Datta, 1976)
- $313$ µm$^2$ $\sim 14$ traversals = mean lethal dose (Lloyd, 1979)

Inactivation cross section $= 1/D_0 = 22$ µm$^2$/track (“very similar” to Barendsen, Datta).

The authors concluded that total track length deposited in nucleus is the critical factor.

- Flatter cells can withstand more traversals.

Speculation: cells flattened against bone surfaces may survive alpha particle traversals (from bone-seekers: $^{226}$Ra, $^{239}$Pu) but transform into osteosarcomas.
Earlier studies used wide beams from isotope sources or accelerators.

Objective: development of a model system for alpha particle irradiations of cells under more controlled conditions.

**Radiobiology of α Particles**

I. Exposure System and Dosimetry

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[Radiation Research, 123, 304-310, 1990]

**TABLE I**

Technical Data Describing the α-Particle Exposure System

<table>
<thead>
<tr>
<th>Source</th>
<th>238Pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclide</td>
<td>238Pu</td>
</tr>
<tr>
<td>Half-life</td>
<td>87.74 years</td>
</tr>
<tr>
<td>Specific activity</td>
<td>630 GBq g⁻¹</td>
</tr>
<tr>
<td>α-Particle energies</td>
<td>5.495 MeV 71.6%</td>
</tr>
<tr>
<td></td>
<td>5.452 MeV 28.3%</td>
</tr>
<tr>
<td></td>
<td>5.352 MeV 0.1%</td>
</tr>
<tr>
<td>Activity</td>
<td>144 MBq</td>
</tr>
<tr>
<td>Diameter</td>
<td>70 mm</td>
</tr>
<tr>
<td>Construction</td>
<td>Electro-deposition on stainless steel</td>
</tr>
<tr>
<td>Supplier</td>
<td>Los Alamos National Laboratory</td>
</tr>
</tbody>
</table>

Honeycomb collimator

<table>
<thead>
<tr>
<th>Material</th>
<th>Aluminum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td>25 μm</td>
</tr>
<tr>
<td>Channel length</td>
<td>7.4 mm</td>
</tr>
<tr>
<td>Channel diameter</td>
<td>3.0 mm</td>
</tr>
</tbody>
</table>

Exit window

<table>
<thead>
<tr>
<th>Material</th>
<th>Mylar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1.39 g cm⁻³</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.5 μm (2.1 × 10⁻⁴ g cm⁻²)</td>
</tr>
<tr>
<td>Diameter</td>
<td>27.8 mm</td>
</tr>
<tr>
<td>Distance to source</td>
<td>16.3 mm</td>
</tr>
</tbody>
</table>

Disk base

<table>
<thead>
<tr>
<th>Material</th>
<th>Mylar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1.39 g cm⁻³</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.5 μm (2.1 × 10⁻⁴ g cm⁻²)</td>
</tr>
<tr>
<td>Distance to source (no filter)</td>
<td>17.4 mm</td>
</tr>
<tr>
<td>Distance to source (filter)</td>
<td>18.4 mm</td>
</tr>
</tbody>
</table>
The charge on the alpha particle decreases as the velocity decreases.

Early models did not take this into account.
• Changing the thickness of the mylar filter produces different energy spectra reaching the cells.
• Spectra become quite broad at lower energies.

### TABLE III
Energy, Range and Absorbed Dose Rates Produced from Mylar Energy Degradation Filters for the $^{238}$Pu $\alpha$-Particle Source

<table>
<thead>
<tr>
<th>Mylar filter thickness ($\mu$m)</th>
<th>Mean energy (MeV)</th>
<th>Energy-weighted stopping power (keV/$\mu$m)</th>
<th>Mean energy range in tissue ($\mu$m)</th>
<th>Cell surface dose rate (cGy/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>3.53</td>
<td>116</td>
<td>21</td>
<td>3.59 ± 0.16</td>
</tr>
<tr>
<td>1.5</td>
<td>3.23</td>
<td>123</td>
<td>18</td>
<td>3.81 ± 0.17</td>
</tr>
<tr>
<td>3.0</td>
<td>3.05</td>
<td>128</td>
<td>17</td>
<td>3.95 ± 0.17</td>
</tr>
<tr>
<td>6.0</td>
<td>2.42</td>
<td>148</td>
<td>13</td>
<td>4.60 ± 0.20</td>
</tr>
<tr>
<td>9.0</td>
<td>1.78</td>
<td>178</td>
<td>9</td>
<td>5.56 ± 0.24</td>
</tr>
<tr>
<td>12.7*</td>
<td>0.99</td>
<td>232</td>
<td>5</td>
<td>6.51 ± 0.35</td>
</tr>
</tbody>
</table>

* Values for 12.7 $\mu$m Mylar energy degradation filter were not measured but estimated from 9-$\mu$m data and range-energy relationships.

Track etch measurements in CR-39 indicate $\sim$1900 tracks/mm$^2$/sec.
Radiobiology of α Particles

III. Cell Inactivation by α-Particle Traversals of the Cell Nucleus

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[Radiation Research, 128, 204-209, 1991]

Collimated 3.5 MeV alpha particles
Careful estimation of nuclear cross section, nuclear thickness, together with keV/µm, allows calculation of dose to the nucleus.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean nuclear thickness (µm)</th>
<th>Mean nuclear area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-10B</td>
<td>3.7</td>
<td>105 ± 30</td>
</tr>
<tr>
<td>HSTE-23 (round shape)</td>
<td>7.1</td>
<td>65 ± 19</td>
</tr>
<tr>
<td>C3H 10T1/2</td>
<td>2.1</td>
<td>203 ± 49</td>
</tr>
<tr>
<td>V79</td>
<td>3.8</td>
<td>77 ± 25</td>
</tr>
<tr>
<td>AG1522</td>
<td>1.2</td>
<td>144 ± 45</td>
</tr>
</tbody>
</table>

*These mean nuclear thicknesses are from the following references: CHO-10B and HSTE (13), C3H 10T1/2 (14), V79 (11), and AG1522 (10).
### Table II

<table>
<thead>
<tr>
<th>Cell line</th>
<th>$D_0$ (cGy)$^a$</th>
<th>$D_0^N$ (cGy)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-10B</td>
<td>71 ± 7</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>HS-23</td>
<td>63 ± 10</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>C3H 10T1/2</td>
<td>57 ± 13</td>
<td>60 ± 13</td>
</tr>
<tr>
<td>V79</td>
<td>85 ± 7</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>AG1522</td>
<td>35 ± 5</td>
<td>36 ± 5</td>
</tr>
</tbody>
</table>

$^a D_0$ is the dose in tissue at the cell–Mylar interface corresponding to 37% survival.

$^b D_0^N$ is the corresponding value to $D_0$ in the cell nucleus region.

$D_0^N$ calculated at the center (5th layer) of the nucleus.
$E^N_\alpha$ is the calculated energy deposited in the nucleus by the traversal of a single alpha particle.

$D^N_\alpha$ is the absorbed dose to the nucleus from a single alpha particle.

Number of alpha particle traversals per nucleus was calculated three ways:

1. number of traversals = $N_T$
   
   alpha particle fluence = 1900/mm$^2$. sec (measured in CR-39 track detector)
   
   dose rate = 3.8 cGy/sec (measured with ionization chamber)
   
   $A$ = nuclear cross section ($\mu$m$^2$)
   
   $$N_T = \frac{D_0 \cdot 1900 \cdot A}{3.8}$$

2. $N_T = \frac{D^N_0}{D^N_\alpha}$

   where $D^N_\alpha$ = the nuclear dose from a single alpha particle,
   
   and $D^N_0$ = the dose to the nucleus at $D_0$.

3. By simply dividing the number of tracks/µm$^2$, (as read from CR-39 track detector) at a dose equal to $D_0$, by the nuclear area.

### Table III

<table>
<thead>
<tr>
<th>Cell line</th>
<th>$E^N_\alpha$ (keV)</th>
<th>$D^N_\alpha$ (cGy)</th>
<th>$\frac{dE}{dx}$ (keV/µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-10B</td>
<td>471</td>
<td>18.5</td>
<td>125</td>
</tr>
<tr>
<td>HS-23</td>
<td>995</td>
<td>33.3</td>
<td>136</td>
</tr>
<tr>
<td>C3H 10T1/2</td>
<td>260</td>
<td>9.4</td>
<td>121</td>
</tr>
<tr>
<td>V79</td>
<td>491</td>
<td>26.2</td>
<td>124</td>
</tr>
<tr>
<td>AG1522</td>
<td>144</td>
<td>13.0</td>
<td>121</td>
</tr>
</tbody>
</table>
Cross section: the relative probabilities of inducing lethal events at a given fluence.

\[
\sigma (\mu m^2) = k \frac{L \cdot \alpha}{\rho} = \frac{L (keV/\mu m)}{0.065 \cdot D_0^N (cGy)}
\]

\(L\) = mean stopping power of alpha particle traversing the nucleus
\(\rho\) = the density of tissue
\(\alpha = 1/ D_0^N \) (cGy)
\(k\) = a constant to match units

The inactivation cross sections are fairly constant, with the exception of the (very flat) AG1522 cells.

Inactivation cross sections agree with literature values.

Total track length: the number of traversals x nuclear thickness.

The total track length for inactivation is nearly constant, with the exception of the AG1522 cells.
Observations:

- The sensitivity to $^{60}$Co varies with cell line.
- RBEs are nearly constant.
- No correlation between $N_T$ and RBE.

Conclusions:

- Average number of alpha particles leading to a lethal lesion is greater than 1 (range 2-6) and dependent on the cell line used.

- Total track length in the nucleus is a better measure to compare the sensitivities of different cell lines.

- Non-lethal lesions may be responsible for the greater carcinogenicity of alpha particles.
Environmental exposure to radon produces a low dose, where very few cells are hit at all. However the few cells that are hit receive a substantial dose (on the order of 0.5 Gy).

Irradiation with a wide field of alpha particles from an accelerator or an isotope source means that Poisson statistics must be used in the interpretation of dose.

\[
P(n) = \frac{(e^{-\bar{n}} \cdot \bar{n}^n)}{n!}
\]

where \( \bar{n} \) = the average number of hits/target, and \( n \) = the specific number of hits/target

For an average dose of one alpha particle per cell, the actual distribution is predicted to be:

- 0 particles 37%
- 1 particle 37%
- 2 particles 18%
- 3 particles 6%
- 4 particles 1.5%
- 5 particles 0.3%

Clearly this complicates the interpretation of the results.

Experimental approaches to deliver exactly defined numbers of alpha particles include

- Microbeams
- Retrospective imaging of cells grown on a track detector
• Cell culture dish has LR115 track detector as a base.
• Cells plated, locations recorded.
• Irradiation from below with alphas from a $^{210}$Po source
• Allow cells to form colonies (6 days).
• Re-scan dish, re-visit original locations, colony criteria = 50 cells.
• Etch LR115 track detector by floating on alkaline etching solution.
• Re-scan dish, record track locations.
• Spatial resolution of the relocation hit determination = 0.9 µm.
Survival is similar to other reports using microbeams.

No dependence of survival on membrane or cytoplasm hits observed, at the dose level used.
Comparison of the predicted Poisson distribution of tracks per nucleus to the actual number of tracks observed per nucleus.

[Image removed due to copyright considerations]
Determination of penetration depth using mylar thickness.

Track diameter increases at “track ends”.

[Image removed due to copyright considerations]
Conventionally assumed characteristics of alpha particle irradiation.
- No matter how low the dose, any cell traversed receives a relatively high dose (~0.5 Gy).
- Cells not traversed are unaffected.

Previous work:
- Chromosomal instability in progeny.
- Number of cells showing instability was greater than predicted based on Poisson statistics.

Possible problems with interpreting these results.
- There is always a Poisson distribution of cells hit and non-hit.
- Is the instability observed in the progeny of a hit cell or a non-hit cell (i.e., is there a “bystander effect”?)

Objective:
Create experimental conditions where the surviving population was largely non-hit, but was present (“bystanders”) during the irradiation.

Approach:
- Interpose a grid between the cells and the alpha source.
- This will shield cells from irradiation.
- CR-39 track etch techniques to verify grid effect.
- Harvest cells
  - determine survival
  - determine genetic effect (instability: non-clonal aberrations in descendants in a colony derived from one of the original cells)
The maximum expected proportion of cells exhibiting instability was calculated.

- The particle fluence is known,
- Using Poisson statistics the proportion of cells surviving a particle traversal is estimated from the cell survival curve.

With a dose of 1 Gy, the majority of cells irradiated are inactivated.

- With no grid: 20% of survivors were irradiated
- With grid present: 3% of survivors were irradiated.
Table 1. Significant discrepancy in the observed and expected frequencies of haemopoietic colonies exhibiting chromosomal instability defined by the incidence of nonclonal cytogenetic aberrations (7, 8)

<table>
<thead>
<tr>
<th>α-Particle irradiation dose, Gy</th>
<th>Colonies exhibiting instability, n/n</th>
<th>Expected $P_A(&gt;0)$</th>
<th>Exact binomial test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>$2/5 = 0.400^*$</td>
<td>0.055</td>
<td>$2.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>0.5</td>
<td>$6/12 = 0.500^*$</td>
<td>0.105</td>
<td>$7.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>0.5 combined data</td>
<td>$16/26 = 0.615^+$</td>
<td>0.105</td>
<td>$4.1 \times 10^{-10}$</td>
</tr>
<tr>
<td>0.5 combined data</td>
<td>$22/38 = 0.579$</td>
<td>0.105</td>
<td>$1.2 \times 10^{-12}$</td>
</tr>
<tr>
<td>1.0</td>
<td>$4/10 = 0.400^+$</td>
<td>0.200</td>
<td>0.12</td>
</tr>
<tr>
<td>1.0</td>
<td>$41/64 = 0.640^+$</td>
<td>0.200</td>
<td>$1.5 \times 10^{-13}$</td>
</tr>
<tr>
<td>1.0 combined data</td>
<td>$45/74 = 0.608$</td>
<td>0.200</td>
<td>$1.2 \times 10^{-13}$</td>
</tr>
</tbody>
</table>

Probabilities of obtaining the observed results if the expected values represent the true values was calculated by using an exact binomial test (10).

*From ref. 7.
†From ref. 8.
‡From the current study.

Table 2. Significant discrepancy in the observed and expected frequencies of haemopoietic colonies exhibiting chromosomal instability with and without the grid interposed between the α-particle source and the cells (in the latter case, 1 Gy of absorbed dose is delivered to the unshielded cells only)

<table>
<thead>
<tr>
<th>α-Particle irradiation dose, Gy</th>
<th>No. colonies with aberrant cells/total no.</th>
<th>No. aberrant cells/total no.</th>
<th>No. chromatid breaks</th>
<th>No. chromatid exchanges and minutes</th>
<th>No. chromosome fragments</th>
<th>No. ring chromosomes</th>
<th>No. translocations</th>
<th>Mean no. aberrations per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22/55</td>
<td>36 /662</td>
<td>36</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>1 Gy</td>
<td>41/64</td>
<td>137/1099</td>
<td>191</td>
<td>4</td>
<td>24</td>
<td>3</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>1 Gy with grid</td>
<td>41/63</td>
<td>115 /871</td>
<td>128</td>
<td>15</td>
<td>29</td>
<td>5</td>
<td>6</td>
<td>0.21</td>
</tr>
</tbody>
</table>

$P = 1.5 \times 10^{-6}$ (control vs. 1 Gy); $P = 3.4 \times 10^{-7}$ (control vs. 1 Gy + grid); $P = 0.63$ (1 Gy vs. 1 Gy + grid).

Results:
Adding a grid reduced the number of irradiated cells present
The mean number of aberrations was the same with or without the grid.

Implications:
- Mechanism not understood (reactive oxygen species involved?)
- Risk from exposure to alpha particles not limited to cells actually hit.
- Potential role in carcinogenesis.
- Does risk estimation at low doses underestimate the effect?
Exposure to high levels of radon has been linked to an increased incidence of lung cancer in uranium miners.

Epidemiological studies estimate that 16,000 cases of lung cancer per year in the U.S. are caused by radon.

Uncertainties exist in the conversion of exposure, measured in working level months (WLM) to a dose (Gy).

The biological stages of carcinogenesis following radon exposure and leading to a carcinoma of the lung are not known.

Objective: to generate a relationship between exposure (WLM) and dose (mGy) to target cells in the lung: deep-lung fibroblasts.

Approach:
- The rat is used as a model system.
- Deep lung-fibroblasts are the target cell.
- Exposures in vitro use cells isolated from the rat, then exposed to radon gas and progeny in vitro. [RnCl₂ source as a generator. ²²²Rn equilibrated with cell culture medium, then the cells are added.]
- Exposure in vivo to radon aerosol (uranium ore dust 5.3 mg/m³).
- Endpoint is micronucleus formation in cells during first division after irradiation.
In vivo exposures:
- In vivo exposures of 0, 115, 213, and 323 WLM.
- Concentration was 1015 WL!!
- Exposure over 1, 2 or 3 days at ~ 100 WLM/day.
- Rats sacrificed 4 hrs after end of exposure,
- lung fibroblasts isolated, grown in cytochalasin B for 66-72 hrs
- score micronuclei/1000 binucleated cells

### TABLE I

<table>
<thead>
<tr>
<th>Exposure (WLM)</th>
<th>Binucleated cells</th>
<th>Number of micronuclei in binucleated cells</th>
<th>Total micronuclei</th>
<th>Micronuclei/1000 binucleated cells</th>
<th>Variance/mean</th>
<th>μ^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2014</td>
<td>1955 56 3 0 0</td>
<td>62 31</td>
<td>1.00 0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>3010</td>
<td>2779 205 22 4 0</td>
<td>261 87</td>
<td>1.11 4.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>213</td>
<td>3001</td>
<td>2702 257 35 6 1</td>
<td>348 116</td>
<td>1.17 6.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1007</td>
<td>843 128 31 4 1</td>
<td>206 205</td>
<td>1.30 6.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1001</td>
<td>859 119 16 5 2</td>
<td>174 174</td>
<td>1.35 7.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1000</td>
<td>849 133 16 2 0</td>
<td>171 171</td>
<td>1.12 2.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1021</td>
<td>890 115 14 2 0</td>
<td>149 146</td>
<td>1.07 1.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1000</td>
<td>866 106 27 1 0</td>
<td>163 163</td>
<td>1.25 5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>1000</td>
<td>784 178 31 5 2</td>
<td>263 263</td>
<td>1.19 4.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^*Coefficient of distribution; if value is >1.96, the distribution is overdispersed relative to Poisson distribution.
*In vitro* exposures:

- Harvest rat lung fibroblasts
- Grow in culture for 16 hrs or 96 hours; irradiate
- 16 hours cells still in G₀/G₁, i.e., not dividing
- 96 hours, > 50% have entered S or G₂/M
- after irradiation, grow in cytochalasin B for an additional 66-72 hours
- score micronuclei/1000 binucleated cells.

The dose response to $^{222}\text{Rn}$ *in vitro* is not different for dividing or non-dividing cells.

**Significance:**

- This study is the first to use deep lung fibroblasts (slow turnover in vivo, can be stimulated to divide in vitro).
- In vivo dose-response was **linear** despite the long time of the irradiations 1,2, 3 days; i.e., no loss of damaged cells during the experiment.
- This study produced a model for a “biological indicator of dose”: 1 WLM produced damage equivalent to 0.79 mGy.
Previous work has been done with alveolar macrophages.
  - Can isolate by “lavage”
  - Much higher exposures used (1000 WLM!!)
  - Cytochalasin B or BrdUrd not used in the macrophage study
  - Induction of micronuclei was 5 x lower in macrophages: 0.012 MN/100 cells/WLM compared to the lung fibroblast study: 0.058 MN/100 cells/WLM.
  - Macrophages migrate: may not have been present for the whole irradiation.

Previous work looked at the cells of the upper respiratory tract. This group and others looking at different cell types, different endpoints 1.7 – 2.5 mGy/WLM in rat tracheal epithelial cells—chromosome aberrations 2.6 mGy/WLM in deep lung fibroblasts—colony forming assay.

The current study used the same cell type, endpoint, non-dividing cells, both in vivo and in vitro.
Radon produces 55% of the effective dose equivalent to the general population.
Most of this dose is delivered to bronchial epithelial cells.
Estimation of risk from radon exposure uses the epidemiological approach.
Data are not sufficient for a biological approach using RBEs and specific biological endpoints.

Alpha particles penetrating through lung tissue have a variety of different LETs. Complicates the calculation of dose to a particular site.

**Objective:** investigation of the early steps in the chain of events leading from radon exposure to tumor formation

**Approach:**
- The rat is used as a model system.
- Deep lung-fibroblasts are the target cell.
- Also used a Chinese Hamster cell line, CHO, also a fibroblast cell line.
- Exposures *in vitro* use cells isolated from the rat, then exposed to radon gas and progeny *in vitro*. [RnCl₂ source as a generator. ²²²Rn equilibrated with cell culture medium, then the cells are added.]
- Exposure *in vivo* to radon aerosol (uranium ore dust 5.3 mg/m³).
- Endpoint is micronucleus formation in cells during first division after irradiation.
- ⁶⁰Co irradiations used as a photon control both *in vitro* and *in vivo.*
Dose-response for micronuclei formation in rat lung fibroblasts is linear for $^{60}$Co, both $\textit{in vitro}$ and $\textit{in vivo}$. 

[Image removed due to copyright considerations]
The dose-response for micronucleus formation is similar \textit{in vitro} and \textit{in vivo}, for both CHO (fibroblast) cells and for primary lung fibroblasts.

The dosimetry is accurate for the \textit{in vitro} exposures

RBEs are approximately 10.
Significance:
The isolation procedures did not affect the dose-response relationship

Two important physical factors can influence RBE
- dose rate
- LET

Differences in dose rate between the $^{60}$Co controls (0.5 Gy/min) and the radon exposures (dose delivered over hours in vitro and over several days in vivo) did not seem to change the dose response relationship.

The LET spectrum of the alpha particles reaching the fibroblasts in vivo is unknown. There may be a spread of LET both above and below the 100 keV/µm “maximum”.

Table 1. Effectiveness of radon relative to $^{60}$Co in the production of micronuclei following in vitro and in vivo exposure

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Exposure type</th>
<th>Intercept ± SE (1000 cells)</th>
<th>Slope ± SE (1000 cells)</th>
<th>Coefficient of determination</th>
<th>RBE ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>$^{60}$Co</td>
<td>32.4 ± 7.9</td>
<td>40.4 ± 3.8</td>
<td>0.93</td>
<td>12.5 ± 2.4</td>
</tr>
<tr>
<td>CHO</td>
<td>Radon</td>
<td>72.3 ± 25.0</td>
<td>503.5 ± 83.2</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>RLF</td>
<td>$^{60}$Co</td>
<td>15.4 ± 26.0</td>
<td>54.6 ± 11.4</td>
<td>0.92</td>
<td>10.9 ± 2.6</td>
</tr>
<tr>
<td>RLF</td>
<td>Radon</td>
<td>33.1 ± 39.8</td>
<td>593.3 ± 68.3</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>$^{60}$Co</td>
<td>1.6 ± 6.5</td>
<td>62.0 ± 2.7</td>
<td>0.99</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>RLF</td>
<td>Radon</td>
<td>16.9 ± 8.9</td>
<td>658.5 ± 56.3</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>
“The Bystander Effect”

Little and colleagues have developed a plutonium-based alpha particle irradiator, similar to the one at Los Alamos described by Raju.

The range of the alpha particles was calibrated using the track-etch approach with increasing thicknesses of mylar.
[Image removed due to copyright considerations]
Results indicate the alpha particles are 3.65 MeV at the cell surface, with an LET of 112 keV/µm. The particle fluence is 0.0054 tracks/µm³, which produces a dose rate of 9.9 cGy/min. A camera shutter mechanism allows exposures down to 0.067 sec.

<table>
<thead>
<tr>
<th>Exposure time (s)</th>
<th>Absorbed dose (mGy)</th>
<th>Mean no. of α-tracks/nucleus a</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.067</td>
<td>0.16</td>
<td>0.0004</td>
</tr>
<tr>
<td>0.125</td>
<td>0.31</td>
<td>0.0007</td>
</tr>
<tr>
<td>0.250</td>
<td>0.61</td>
<td>0.0014</td>
</tr>
<tr>
<td>0.500</td>
<td>1.23</td>
<td>0.0028</td>
</tr>
<tr>
<td>1.000</td>
<td>2.45</td>
<td>0.0056</td>
</tr>
<tr>
<td>2.000</td>
<td>4.90</td>
<td>0.0112</td>
</tr>
</tbody>
</table>

* a CR-39 track etch plastic was used for measurements of particle fluence and field uniformity. The dose rate was 0.0054 α-track/mm²/min, and the average size of the nucleus was 62.2 µm², yielding 0.366 tracks/nucleus/min.

With a dose rate of 0.366 tracks/nucleus/min, exposures of 2 sec or less produced, on average, very low numbers of alpha particle tracks/nucleus (0.0004 – 0.0112).

- Very few nuclei (< 1%) were actually traversed by an alpha particle at these doses.

**SCE**

Cells irradiated, synchronized in G₁. Bromodeoxyuridine added, cells allowed to grow for 2 cell cycles, chromatids stain differently. SCE can be detected. SCE is a crossing over event between the chromosomes attached at the centromere.
Sister chromatid exchange (SCE) versus exposure time (dose). Background rate has been subtracted.

The SCE rate increases rapidly and plateaus. At plateau, the rate is 1.4 times the background rate.
Scoring of SCE expressed as SCE per chromosome.

- At 0.31 mGy, 30% of cells showed increased SCE.
- At 2.45 mGy, 43% of cells showed increased SCE.
- 13% of cells showed SCE per chromosome > 0.6, a level rarely seen in control cells.
- At these doses only 0.1 to 0.5% of cell nuclei were traversed by an alpha particle.

A comparable level of exchange with x-rays requires 1-2 Gy (RBE >100!!).

Significance of SCE in mammalian cells is not clear.

The doses used here are too low to produce detectable levels of cell killing.
Implications:

Cells not directly traversed show a chromosomal change.

The risk to individuals may not be easily extrapolated from epidemiological data at high doses with low LET radiation.

Alpha particle dose in the range used in this study (0.31-4.9 mGy) are well within the range of exposure reported to occur from radon in homes.
More indirect evidence from the Little lab on the existence of a "Bystander Effect" using a different endpoint: HPRT mutation.

Objective: measure effects of very low doses of alpha particles: doses relevant to environmental exposures.
Requires a more sensitive endpoint.
N.B. Dose = 0.174 Gy (17.4 cGy) for each track through the nucleus. Increasing the exposure results in more cells receiving the same dose. At high doses, when there is more than one track per cell, the bystander effect is overshadowed by direct dose.
• Dose response becomes non-linear at low doses.
• Unexpectedly higher response than predicted from extrapolation of the high dose results down to the low dose region.
Mutation frequency per alpha particle track increased 5-fold at the lowest fluence.

Conclusion is that the enhanced mutation rate at low particle fluences was the result of mutations in non-irradiated bystander cells.

“The Bystander Effect”

- probably a variety of different mechanisms
- some reports say gap junction, intracellular communication is required,
- other reports say it is not
- does it occur in vivo?
- Implications for radiation protection and the current debate over the “linear no-threshold” hypothesis.