

LET, RBE and Damage to DNA

Linear Energy Transfer (LET)

When is stopping power not equal to LET?

Stopping power ($-dE/dx$) gives the energy **lost** by a charged particle in a medium.

LET gives the energy **absorbed** in the target.

Secondary **δ -electrons** can transport a fraction of the energy **out of the target volume**.

- Particularly true with heavy charged particles if the target is small compared with the ranges of the secondary electrons (delta rays).
- On the biological scale, the target dimensions are on the order of microns (cells), nanometers (chromatin), or angstroms (DNA).

Restricted stopping power $LET_{\Delta} = -\left(\frac{dE}{dx}\right)_{\Delta}$

- Energy transfers $> \Delta = 100\text{eV}$ are considered as imparted to **δ -electrons**.
- The symbol LET_{∞} denotes the **unrestricted stopping power**.
- LET is commonly found in the biological literature with no subscript. It is assumed that the unrestricted stopping power is implied.

[This is really an issue only with HZE particles and tracks.]

Examples:

<u>Radiation</u>	<u>Typical LET values</u>
1.2 MeV ^{60}Co gamma	0.3 keV/ μm
250 kVp x rays	2 keV/ μm
10 MeV protons	4.7 keV/ μm
150 MeV protons	0.5 keV/ μm
14 MeV neutrons	12 keV/ μm
Heavy charged particles	100-2000 keV/ μm
2.5 MeV alpha particles	166 keV/ μm
2 GeV Fe ions	1,000 keV/ μm

“Microdosimetric Structure of Heavy Ion Tracks in Tissue”,

Chatterjee, A. and Schaefer, H.J.

Radiation and Environmental Biophysics, 13, 215-227, 1976.

It is possible to have two particles of **different Z**, and **different E** (velocity), but the **same LET**. The microscopic distribution of energy around the track **will be different**.

- **Glancing collisions: the track core**
 - E transferred is small
 - the number of these interactions is large
- **Close collisions: the track penumbra**
 - E transferred is large, up to a maximum
 - The number of these interactions is small.

The *core radius* is given by the formula: $r_c = 0.0116 \beta$ (μm)

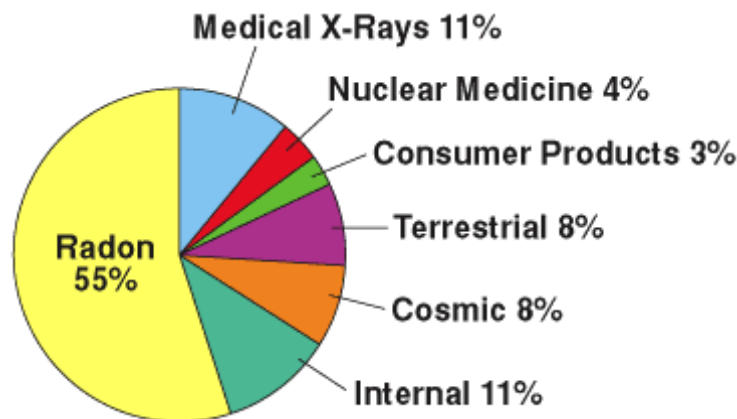
where $\beta = v/c$, i.e., the velocity of the particle relative to the speed of light.

The *penumbra radius*, r_p , is given by the formula:

$$r_p = 0.768 E - 1.925 \sqrt{E} + 1.257 \quad (\mu\text{m})$$

where E is the kinetic energy of the particle in MeV/**nucleon**.

Data for three nuclei with different Z numbers and energies yet same LET_∞



“Radiation effects for HZE particles cannot be measured with conventional dosimetric units.”

“...the core must be seen radiobiologically as a submicroscopic region of complete saturation and destruction.” !!!

Relative Biological Effectiveness (RBE)

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Given: $1 \text{ Gy} = 1 \text{ J/kg}$; $1 \text{ eV} = 1.6 \times 10^{-19} \text{ J}$

Assume: $1 \text{ ionization} = 33 \text{ eV}$; $1 \text{ nucleus} = 10^{-10} \text{ gram}$ or about $5 \mu\text{m}^3$

Therefore: $1 \text{ Gy} \approx 20,000 \text{ ionizations} / 10^{-10} \text{ g}$

In crossing the $5 \mu\text{m}$ nucleus:

1 MeV electrons lose 200 eV in 6 ionizations/ μm ,
700 tracks $\approx 20,000$ ionizations $\approx 1 \text{ Gy}$

30 keV electrons lose 1 keV in 30 ionizations/ μm ,
140 tracks $\approx 20,000$ ionizations $\approx 1 \text{ Gy}$

4 MeV protons lose 10keV in 300 ionizations/ μm
14 tracks $\approx 20,000$ ionizations $\approx 1 \text{ Gy}$

The dose to the nucleus is the same.

The biological effect is very different.

In comparing different types of radiation, it is customary to use x rays (classically 250 kVp x rays, currently shifting to ^{60}Co gamma or > 1 MeV x-rays) as the reference standard.

- Relative biological effectiveness (RBE) is defined as:

$$RBE = \frac{D_{ref}}{D_{test}}, \quad \text{where}$$

- D_{ref} is the dose of x rays, and
- D_{test} is the dose of the test radiation needed to produce **the same biological effect**.

This requires a biologic system where the effects of radiation may be **scored quantitatively**.

Many different endpoints are possible (in vitro, in vivo, normal tissue, tumors).

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Fig. 1-7 in Turner J. E. *Atoms, Radiation, and Radiation Protection*, 1st ed. New York, NY: Pergamon, 1986.

Relation between RBE and adsorbed dose

The different shapes of low-LET and high-LET survival curves lead to a variation in RBE as a function of endpoint (survival).

The RBE depends on the level of biologic damage chosen as the endpoint.

RBE varies as a function of dose.

- Large doses: RBE proportional to the ratio of the final slopes
- Intermediate doses: shoulder region, RBE \uparrow as dose \downarrow due to the shoulder of the reference curve.
- Low doses: RBE tends towards a ratio of the initial tangents

Factors that influence RBE

- Radiation quality (LET)
- Radiation dose
- Number of dose fractions
- Dose rate
- Biological system or end point

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Fig. 7.3a in Hall, Eric J. *Radiobiology for the Radiologist*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000.

Relation between RBE and LET

- RBE is strongly influenced by the LET of the particular radiation.
- As the LET increases, the slope becomes steeper, and the extrapolation number, n , tends towards unity.

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The “optimum” LET?

- The maximum value of 100 keV/ μm is similar for a wide variety of mammalian cells and for different endpoints (mutation, cell killing).
- It has been suggested that this reflects the “target” size and is related to DNA structure, which is similar for all mammalian cells.

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Fig. 7.6 in [Hall].

The “overkill” effect

- Based on target theory.
- Two inactivated sites required for cell kill.
- Densely ionizing radiation is inefficient in producing the maximum amount of cell kill.
- Low-LET radiation produces a sparsely ionizing track.
- It is a rare event for two tracks to deposit energy in the same cell.
- LET values above 100 keV/ μm ”waste” dose

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The molecular theory

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Fig. 7.7 in [Hall].

This effect could also be related to repair.

- Low LET damage is easier for the cell to repair.
- Some fraction of the *high-LET damage is more difficult or impossible to repair.*
- The overall result would be similar.
- At some point dose is wasted.

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The Oxygen Enhancement Ratio (OER)

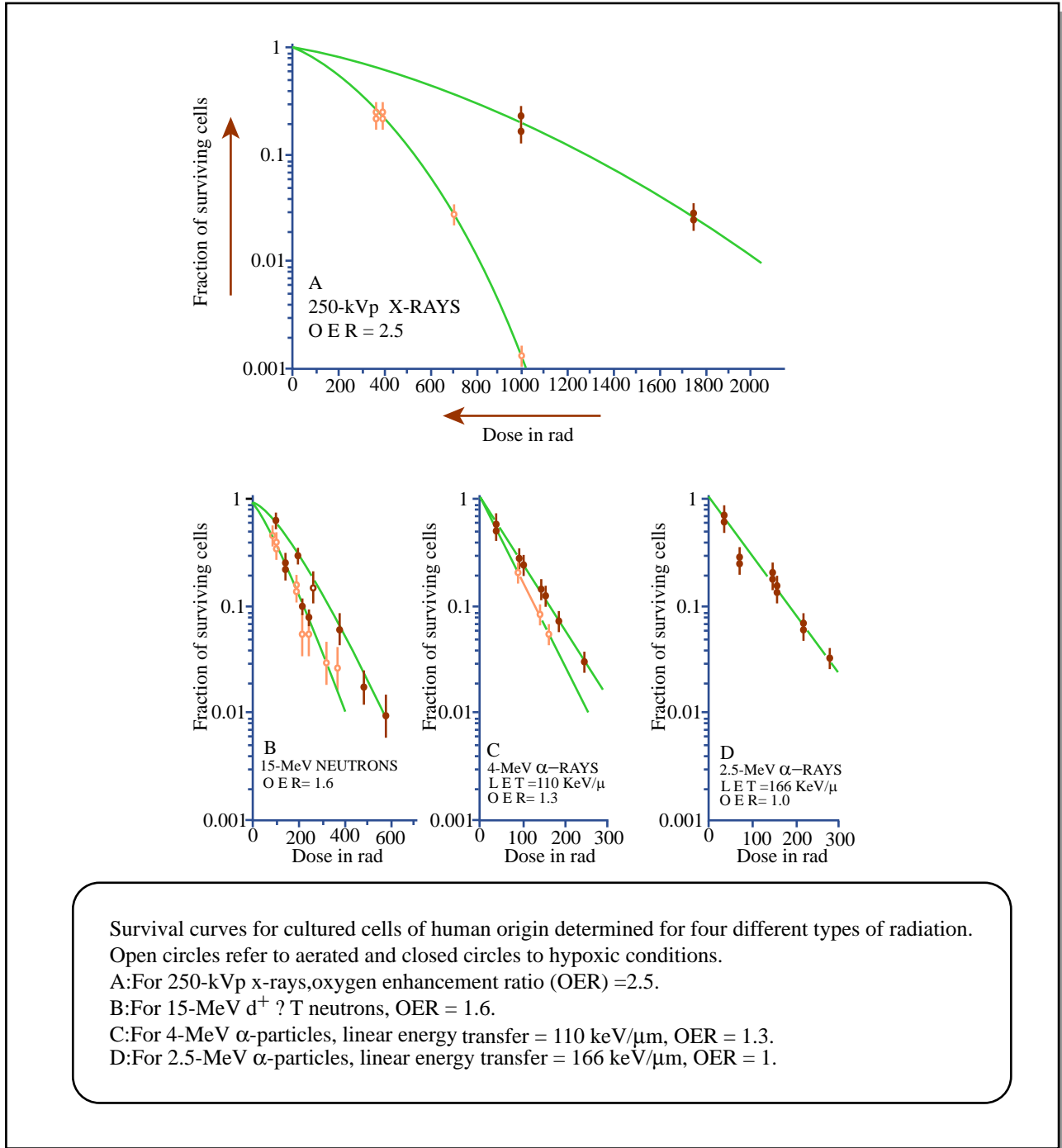


Figure by MIT OCW.

Significant implications for tumor therapy...

OER vs LET

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Fig. 7.8, 7.9 in [Hall].

What is the evidence that DNA is the target?

Selectively irradiate the nucleus or the cytoplasm. Results show the nucleus to be much more sensitive than the cytoplasm.

- Polonium needle: alpha particle range $\sim 40\mu\text{m}$.

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Fig. 3.4 in [Hall].

- Microbeams capable of delivering particles (protons or alpha particles) with μm resolution.

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Microbeam Experiments

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Graphs showing that cell survival rates plummet if the nucleus is irradiated, but survival rates are largely unaffected by irradiation of the cytoplasm alone.

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Radioisotope Incorporation

- Incorporation of halogenated base analogues into DNA sensitizes cells.

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Incorporation makes the DNA more susceptible to damage, including radiation damage.

- Radioisotopes incorporated into DNA kill cells much more efficiently than radioisotopes in RNA or in proteins. ^{125}I in the DNA is 200-300 times more effective than ^{125}I in the cytoplasm or on the cell membrane.
- Tritium is particularly useful: emits beta particles of 18 keV, range in tissue is less than 1-2 μm .
 - Tritiated thymidine....labels DNA
 - Tritiated uracillabels RNA
 - Tritiated amino acids to label proteins
 - Tritiated water (uniform distribution) is 1000 times less effective than tritiated thymidine (DNA localization).
 - [^3H]Thd incorporation caused chromosome breaks, correlating with the point of attachment visualized by autoradiography.
- Cells deficient in DNA repair enzymes are generally more radiosensitive.
- Drugs that inhibit DNA repair are sensitizers.

Types of DNA Damage

Radiation can produce a variety of lesions in DNA

- Single strand break
- Double strand break
- Alteration to bases
- Destruction of sugars
- Crosslinks and formation of dimers

But what about the background levels of DNA damage that occur every day from “natural” sources, primarily oxygen and reactive oxygen species?

We have evolved sophisticated DNA damage detection and repair mechanisms to deal with this damage.

Does radiation produce unique damage?

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DNA Strand Breaks

Single strand breaks:

- Can take place at the phosphodiester bond, or at the bond between the base and the sugar.
- A large proportion of the single strand breaks are caused by hydroxyl radicals (OH•). Radical scavenging experiments have demonstrated this.

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Single strand breaks are easily repaired.

Double strand breaks are repaired more slowly and with more errors.

Measured Numbers of Damaged Sites per Cell per Gray		
<i>Type</i>	<i>Yield</i>	<i>Reference</i>
Single-strand breaks	1000	17
8-Hydroxyadenine	700	18
T* (thymine damage)	250	19
Double-strand breaks	40	17
DNA-protein cross-links	150	20

- An X-ray dose of ~1 Gy produces about 1000 single strand breaks and about 40 double strand breaks in a typical mammalian cell.
- This dose causes about 50% cell death.
- DSBs are not necessarily lethal.

Double strand breaks

- Involves breakage of both strands at points less than 3 nucleotides apart.
- Double strand breaks have shown a direct proportionality to radiation dose,
- ...*but NOT to LET.*

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See Holley, W.R., I. S. Mian, S. J. Park, B. Rydberg, and A. Chatterjee. "A Model for Interphase Chromosomes and Evaluation of Radiation-Induced Aberrations" *Radiation Research* 158 (2002) 568-580

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So What Kills Cells?

Chromosome aberrations correlate with dose.

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High LET radiation is more effective than low-LET for production of aberrations.

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Chromosome aberrations correlate with cell death.

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Fig. 3.5 in [Hall].

- Observation of individual cells following irradiation: if a micronucleus was present in the daughter cell, no colony was formed.
- *N.B.*, Aberrations present at mitosis, may be far removed from initial damage.
- Repair, cell cycle, changes in chromatin structure all can affect the results.
- Chromosome aberrations also show the LET dependency with a maximum at about 100 keV/μm. At high LET, the damage could be too severe for survival.

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Chromosome aberrations require a dsb.

The fact that ssbs and dsbs do not show the same LET dependence as other endpoints is unexplained.

Recent work indicates that clustered damage is involved.