The Oxygen Effect;

Re-oxygenation; Linear Energy Transfer; and Relative Biological Effectiveness

(Chapters 6, and 7)

Feb 2017

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Chapter 6 – Lecture Topics

- Nature of the oxygen effect
- Time of occurrence & mechanism
- Required O_2 concentrations
- Chronic and acute hypoxia
- Experimental evidence for hypoxia
- Re-oxygenation
- Time sequence; importance in radiotherapy
- Mechanism of re-oxygenation
- The importance of re-oxygenation in radiotherapy
- Hypoxia and tumor progression

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Modifying the Effect of Radiation

- It is possible to modify radiation impacts:
  - altering dose rate (same total dose)
  - changing time between doses (fractionation)
  - modifying post-irradiation environment
- Previously mentioned O_2 effect
- Was noted early, 1923 on vegetable seeds
  - Correlation bet radiosensetivity and O2 by Petry
- Extended to tumor systems in 1930s
  - Mottram - England

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Oxygen Enhancement Ratio (OER)

- Ratio of hypoxic to aerated doses needed for same biological endpoint
- Oxygen presence (aerated cells) increases radiation effectiveness for cell killing
- Lack of oxygen (hypoxic cells) results in more radioresistant cells
- Cell surviving fractions are lower in the presence of oxygen vs. hypoxic conditions

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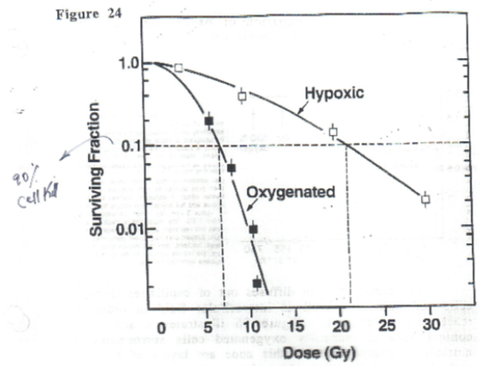
The Concept of OER

Figure 24



a function of radiation dose under **hypoxic** and oxygenated conditions. The hypoxic cells demonstrate marked radioresistance as compared to the oxygenated cells. Note that in this example in order to achieve 90% cell kill (surviving fraction = 0.1) a dose of only 7 Gy is required for **oxygenated** cells whereas a dose of 21 Gy is required to achieve the same level of cell kill under **hypoxic** conditions. The ratio between these two radiation doses necessary to achieve the same level of cellular lethality (hypoxic/oxic) is called the **OXYGEN ENHANCEMENT RATIO (OER)**. In this example OER = 21/7 = 3.0. For sparsely ionizing (**low LET**) radiations such as X- & gamma-rays, the OER at high doses typically lies between **2.5** and **3.0**. The OER for densely ionizing (**high LET**) radiations such as alpha-particles is **1.0** (there is no significant oxygen effect). The OER for fast neutrons has an intermediate value.

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OER

- OER varies from 2-3, increasing with dose
- For low-LET radiations
  - oxygen effect is more pronounced
- For high-LET radiations
  - oxygen effect is non-existent (OER = 1)
- Why?

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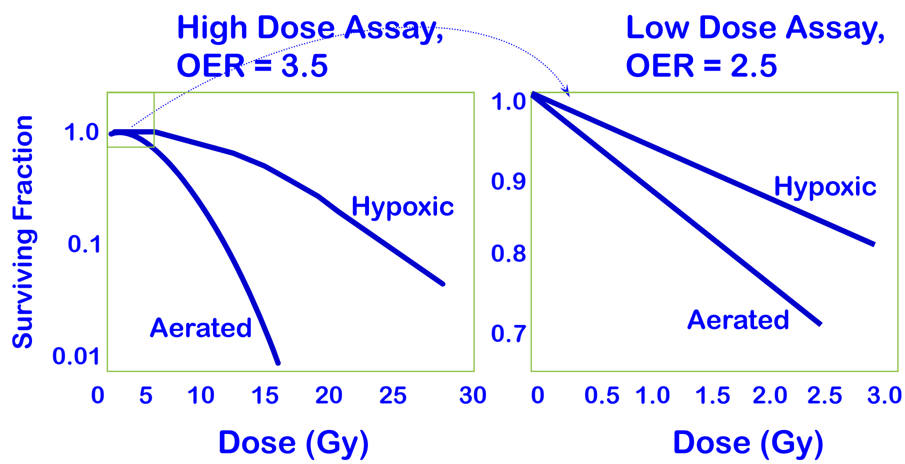
Nature of the Oxygen Effect

Low-LET radiation



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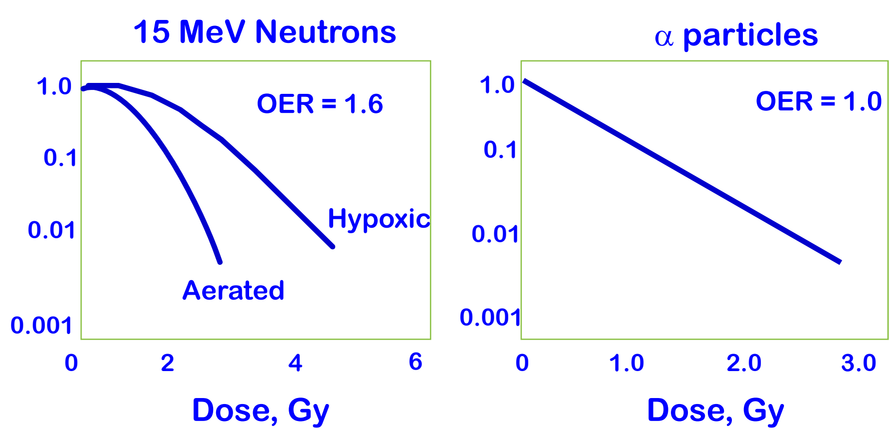
Other Radiations and the OER

High-LET radiation



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OER

- For Oxygen effect to be observed, O_2 is required either
  - during irradiation, or
  - soon (*very!*) after irradiation
    - accelerator pulse of e^- followed by “exploding” O_2
    - O_2 required within a few μs after exposure

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OER, timing

- Sophisticated experiments have been performed in which oxygen, contained in a chamber at high pressure, was allowed to "explode" onto a single layer of bacteria (and later mammalian cells) at various times before or after irradiation with a 2-μS electron pulse from a linear accelerator.
- It was found that oxygen need not be present during the irradiation to sensitize but could be added *afterward,* provided the delay was not too long.
- Some sensitization occurred with oxygen added as late as 5 ms after irradiation.

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Mechanism of Oxygen Enhancement

- Radiochemistry of radiation effect:
  - radiation absorption --> energetic (i.e. fast) charged particle --> ion pair created
  - $T_{1/2}$ of ion pair is short (10^{-10} sec)
  - ion pair produces free radical: OH•
  - $T_{1/2}$ of free radical is short (10^{-5} sec)
  - ion pair --> indirect effect --> break bonds --> chemical changes

"Oxygen Fixation Hypothesis"

—CH_3 (functional group) —ion pairs / free radicals→ —$CH_2^{\bullet}$ (free radical, unpaired electron)

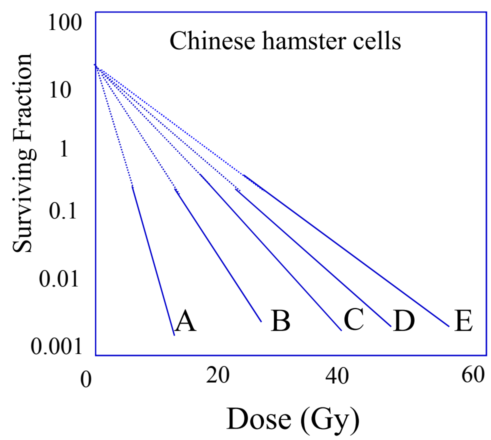
Generally, the free-radical reactions go like this:

—$CH_2^{\bullet} + O_2 \longrightarrow CH_2O_2$ an organic peroxide "fixes" the indirect damage

(oxygen has no impact on <u>direct</u> damage)

O_2 Concentration Effects

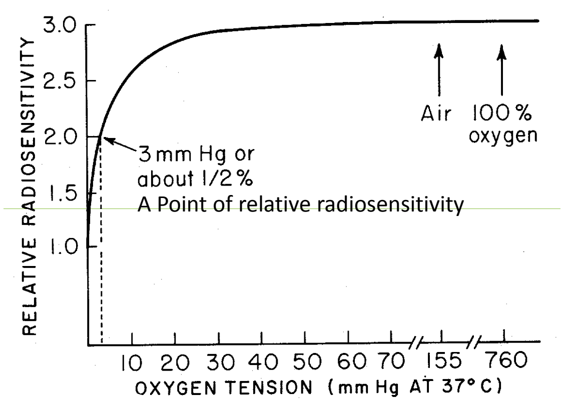
- Bacteria and mammalian systems show similar effects
- A: 210,000 ppm (air)
- B: 2200 ppm O_2
- C: 355 ppm O_2
- D: 100 ppm O_2
- E: 10 ppm O_2



Photon Irradiation

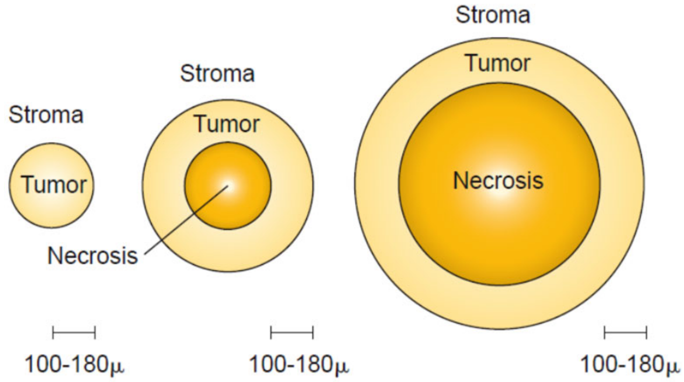
Radiosensitivity and O_2 Concentration

- O_2 levels
  - 20% normal
  - 16% dizziness
  - 10% immediate unconsciousness
- 2-4% is ~ plateau
- ~ 0.5% oxygen doubles effect
- Doesn't take much for impact



If radiosensitivity is given a value of 1 for anoxic, it is about 3 for aerated. Most of this change happens from 0-30 mm-HG of O2. A relative radio-sensitivity happens half way at about 3 mm-hg, which is about 0.5% O2 concentration.

Thomlinson & Gray (1955)



The conclusions reached by Thomlinson and Gray from a study of histologic sections of human bronchial carcinoma. No necrosis was seen in small tumor cords with a radius of less than about 160 μm. No tumor cord with a radius exceeding 200 μm was without a necrotic center. As the diameter of the necrotic area increased, the thickness of the sheath of viable tumor cells remained essentially constant at 100 to 180 μm.

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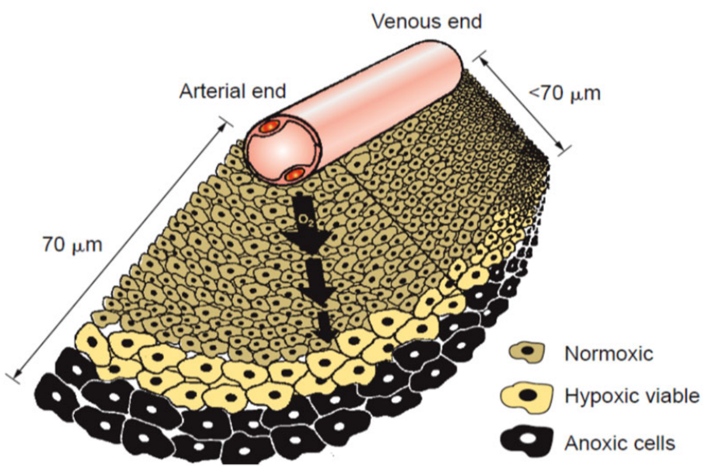
Hypoxia and Radiosensitivity

Diffusion of oxygen through a capillary in tumor tissue



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Two Conditions of Hypoxia

- Chronic hypoxia
  - limited diffusion distance of O_2 through respiring tissues
  - tumors may outgrow blood supply, have O_2 starved regions
- Acute hypoxia
  - Develops when blood vessels has blockage or are temporarily shut down.
  - There is evidence that tumor blood vessels open and close randomly. So this condition occurs when there is transient fluctuations in blood flow due to malformed vasculature.

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Acute and Chronic Hypoxia in Tissue

Difference bet chronic and acute hypoxia is shown…

Chronic results from limited diffusion distance of O2 respiring tissue.

Acute results from temporary closing of tumor blood vessels.

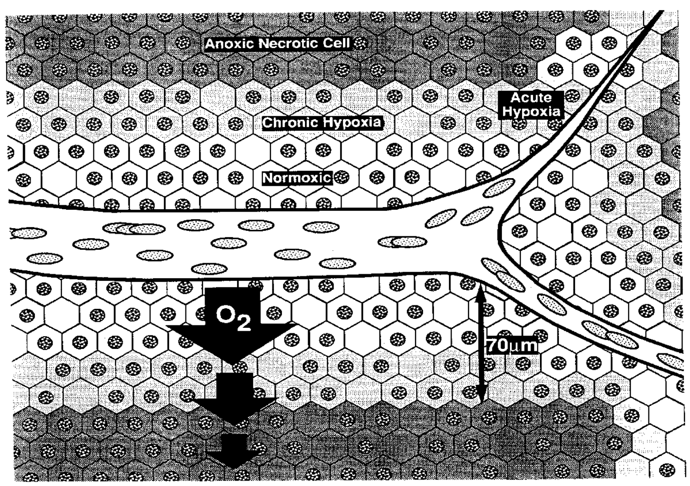

Figure 8-9. Diagram illustrating the difference between *chronic* and *acute* hypoxia. *Chronic hypoxia* results from the limited diffusion distance of oxygen in respiring tissue that is actively using up oxygen. Cells that become hypoxic in this way remain hypoxic for long periods of time until they die and become necrotic. *Acute hypoxia* results from the temporary closing of tumor blood vessels. The cells are intermittently hypoxic since normoxia is restored each time the blood vessel opens up again. (Redrawn from Brown JM: JNCI 82, 1990)

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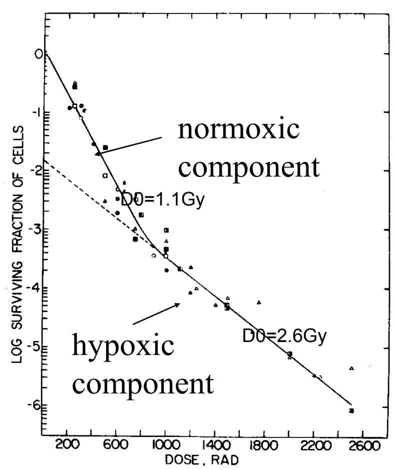
Solid Tumors

- As tumor grows, necrotic center expands
- Thickness of healthy sheath remains constant
- Estimated diffusion distance of O_2 in respiring tissue is 70µm
- In 1950s, radiobiologists focused on
  - O_2 and effect of tumor cell killing
  - alternative treatments with high-LET particles
    - no O_2 effect

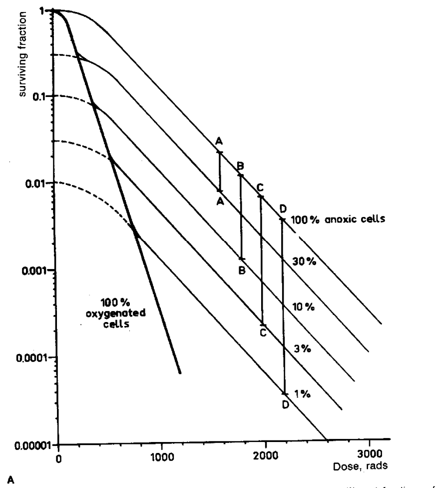
Solid Subcutaneous Lymphosarcoma



- Two-component curve
- Extrapolating back:
  - Surviving Fraction ~ 1%; implies 99% of cells were well oxygenated
  - low-dose response: killing of aerated cells
  - begin killing hypoxic cells >9 Gy
- Solid tumor has protected hypoxic cells (i.e., clonogenic)

Fraction of surviving cells as a fn of dose for a solid lymphosarcoma in the mouse irradiated in-vivo. Different slopes indicate that these cells are hypoxic

Hypoxic Cell Fraction in Tumors

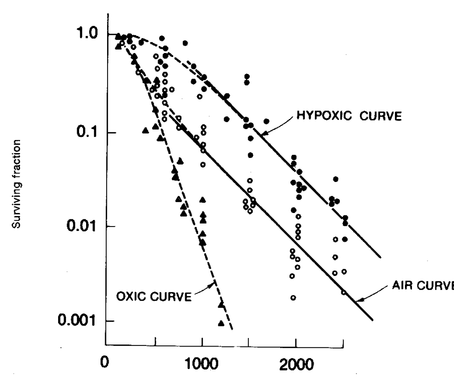


- Paired survival curves
- Distance between SC's indicates % of hypoxia
- This is a theoretical determination; what does real data look like?

Theoretical survival curves for cell populations containing different fractions of hypoxic cells. The fraction of hypoxic cells in each population determines the distance between its survival curve and the curve for the completely hypoxic population.

Hypoxic Cell Fraction in Mouse Tumor

- Air curve
  - mice breathing air
  - mixture of hypoxic and aerated cells
- Hypoxic curve
  - mice asphyxiated by breathing N_2 or cells irradiated *in vitro* in N_2
- Oxic curve
  - cells *in vitro* in O_2



Reoxygenation

- During fractionated treatments, oxygen status varies in cells and is dynamic
- As cells die, hypoxic cells within the tumor obtain more oxygen to improve their oxygen status
  - these cells have an increased OER which makes the next dose fraction more effective

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Reoxygenation

- Mouse sarcoma:
  - 14% hypoxic cells, initially
  - 5 dose fractions, 1.9 Gy/day
  - 3 days later, 18% hypoxic cells
- Similar experiment:
  - 4 fractions over 4 days
  - next day, 14% hypoxic cells
- <u>Fraction</u> of hypoxic cells essentially unchanged by therapy

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Reoxygenation

- What's going on?
- Treatment kills oxygenated tumor cells
- Hypoxic ones become oxygenated
- This is good for therapy!
  - oxygenated cells are more radiosensitive
- Reoxygenation:
  - hypoxic cells reoxygenate after radiation therapy

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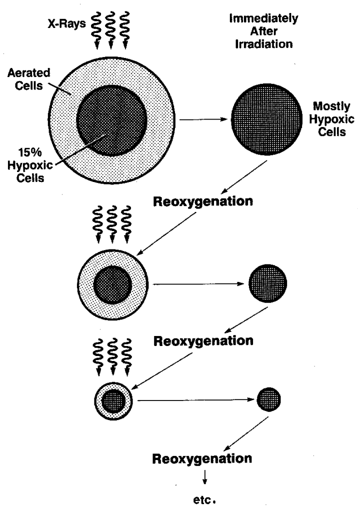
Process of Reoxygenation



- If reoxygenation occurs, the presence of hypoxic cells does not significantly impact the outcome of the multi-fraction dose regime

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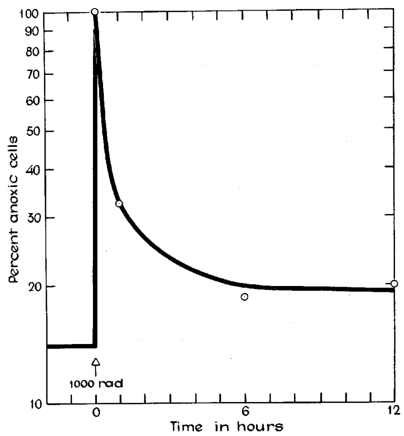
Percentage of Hypoxic Cells



- Kallman and Bleehen experiment: Transplantable mouse sarcoma
- Initially, all aerated cells are killed (10 Gy)
- Rapid reoxygenation
- 6 hours, back to near pre-irradiation levels
- Similar results in other tumor systems

Percentage of hypoxic cells in a transplantable mouse sarcoma as a function of time after a dose of 10 Gy of x-rays. Immediately after irradiation, essentially 100% of the viable cells are hypoxic because such a dose kills a large proportion of the aerated cells. In this tumor, the process of reoxygenation is very rapid. By 6 hours after irradiation, the percentage of hypoxic cells has fallen to a value close to the pre-irradiation level.

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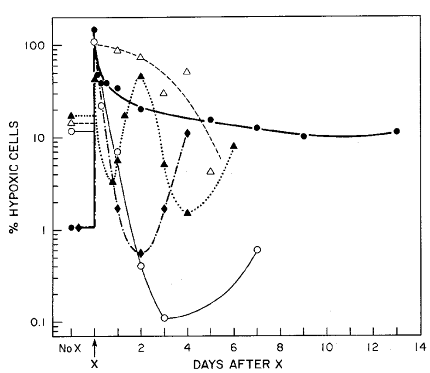
Hypoxic Cells Post-Irradiation



- Sequence for reoxygenation varies with the tumor type
  - mouse osteosarcoma: Δ
  - mouse fibrosarcoma: ●
  - mouse fibrosarcoma: ♦
  - mouse mammary carcinoma: O
  - rat sarcoma (2 waves): ▲
- Extent & rapidity of reoxygenation is extremely variable

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Significance for Radiotherapy

- The presence of O_2 enhances cell killing
- Tumors (animal) include both aerated and hypoxic cells
- Hypoxia confers protection from
  - x-rays (low/moderate LET radiations) and certain chemotherapeutic agents
  - i.e., agents involving free radical mechanisms

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Significance

- Reoxygenation pattern not well known for many systems
  - mouse mammary carcinoma
  - two-days post irradiation
    - proportion of hypoxic cells *lower* than untreated tumor
  - large x-ray doses @ 48 hr intervals would eliminate all hypoxic cells
  - requires in-depth knowledge of tumor reoxygenation patterns

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Significance

- If *human,* tumors reoxygenate quickly
  - multi-fraction therapy could deal with "resistant" subpopulations
  - dosing at later intervals would maximize cell killing
- Human tumor data not available
  - reoxygenation suggested from multi-fraction therapies
  - 60 Gy in 30 treatments eradicates many tumors where "cure" not expected

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Summary

- O_2 content influences impact of low-LET radiations
- OER is the ratio of hypoxic-to-aerated doses
- OER for photons is about 3 at high doses, decreasing at lower doses
- OER decreases as LET increases
- Oxygen must be present during irradiation, or very soon after (microseconds)
- Only a small amount of O_2 is required (< 4%)

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Summary

- Two forms of hypoxia: acute and chronic
- Reoxygenation
  - process by which cells that are hypoxic become oxygenated
  - currently unknown in (most) human tumors
  - tumors that do not respond to radiotherapy may be those that do not reoxygenate

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Linear Energy Transfer (LET), & Relative Biological Effectiveness (RBE)

(Chapter 7)

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Chapter 7 - Lecture Topics

- Linear energy transfer (LET)
- Relative biological effectiveness (RBE)
- RBE and fractionated doses
- RBE in different cells and tissues
- RBE as a function of LET
- Optimal LET and factors that determine RBE
- The Oxygen effect and LET
- Quality factors & radiation weighting factors

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Energy Deposition

- Low-LET (sparsely ionizing radiation)
  - x-rays
  - gamma
  - betas (higher energy)
- High-LET (densely ionizing radiation)
  - alphas
  - betas (lower energy)
  - protons
  - neutrons

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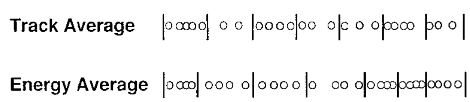
Linear Energy Transfer (LET)

- LET is the average *energy* locally imparted (deposited) per unit track length (keV/μm)
- Different than "stopping power" (energy loss)
- Track averaged vs energy averaged



LET can be averaged in many ways:
TA=Divide track into equal length, then calc the energy deposited in each length and find the mean.
EA=divide track into equal energy increment & avg length of track over which these energy increments are deposited.

Some typical values

RADIATION	LET (keV/μm)	
Cobalt-60 γ-rays	0.2	
250-kV x-rays	2.0	
10-MeV protons	4.7	
150-MeV protons	0.5	
	Track Avg.	Energy Avg.
14-MeV neutrons	12	100
2.5-MeV α-particles	166	
2-GeV Fe ions	1000	

This method makes little diff for x and gamma rays or for mono energetic charged particles but big diff for neutrons.

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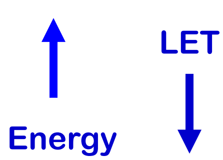
LET of Charged Particles



Particle	Mass (amu)	Charge	Energy (keV)	Average LET (keV/μm)[a]	Tissue Penetration (μm)
Electron	0.00055	-1	1	12.3	0.01
			10	2.3	1
			100	0.42	180
			1,000	0.25	4,000 (0.4 cm)
Proton	1	+1	100	90	3
			2,000	16	80
			5,000	8	350
			10,000	4	1,400
Alpha	4	+2	100	260	1
			5,000	95	35 (.0035 cm)
			200,000	5	200,000

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Photon Energy-Deposition Paths

- Closest in shape and structure to those of betas
- Distance between interactions is often orders of magnitude greater
- Photons are much more penetrating than charged particles

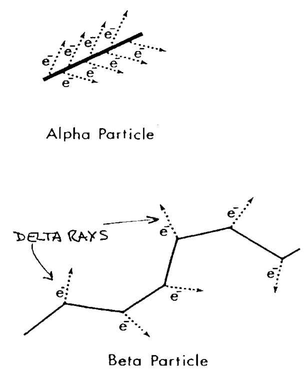
LET of Photons

- LET of photons tends to increase with energy
  - very high energies are an exception

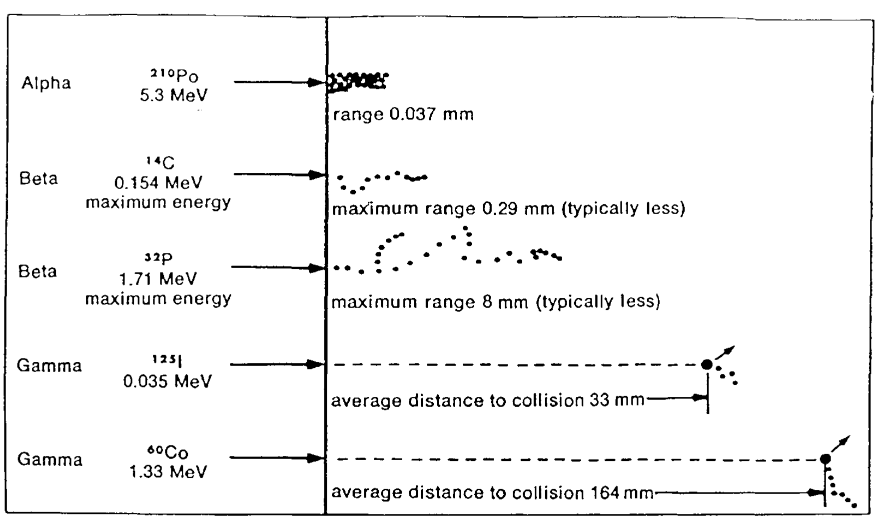
Rays	Energy (MeV)	Average LET (keV/μm) x(10^{-3})[b]
x, gamma	0.080	1.0
	0.120	1.4
	0.140	1.5
	0.511	3.5
	1.000	5.2

Energy Deposition Paths for Alphas and Betas

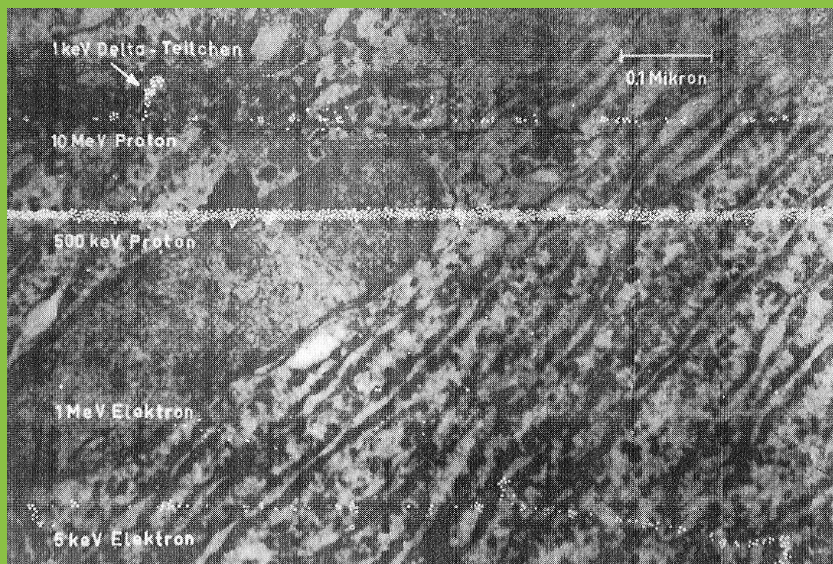
- Alpha paths are generally straight with very concentrated energy deposition
- Beta paths are very random, energy deposition interactions are more dispersed



Typical Energy Deposition Paths for Various Radiations



Variation of ionization density associated with different types of radiation on the background which is an e- micrograph of a human cell. White dots are computer simulation representing ionizations.

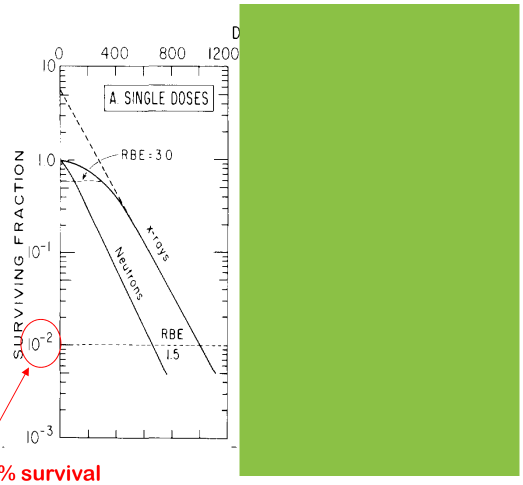


Relative Biological Effectiveness

- Relates biological effect to a "standard"
  - needed because equal energy deposition events (doses) from different radiations do not produce equal effects in biological systems
- Definition:
  - RBE is defined as the ratio of the standard dose to the test dose required for equal biological effect
  - <u>2 standards</u>: 250 kVp x rays; ^{60}Co γ rays
  - for example:
    - LD_{50} for 250 kVp x-rays = 6 Gy (the standard)
    - LD_{50} for 2 MeV neutrons = 3 Gy (the test radiation)
    - thus, the RBE for 2 MeV neutrons is 2

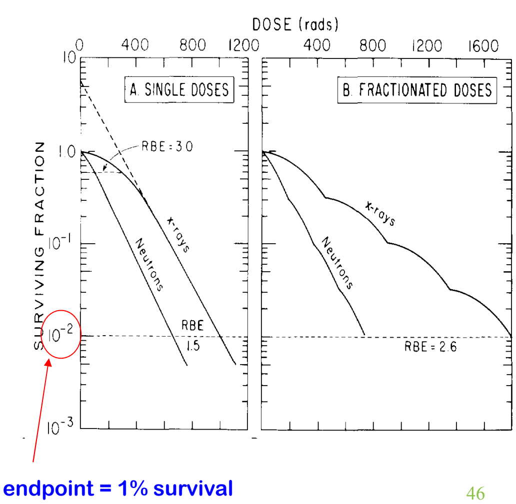
RBE and Fractionated Doses

- What happens to the RBE for neutrons when the dose is fractionated?



RBE and Fractionated Doses

- **Fractionating the dose increases the RBE for neutrons, <u>not</u> because it increases the damage done by neutrons, but because it <u>decreases</u> the effect of x-rays**



RBE for Different Cells/Tissues

- RBE also varies depending on tissue type and biological endpoint
- Cells having a photon survival curve with a large shoulder, indicating that they can incur and repair a large amount of sublethal radiation damage, show a large RBE for neutrons
- Cells having a small shoulder in their photon survival curve have small neutron RBE values
- Photon response impacts neutron RBE

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Variability in RBE

- RBE depends on many more factors:
  - radiation quality
  - biological endpoint
  - biological system
  - choice of radiation "standard"
  - radiation dose and dose rate
  - number of dose fractions (& dose per fraction)

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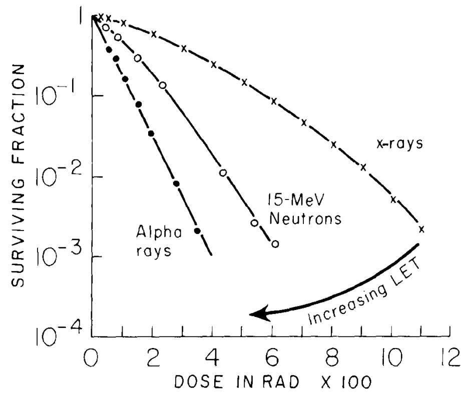
How is RBE related to LET?

- As LET increases, the survival curve slope increases and initial shoulder decreases
- RBE increases with LET up to about ~100 keV/μm



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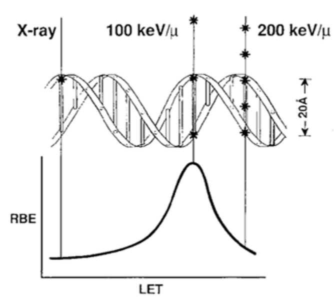
The Optimal LET

- At ~100 keV/μm (~5 MeV α)
  - greatest RBE; producing most biological effect per unit dose
  - separation between ionizing events ~ the diameter of DNA double helix
  - highest probability of double strand break per unit dose
- More densely ionizing radiation is just as effective per track length, but less effective per unit dose
  - sometimes referred to as "overkill"



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Oxygen Enhancement Ratio (OER)

- Recall from previous chapter that,
  - when repair of single-strand breaks is significant, cells are more sensitive in the presence of oxygen
  - molecular oxygen in a cell at the time of free-radical production 'interferes' with the repair process (see next page)
  - the OER is the ratio of doses without and with oxygen present in the cell to produce the same biological effect
  - the OER decreases as LET increases

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"Oxygen Fixation Hypothesis"

$—CH_3$ (functional group) $\xrightarrow[\text{free radicals}]{\text{ion pairs}}$ $—CH_2^{\bullet}$ (free radical, unpaired electron)

Generally, the free-radical reactions go like this:

$—CH_2^{\bullet} + O_2 \longrightarrow CH_2O_2$ an organic peroxide "fixes" the indirect damage

(oxygen has no impact on <u>direct</u> damage)

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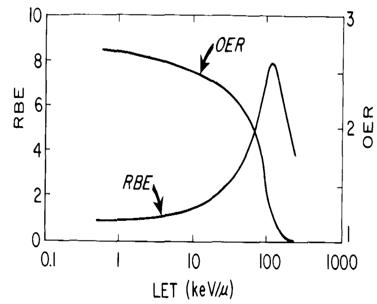
LET, RBE and the Oxygen Effect

- The OER has a value of 2-3 for low-LET radiations
- Decreases with increasing LET above ~30 keV/μm, and reaches unity by an LET of ~160 keV/μm
- As the OER declines, RBE increases until an LET of ~100 keV/μm is reached
- Demonstrates repair process is not significant at higher LET



Radiation Weighting Factor, W_R

- RBE is too specific for use in radiation protection
- Considering differences in biological effectiveness for different radiations, the RBE concept is simplified by using the radiation weighting factor (W_R)
- Very similar to quality factor (QF), with slight exception (average vs point estimate)
- ICRP publishes values for radiation weighting factors

1	**Photons**
1	**Electrons**
5	**Protons**
5-20	**Neutrons**
20	**Alphas**

Summary

- X & γ rays: sparsely ionizing radiations
- α particles, protons, and neutrons: densly ionizing radiation
- Definition of LET & RBE
- RBE ⇧ with LET to a max of ~100keV/μm, thereafter decreases with LET

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Summary

- RBE depends on:
  - Radiation quality (LET)
  - Radiation Dose
  - # of Fx's
  - Dose Rate
  - Biologic system or end point
- The OER =3 for low LET radiation, falls when LET rises to > ~30 keV/μm and reaches unity when LET ~200 keV/μm

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