Radiation Cell Killing

Radiation Cell Killing

- For cells proliferating *in vitro*, define cell death as loss of ***reproductive ability***
- Refers to cell losing its ability to exhibit unlimited cell division
- Clonogenic cell: cell that has reproductive ability, can divide indefinitely to produce a large colony or clone

Radiation Cell Killing

- *In vivo*, predominant form of cell death following irradiation occurs at mitosis, requires a dose of ~ 2 Gy
- Irradiating non-dividing or rarely dividing cells with very high doses, ~ 100 Gy can cause loss of cell function and death, i.e., ***Interphase Death***
- Apoptosis or programmed cell death: involves programmed sequence of events controlled by specific genes. Can occur at low doses of radiation

Construction of an *in vitro* cell survival curve

General Technique

- Add trypsin (enzyme) to a flask containing the cells. Cells will round up and detach from the surface of the flask
- Count the number of cells/mL (manually can use a hemocytometer; electronically can use a Coulter Counter)

Construction of an *in vitro* cell survival curve

- Add a known number of cells to a new flask; incubate for 1-2 weeks
- Each single cell will divide several times to form colonies that are fixed, stained and counted
- Count colonies containing $\geq$ 50 cells (5-6 generations of proliferation) to exclude cells that have limited growth as a result of starting to differentiate or being sub-lethally damaged

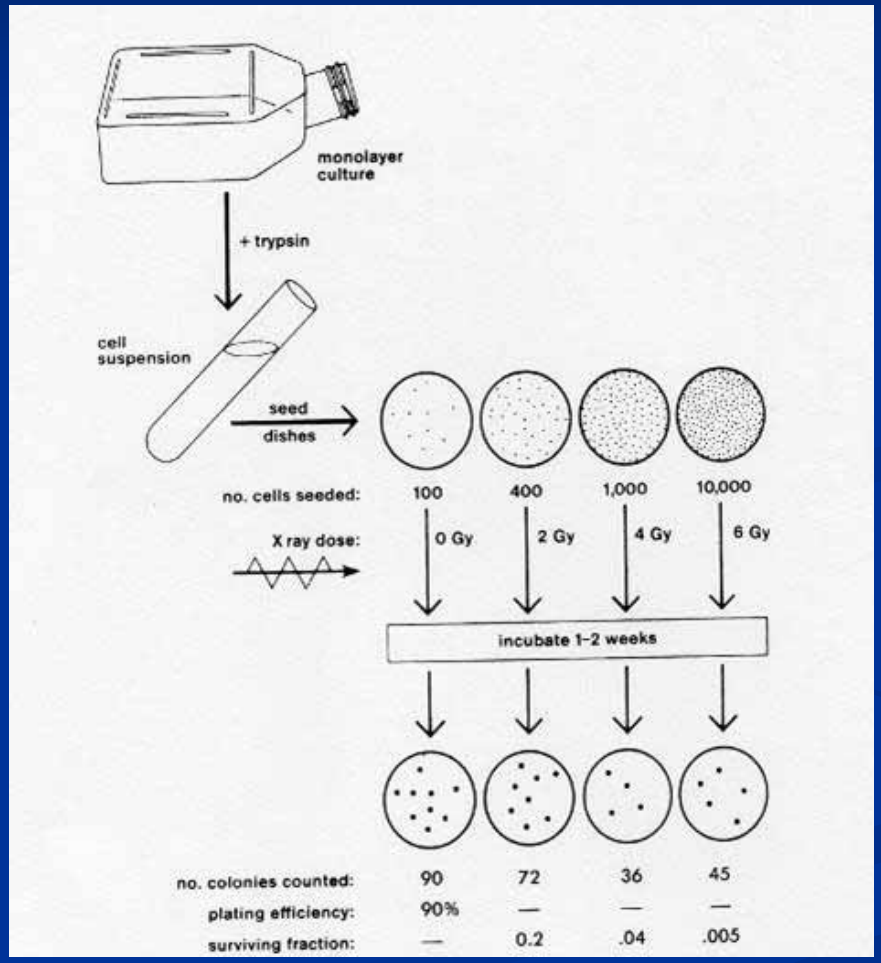
Plating Efficiency

- Not all cells plated out will form colonies
- Inability reflects suboptimal growth medium; errors in cell counting; damage to cells during trypsinization
- Need to determine the Plating Efficiency (PE)

Plating Efficiency

$$PE = \frac{\text{Mean number of colonies/dish}}{\text{Number of cells plated/dish}}$$

Construction of an *in vitro* cell survival curve



Hall, Radiobiology for the Radiologist

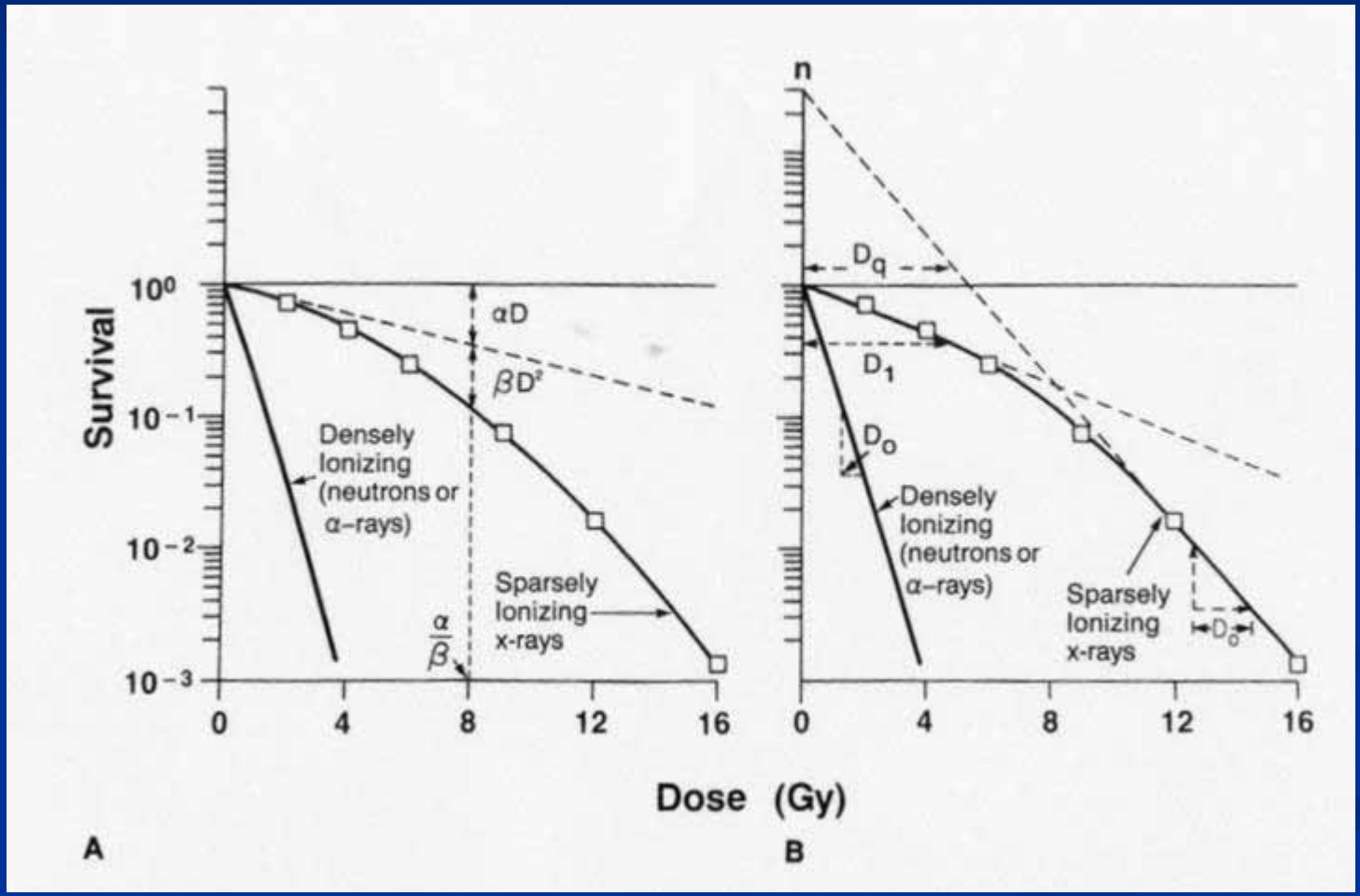
Cell Survival Curve

To construct a cell survival curve use a range of doses and determine the surviving fraction, SF, after each dose

$$\textbf{SF after dose D} = \frac{\textbf{mean number of colonies after dose D/dish}}{\textbf{mean number of cells plated/dish x PE}}$$

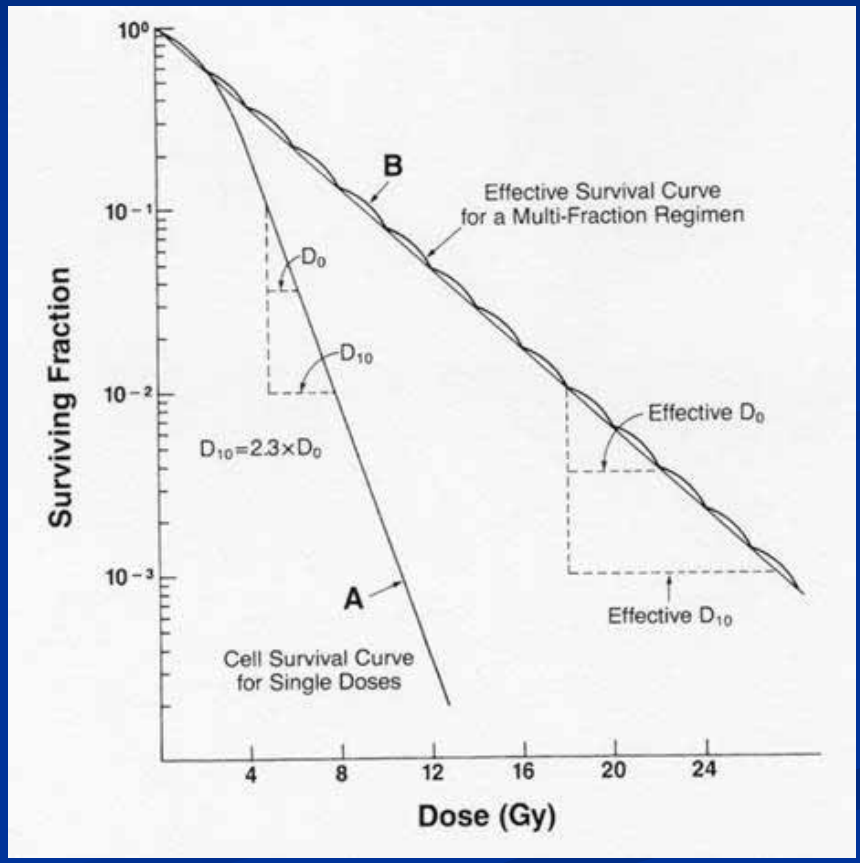
Number of cells seeded per dish needs to be adjusted so that a countable number of colonies is obtained

Mammalian Cell Survival Curves



Effective Cell Survival Curves

- If radiation dose is delivered in a series of equal fractions (F), separated by a time interval that allows complete SLD repair, the effective dose survival curve becomes an exponential function of dose
- Shoulder of the survival curve is repeated many times; the effective survival curve is a straight line from the origin through point on the single-dose survival curve corresponding to the daily dose F
- D_0 (the reciprocal of the slope), has a value close to 3 Gy for human cells.



Radiation Cell Killing

For calculations, useful to use the D_{10}

Dose required to kill 90% of population

$$\mathbf{D_{10} = 2.3 \times D_0}$$

where 2.3 is the natural log of 10

Radiation Cell Killing

Tumor contains 10^9 cells. Effective dose-response curve has no shoulder, $D_0 = 3$Gy

What total dose is required to give 90% chance of tumor cure?

90% probability of tumor control requires 10 decades of cell kill

Dose resulting in one decade of cell kill, D_{10},

$= 2.3 \times D_0 = 2.3 \times 3 = 6.9$ Gy

Therefore, total dose for 10 decades of cell kill

$= 10 \times 6.9 = 69$ Gy

Repair of Radiation Damage

In mammalian cells consider 3 types of radiation damage:

- Lethal damage
- Sublethal damage
- Potentially lethal damage

Lethal Damage

- Irreversible and irreparable
- Leads to cell death

Potentially Lethal Damage

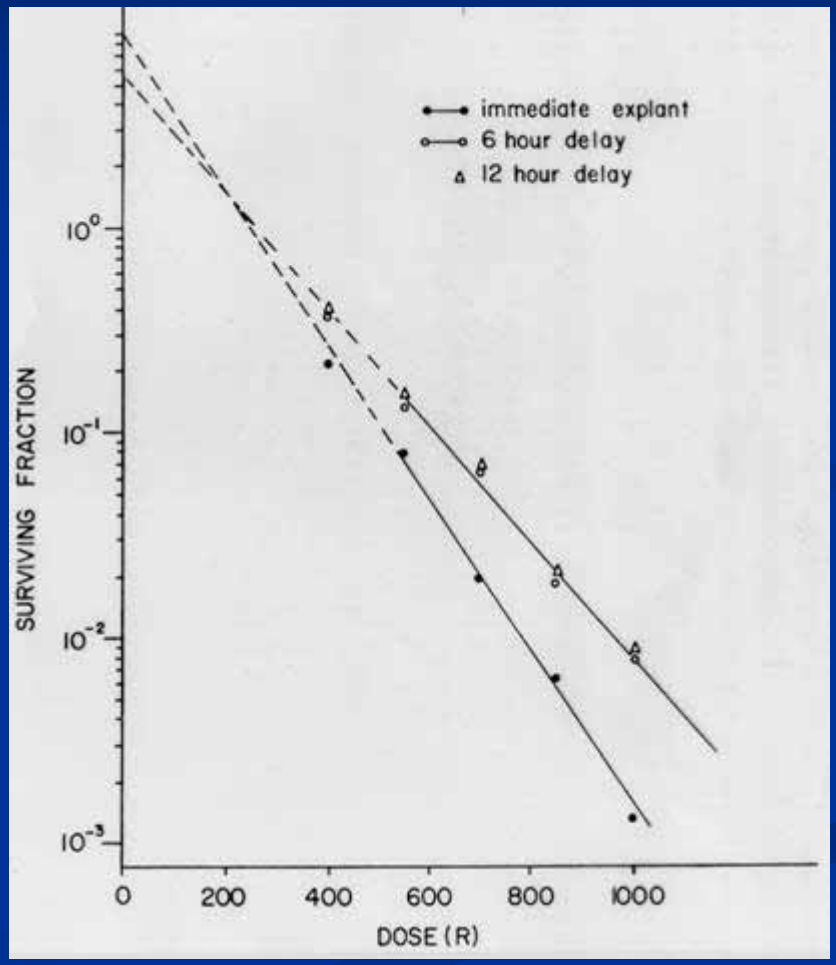
- Component of radiation damage that can be modified by postirradiation environmental conditions

Potentially Lethal Damage

- Varying environmental conditions after exposing cells to X-rays can influence proportion of cells that survive a given dose due to the expression or repair of PLD
- Damage considered to be potentially lethal since under ordinary circumstances leads to cell death
- However, if survival is increased following manipulation of the postirradiation environment, PLD is considered to have been repaired

Potentially Lethal Damage

- X-ray survival curves for density-inhibited stationary-phase cells, subcultured either immediately or 6-12 h after irradiation (Little *et al* Radiol 106:689-94, 1973)



Potentially Lethal Damage

Mechanism

- Cells maintained in sub-optimal conditions do not have to attempt mitosis while chromosomes are expressing radiation-induced injury

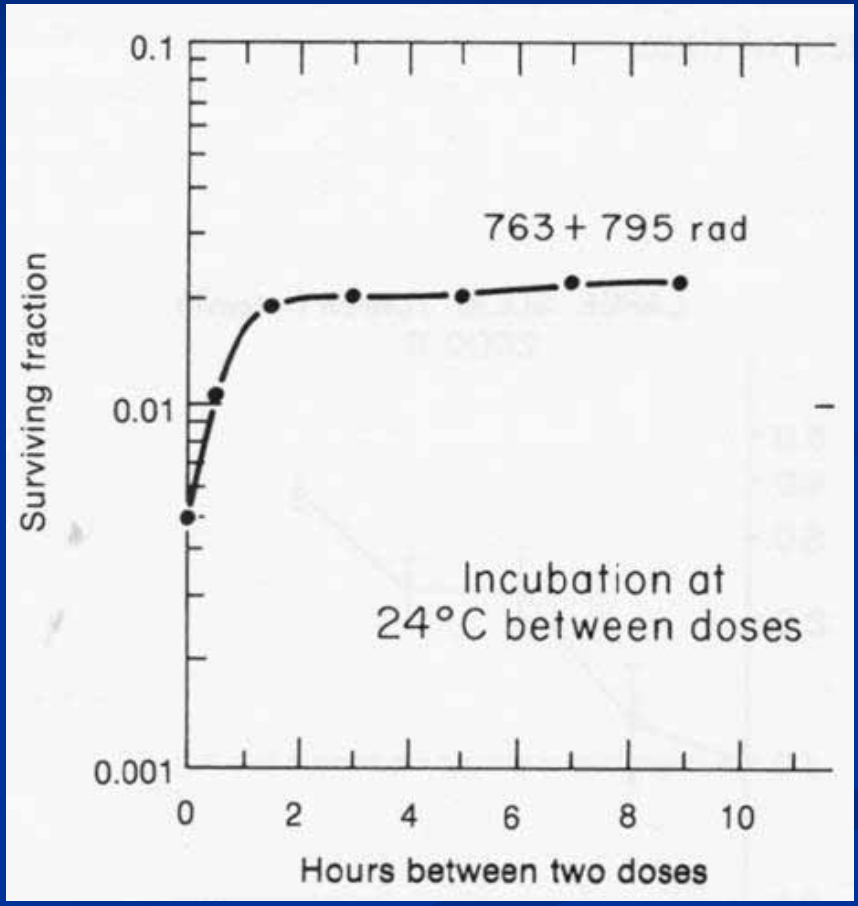
- Delay leads to repair of the DNA damage and increased survival. Relevance to clinical RT remains questionable

Sublethal Damage

- Under normal circumstances can be repaired in hours, usually considered to be complete within 24 h
- If additional sublethal damage added within this time then can interact to form lethal damage
- Sublethal damage repair observed as an increase in survival if a dose of radiation is split into 2 equal fractions separated by a time interval

Sublethal Damage Repair

- Term used to describe the increase in cell survival seen if a given radiation dose is split into 2 equal fractions separated by a time interval.

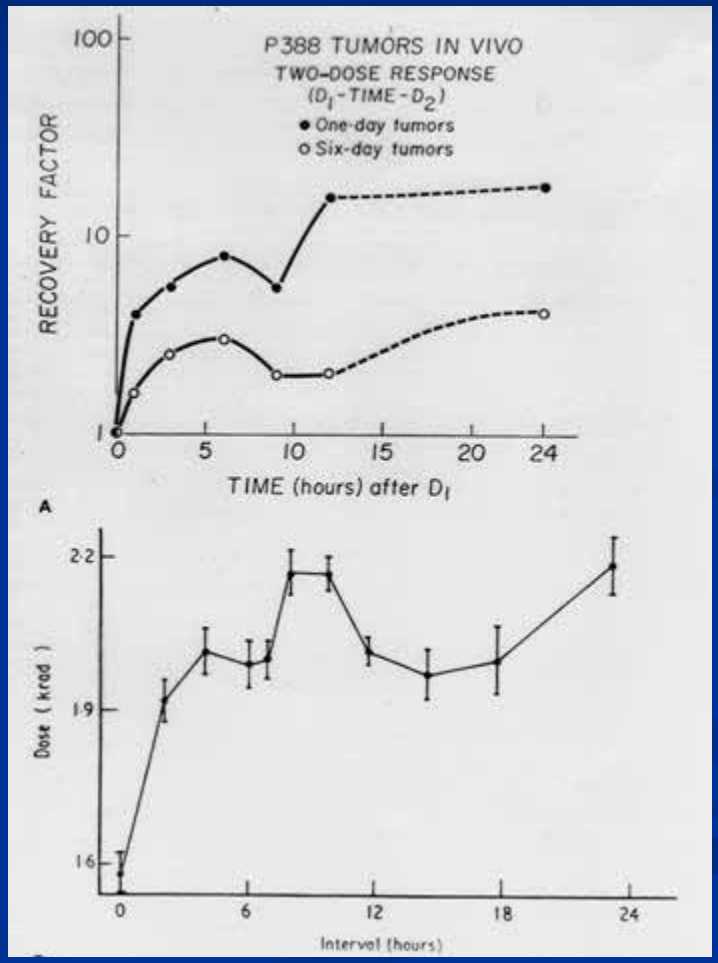


Sublethal Damage Repair

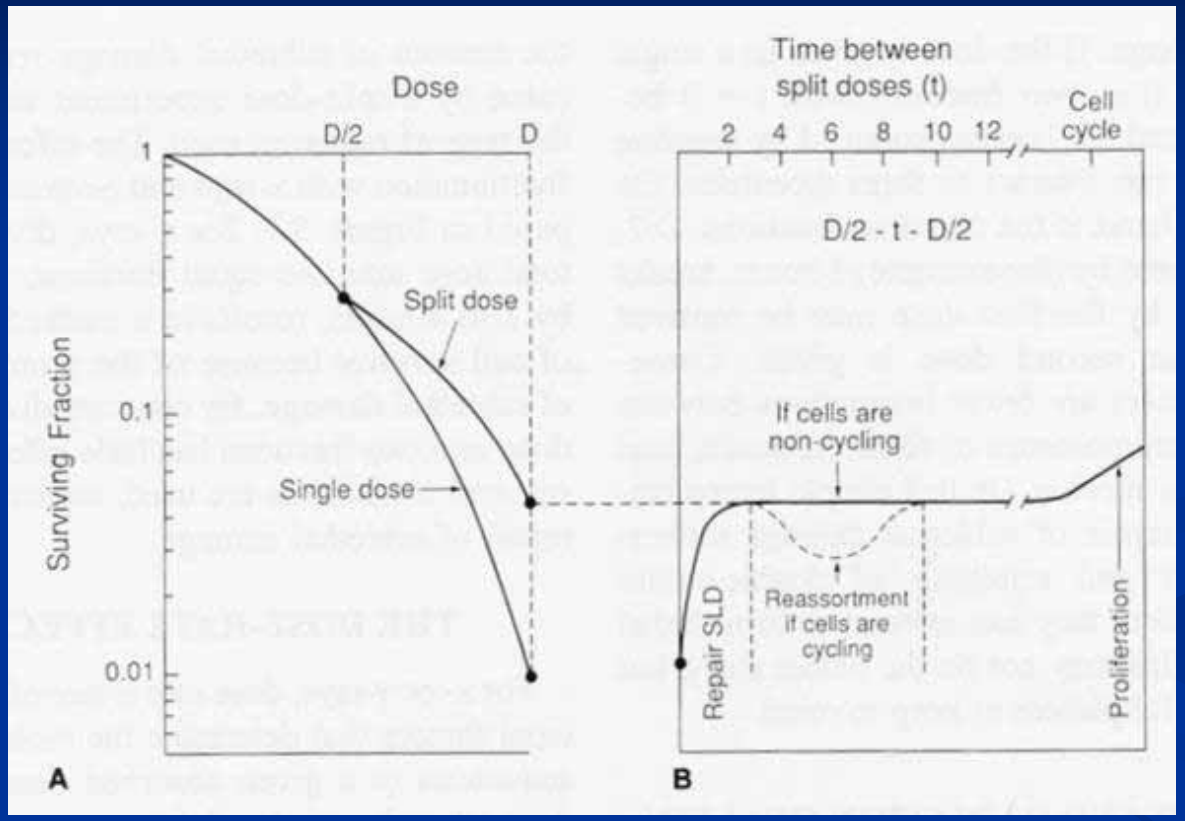
Repair of SLD in 2 *in vivo* mammalian cell systems.

A: Split-dose experiments with P388 lymphocytic leukemia cell in the mouse. One-day tumors contain mainly oxic cells; 6-day old tumors contain hypoxic cells (Belli *et al* JNCI 38:673-82, 1967).

B: Split-dose experiments with skin epithelial cells in the mouse (Emery *et al* Radiat Res 41:450, 1970).



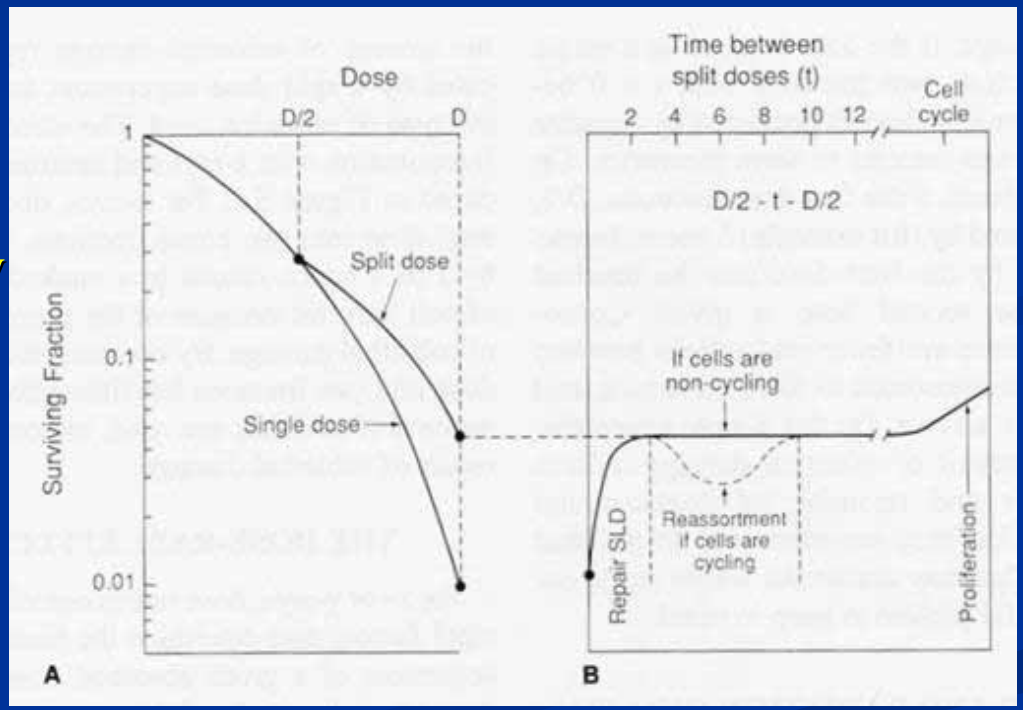
Sublethal Damage Repair



If dose is split into 2 fractions separated by a time interval more cells survive than for the same total dose given in a single fraction, because the shoulder of the curve must be repeated each time.

Sublethal Damage Repair

- As time interval between 2 F increases see rapid increase in SF, usually complete within 2 h in culture but longer *in vivo*, particularly for some late-responding tissues
- As time interval increases may see dip in SF due to movement of surviving cells through the cell cycle; only observed in cycling cells
- If time interval exceeds the cell cycle, see increase in SF due to proliferation.



Repair and Radiation Quality

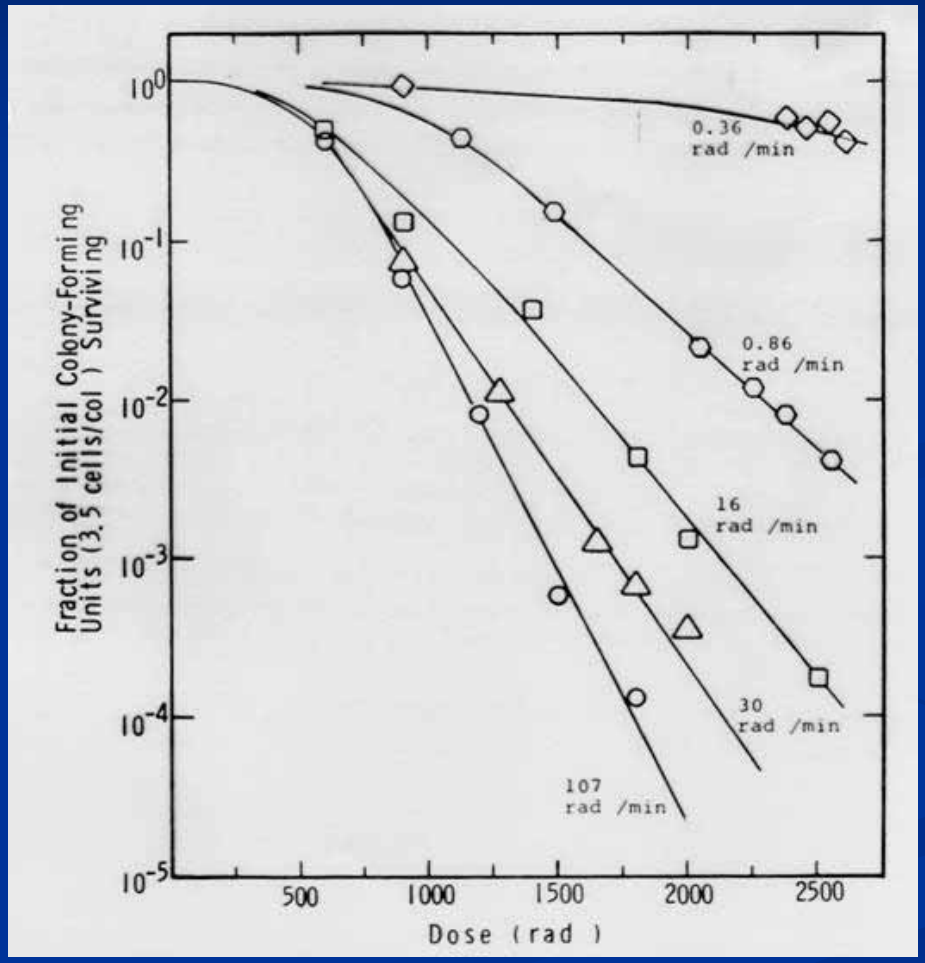
- Since the presence of a shoulder on a cell survival curve is dependent on the quality of radiation used, the amount of SLD repair is similarly dependent on the quality of radiation

- High LET radiation, e.g., neutrons, is associated with little repair of SLD

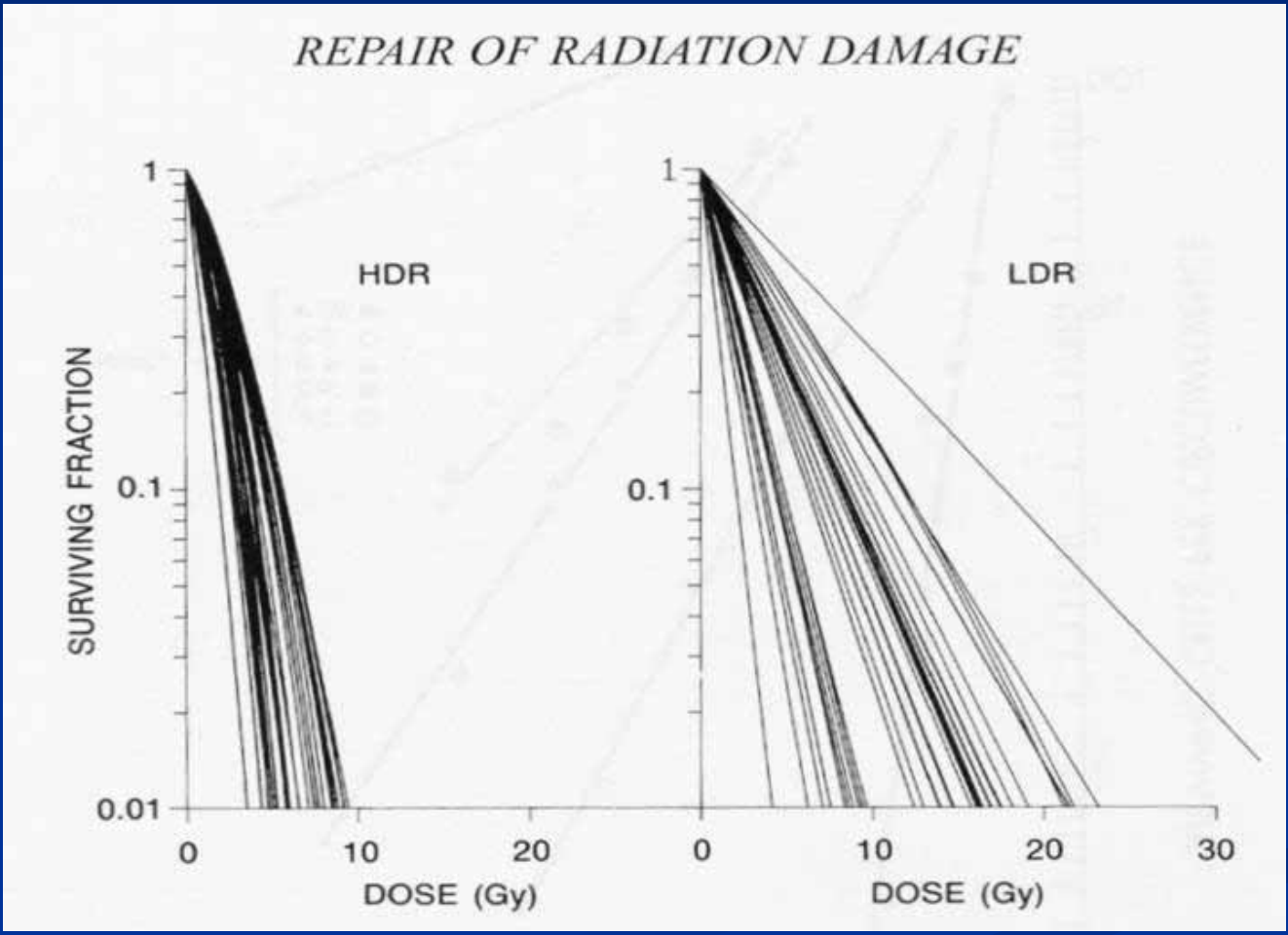
Dose-Rate Effect

- For X- or γ rays, dose rate is one of the most important factors that determine the biologic effect of a given dose
- As dose rate is lowered and exposure time increased, biologic effect is in general reduced
- Seen over a dose range of 1 Gy/min to 0.3 Gy/h; with decreasing dose-rate see loss of shoulder

Dose-Rate Effect



Dose-Rate Effect



Inverse Dose-Rate Effect

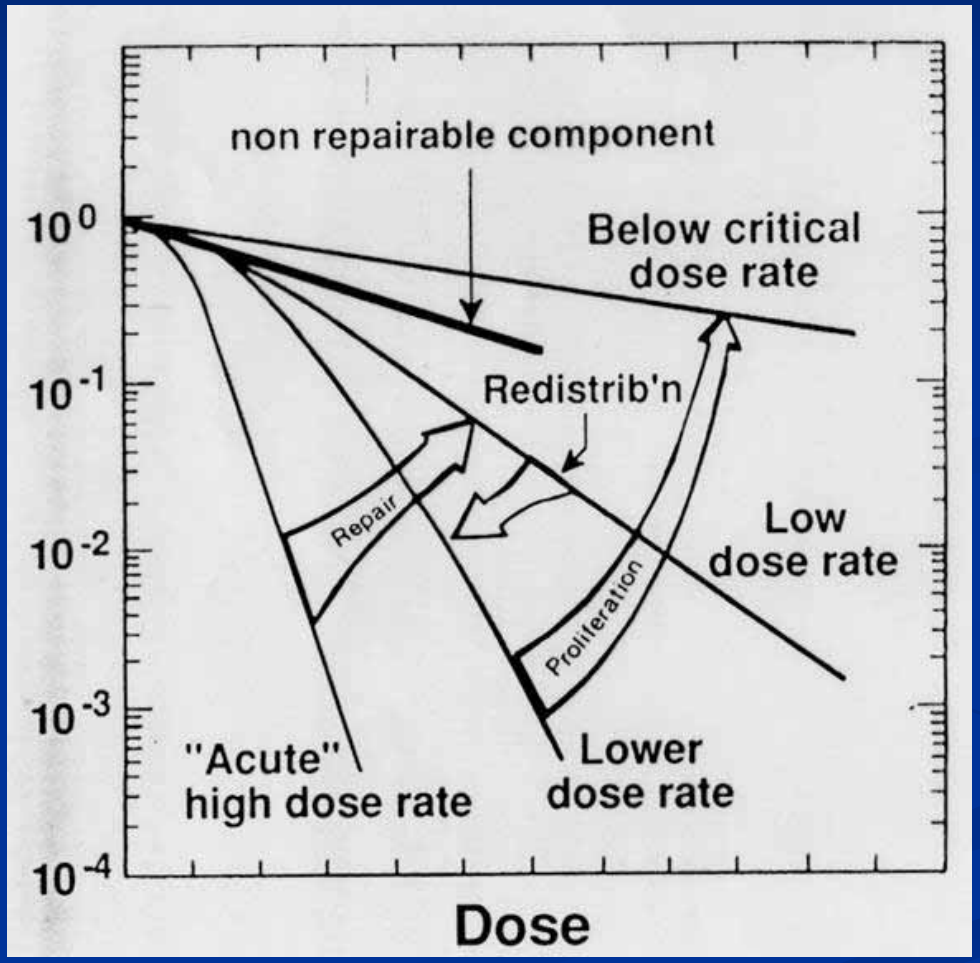
Seen in some situations when lowering the dose rate is associated with an increase in cell kill

Mechanism:

- At dose rates ≤ 0.3 Gy/h cells tend to progress through the cell cycle and become arrested in G_2, a radiosensitive part of the cell cycle

- At higher dose rates the cells stay in the region of the cell cycle they were in at the time of irradiation. In contrast, at low dose rates they continue to cycle into G_2 and thus become more radiosensitive.

Summary of Dose-Rate Effect



Linear Energy Transfer (LET)

- LET is the energy transferred per unit length of track
- Unit is the kiloelectron volt per micrometer (keV/μm) of unit density material
- LET is an average value that can be calculated in different ways

Linear Energy Transfer (LET)

- Track average: obtained by dividing the track into equal lengths, calculating the energy deposited in each length, and finding the mean

- Energy average: obtained by dividing the track into equal energy increments and averaging the lengths of track over which these energy increments are deposited

Linear Energy Transfer (LET)

- For X rays or monoenergetic charged particles the two methods give similar results
- However, very different for 14-MeV neutrons; track average is ~12 keV/μm, energy average LET is ~ 75 keV/μm
- Biological properties of neutrons tend to correlate best with the energy average.

Relative Biological Effectiveness

- The amount of radiation dose is expressed in terms of absorbed energy; dose in Gy is a measure of energy absorbed/unit mass of tissue
- However, equal doses of different types of radiation *DO NOT* produce equal biological effects
- Key to the difference lies in the pattern of energy deposition at the microscopic level

Relative Biological Effectiveness

- To compare the biological effect of different types of radiation use x-rays as the standard

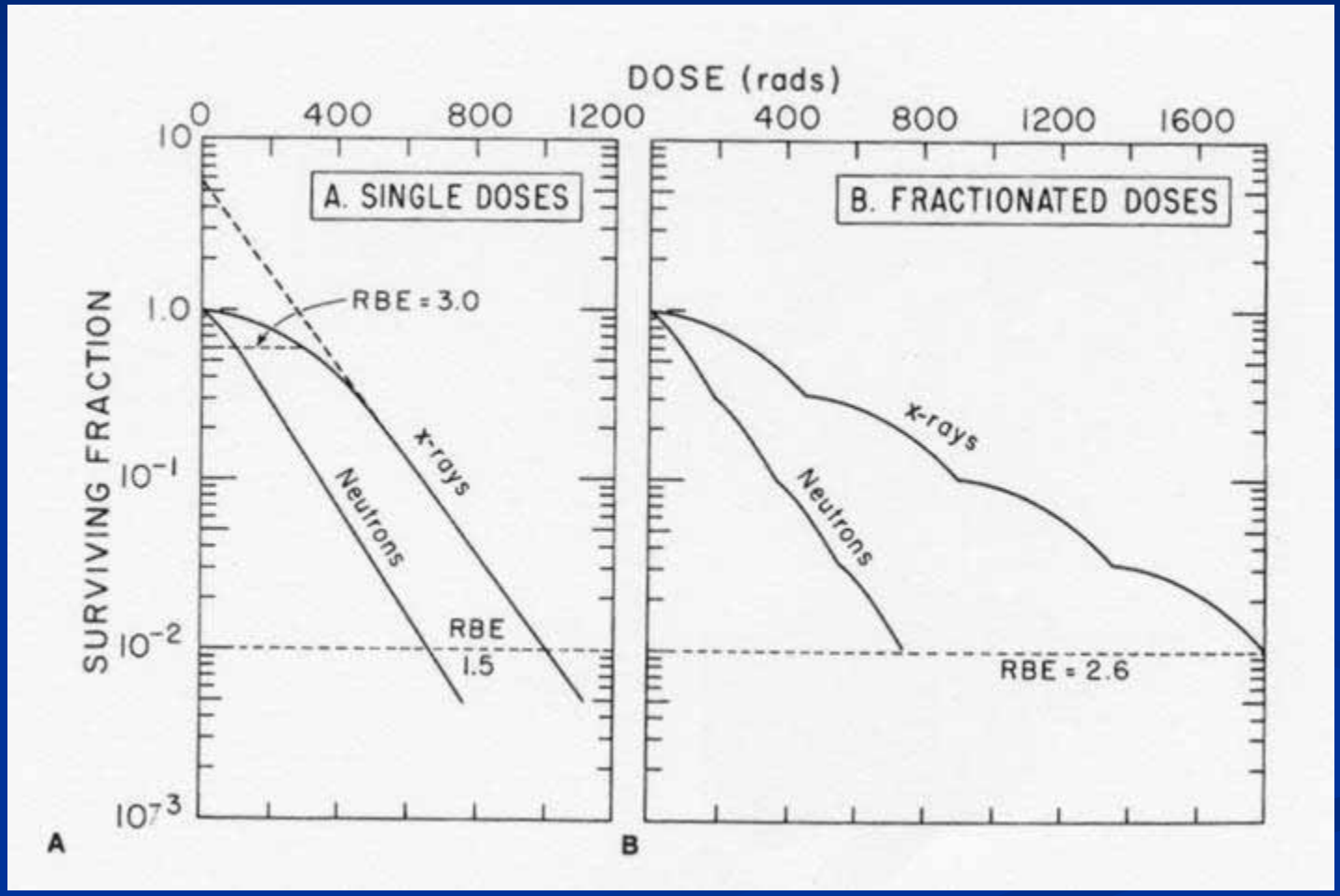
- RBE is formally defined as follows:

$$\text{RBE} = \frac{\text{dose of x-rays to produce a given effect}}{\text{dose of test radiation to produce a given effect}}$$

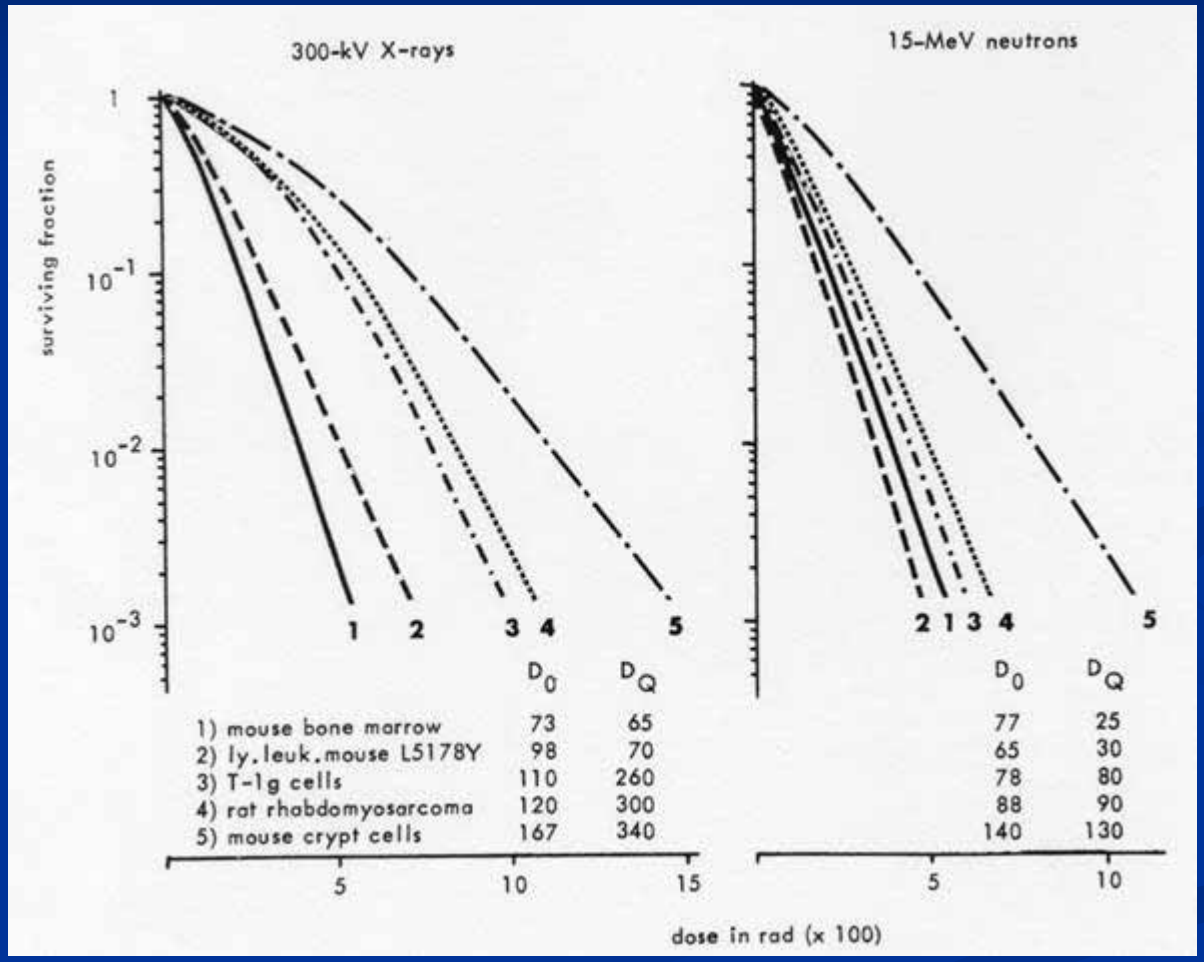
Relative Biological Effectiveness

- RBE is not a single value
- Depends on the level of biological damage (and thus the dose) chosen
- In general, RBE ↑ as dose ↓ until limiting value reached

Relative Biological Effectiveness

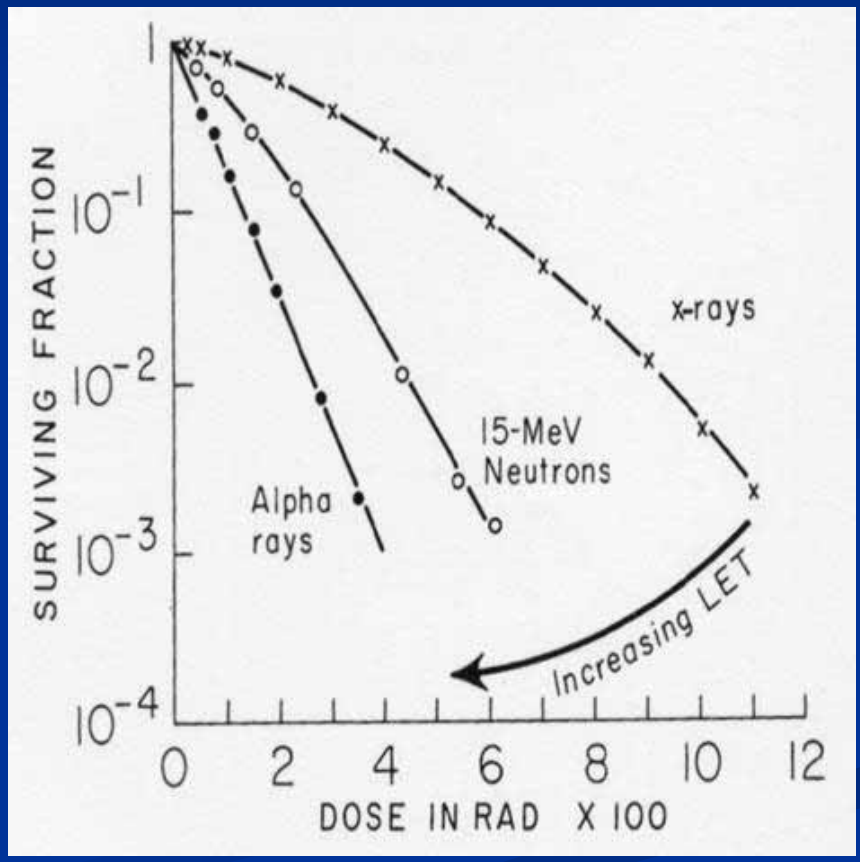


Relative Biological Effectiveness

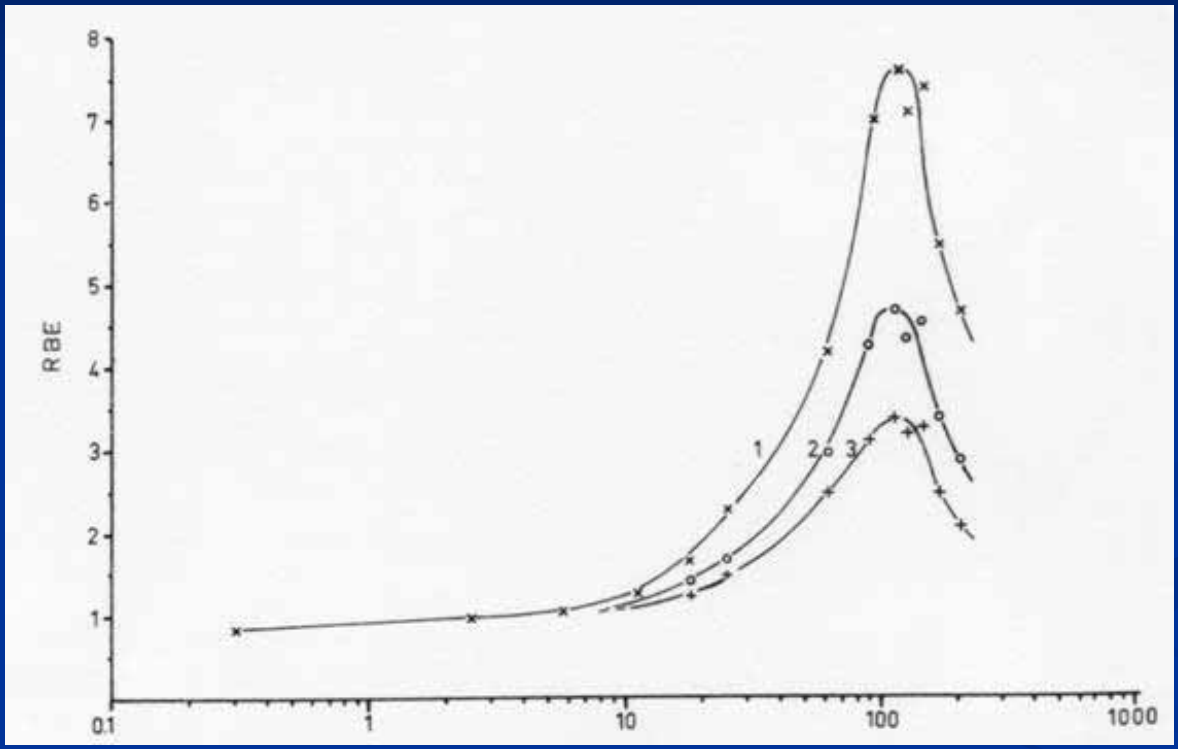


RBE as a function of LET

- As LET increases radiation produces more cell kill per Gy.
- As LET increases survival curves become steeper and the shoulder becomes progressively smaller.



RBE as a function of LET



- If plot RBE as a function of LET, RBE increases slowly at first, then more rapidly as LET > 10 keV/μm.
- RBE then increases rapidly to a peak value of ~ 100 keV/μm, after which RBE decreases rapidly.
- The LET at which RBE peaks is essentially the same for a wide variety of mammalian cells.

Optimal LET

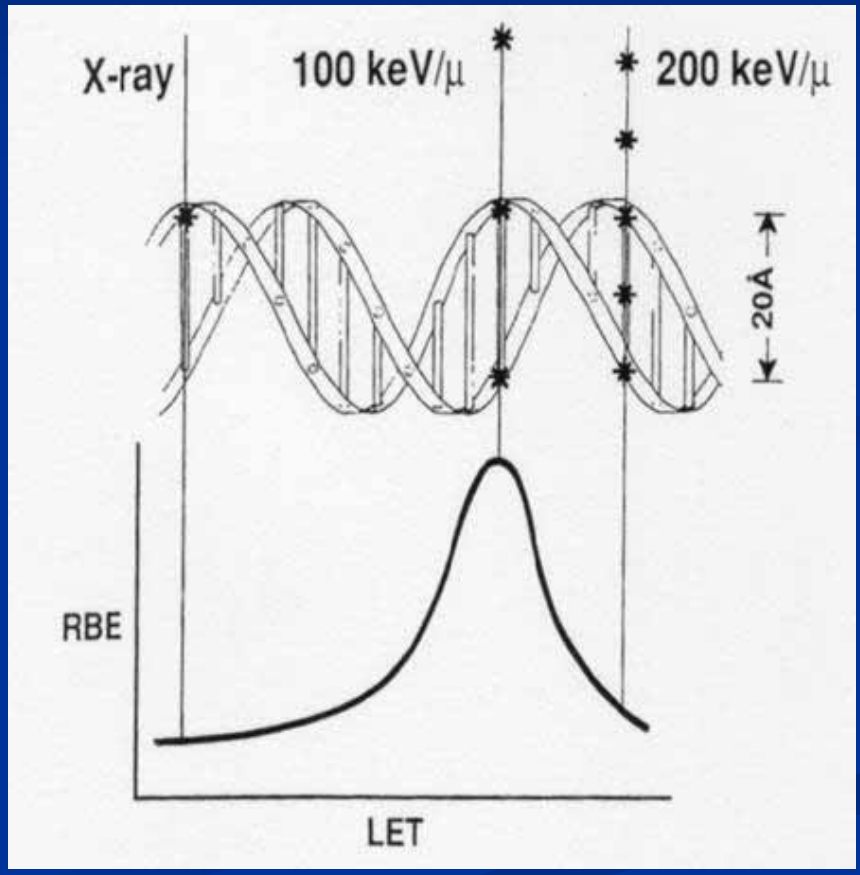
Why is radiation with an LET of 100 keV/μm optimal?

- At this density of ionization, average separation density between ionizing events roughly coincides with diameter of DNA double helix, i.e., 2nm (20A)

- Radiation of this density has the greatest probability of causing a double-strand break by the passage of a single charged particle; double-strand breaks are the basis for most biologic effects.

Optimal LET

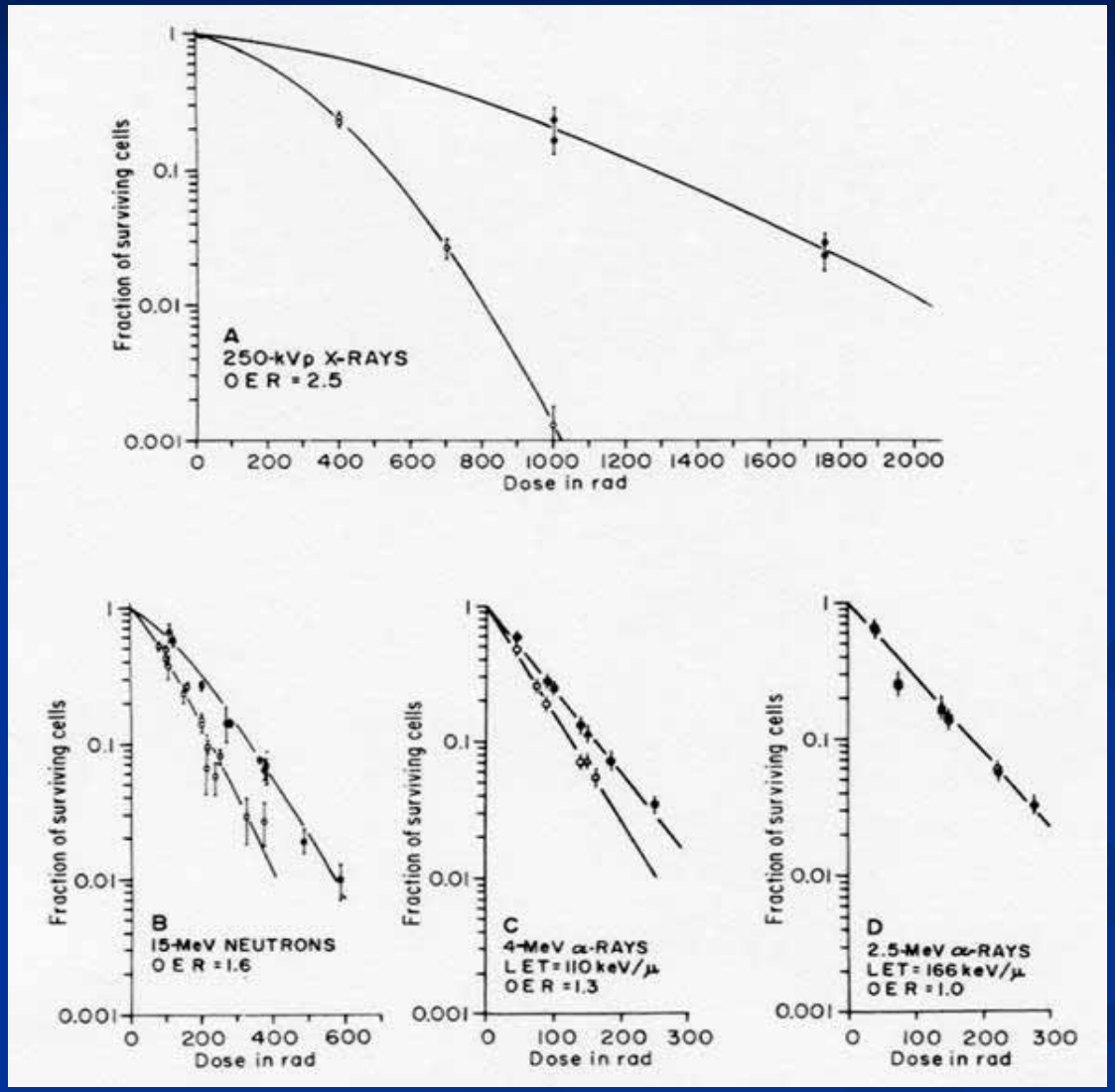
- LET radiation > 100 keV/μm results in wasted energy or overkill
- Very high LET radiation is inefficient since it deposits more energy than needed in critical sites
- These cells are overkilled and /Gy there is less likelihood that other cells will be killed, leading to a reduced biological effect



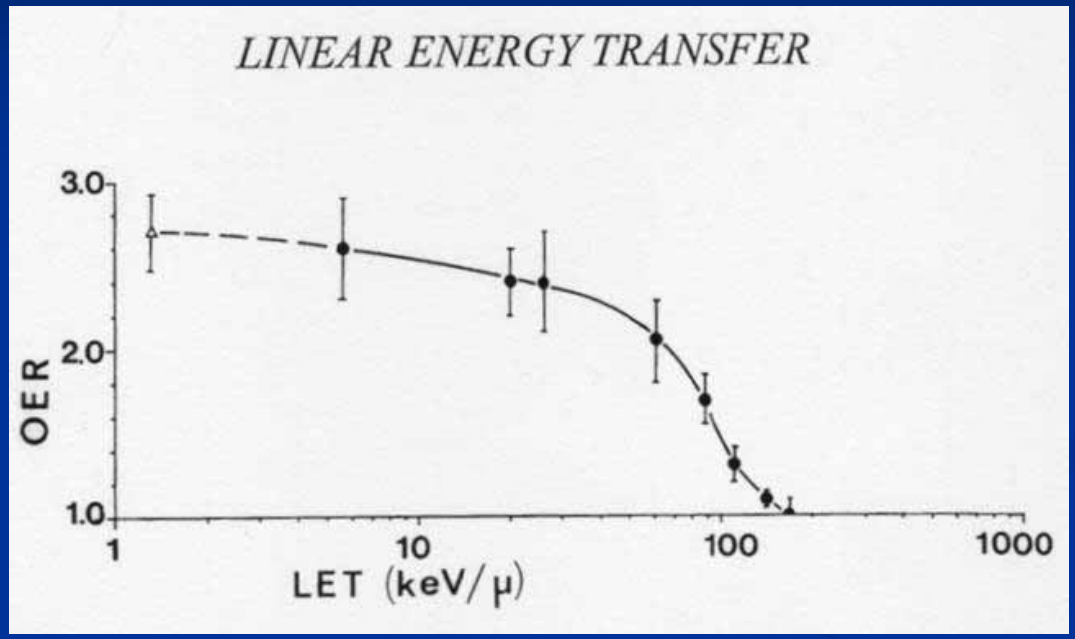
Factors that determine RBE

- Radiation quality (LET)
- Radiation dose
- Number of dose fractions
- Dose rate
- Biologic system or endpoint.

Oxygen Effect and LET



Oxygen Effect and LET



- At low LET, corresponding to x-rays or γ rays, OER is 2.5-3.
- As LET increases, OER decreases slowly until the LET > ~60 keV/μm. OER then falls rapidly, reaching unity when LET around 200 keV/μm.