MPHY3892 Radiobiology

Lecture 2

Cellular Effects,

Target Theories and Survival Curves

Cell Death

INTERPHASE DEATH
(after a dose of several hundred Gy)

- Cessation of metabolic activity
- Disintegration

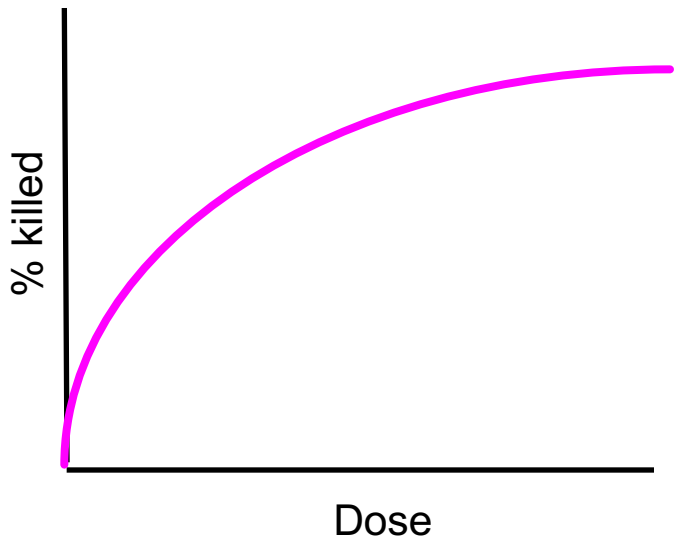
REPRODUCTIVE DEATH
(after a dose of a few Gy)

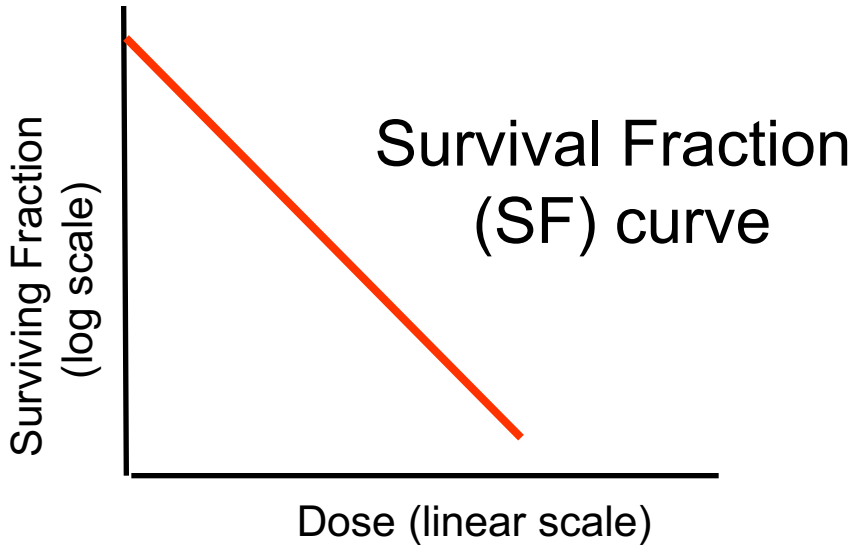
- Inhibition of ability to divide
- Loss of ability to proliferate
- Occurs at the next or subsequent mitosis

Reproductive Death

- In radiobiology there is an emphasis on reproductive death
- Concept of reproductive death is not applicable to differentiated cells that no longer divide and are very resistant to radiation
- In radiotherapy: a tumour is locally controlled when ALL its cells have lost their power for indefinite proliferation
- In radiation protection of acute effects of radiation: regeneration of a damaged normal tissue depends on the number of survived stem cells

Survival Curves for Viruses and Bacteria





Surviving Fraction versus Dose is plotted in semi-logarithmic co-ordinates

Killing is random ⇒ statistical law of Poisson:

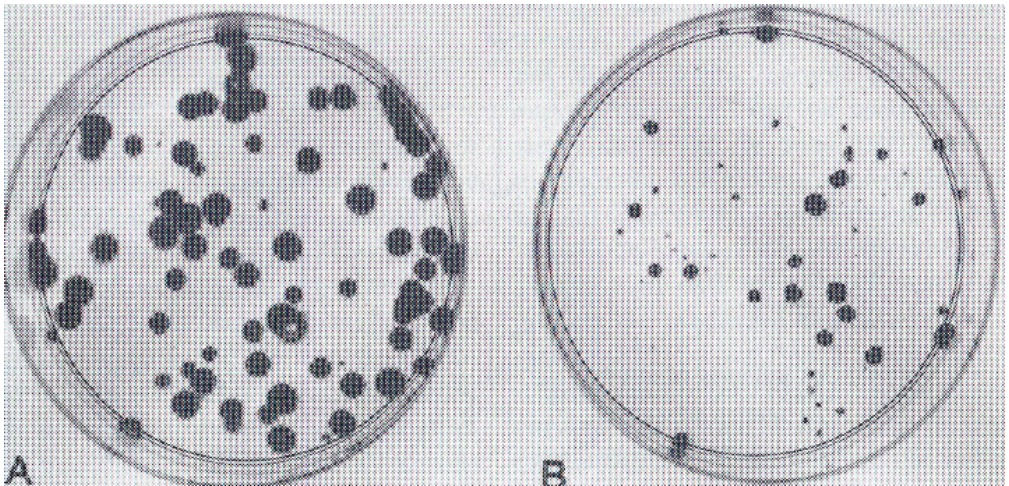
the proportion of surviving cells is e^{-p},

where p is a mean number of lethal events per cell

Generating *in-vitro* Surviving Fraction Curves

- A suspension of single cells in culture is grown at 37^{o}C in Petri dishes in aseptic conditions
- Cells attach to surface
- Cells are irradiated to different doses
- Surviving cells divide and establish cell lines
- After several weeks most cells become exhausted and die
- Some cell lines are able to multiply quasi-indefinitely

After dose D_1



After dose $D_2 > D_1$

Target Theories

- Are developed to link physical facts about radiation absorption with the observed surviving fraction curves
- Propose radiation sensitive targets within cells which have to be hit for the cell to die

Single Hit, Single Target Model

- Assumes a single hit to a single target is enough to kill a cell
- Results in an exponential Survival Fraction curve

$$N = N_0 e^{-D/D_0}$$

Exponential survival curves:
- for viruses, bacteria
- for certain types of mammalian cells

$$N = N_0 e^{-D/D_0}$$

N_0 is the initial number of cells

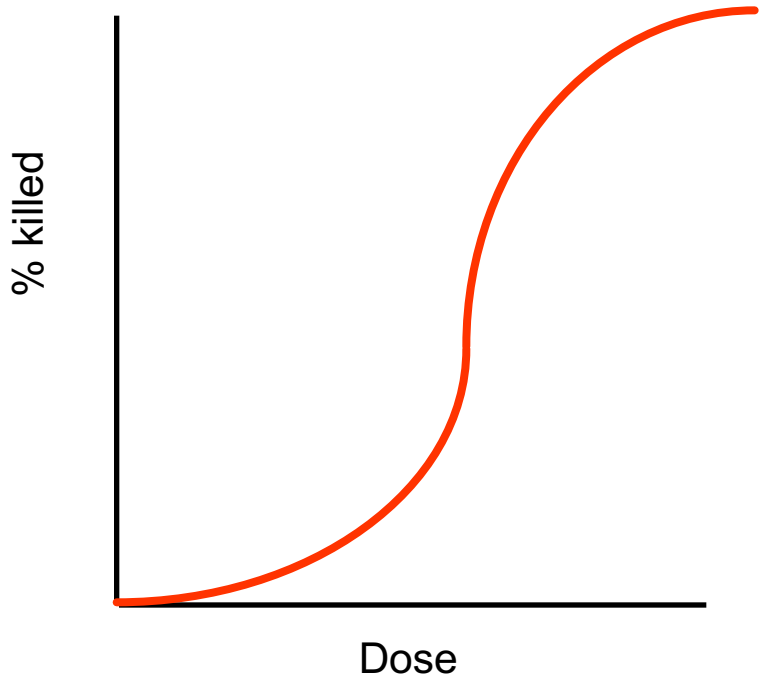
D_0 - mean lethal dose

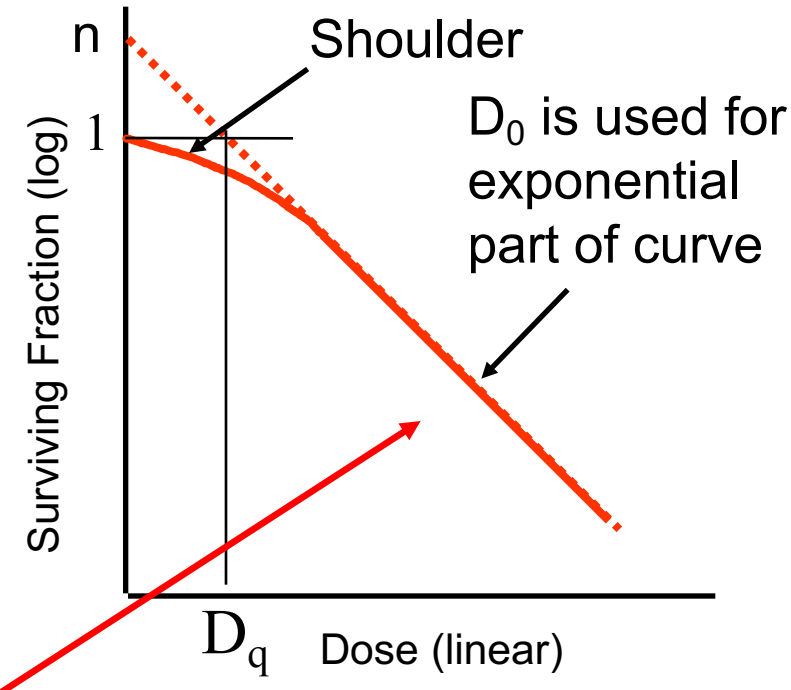
- gives the slope of the line
- is the dose required to reduce the SF to 37%
- is the dose required, on average, to put one hit in each cell
- varies with cell type and type of radiation

For X-rays and γ-rays:

D_0 = 1500 Gy for viruses
100 Gy for bacteria
1 Gy for mammalian bone marrow stem cells

Mammalian Cell SF Curve





* Shoulder at low doses
* Exponential at high doses

Mammalian Cell SF Curve

- D_0 only refers to exponential part

  $D_0 = 1 - 2$ Gy

- Extrapolation number n

  $n = 1 - 5$

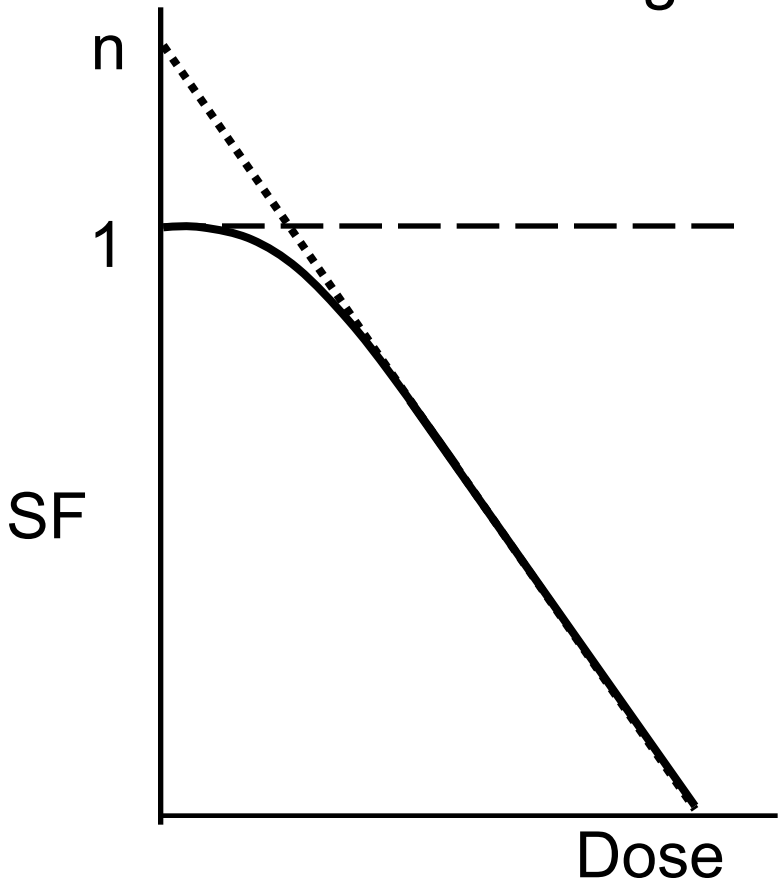
- Quasi threshold dose D_q where extrapolation crosses SF=1 line $\Rightarrow$ is the measure of the size of shoulder

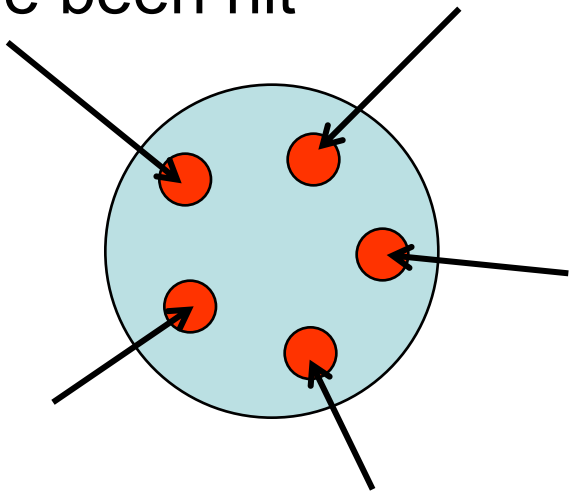
  $D_q = 0.5 - 2.5$ Gy

Not a simple single hit, single target model

Single Hit, Multi-Target Model

- Cell contains **n** identical targets
- Each target is inactivated by ionisation
- Individual target inactivation is not lethal
- Cell is killed when ALL targets have been hit





$$S = 1 - (1 - e^{-D/D_0})^n$$

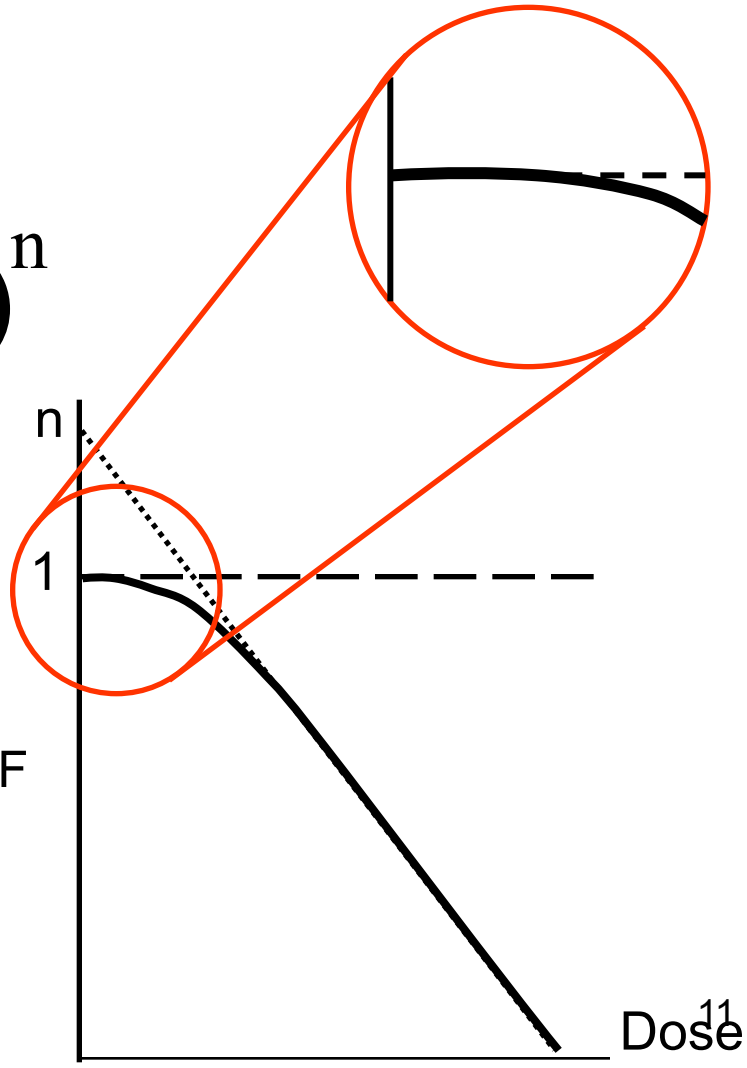
Single Hit, Multi-Target Model

$$S = 1 - (1 - e^{-D/D_0})^n$$

- Assumes random damage
- Probability of target remaining undamaged is e^{-D/D_0}
- 1 hit/cell at dose D_0
- When D is large, $S \rightarrow ne^{-D/D_0}$
- Extrapolation number n = the number of targets per cell
- Quasi threshold dose: $D_q = D_0 \cdot \ln(n)$

Single Hit, Multi-Target Model

$$S = 1 - (1 - e^{-D/D_0})^n$$



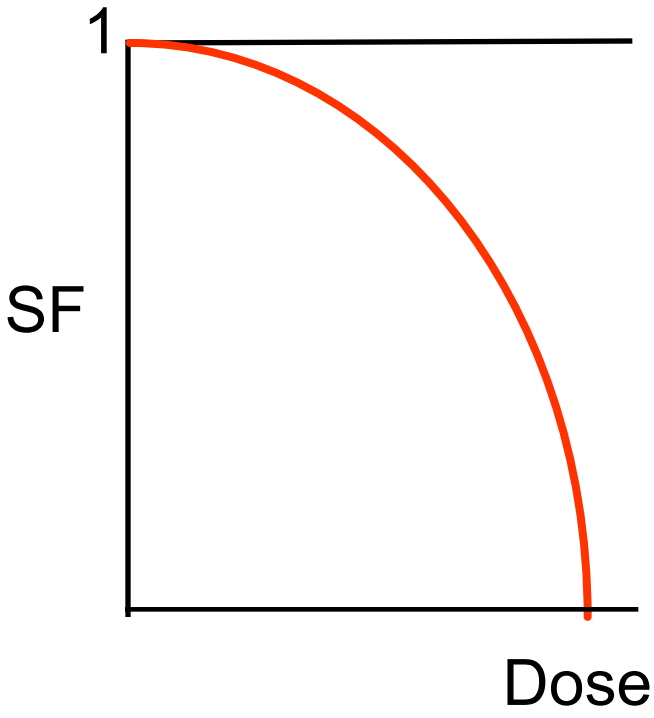
PROBLEM

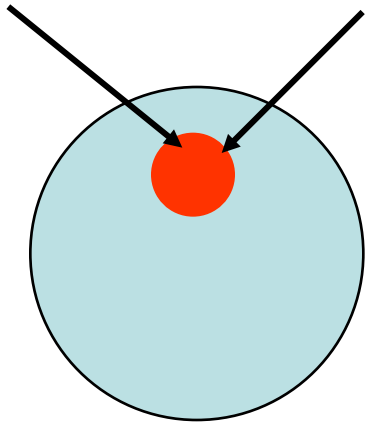
- As D → 0, slope → 0
- Implies there is no cell killing at smallest dose
- Experimental data does not support this

Two Hit, Single Target Model

- A cell contains one target
- A single hit is not sufficient to kill the cell
- Cells are killed if the target is hit twice
- Two hits come from two separate particles
- Mean number of lethal events per cell is proportional to D^2

$$S = e^{-\beta D^2}$$





β is the probability of a sub-lethal event

fails at lowest doses:

as $D \rightarrow 0$, slope $\rightarrow 0$

Two Component Model

- Assumes cells can be killed in two ways:
  - a single lethal event
  - accumulation of sub-lethal events
- Assumes the two methods are independent $\Rightarrow S = S_1 \cdot S_n$
- This model is introduced to get agreement with experimental data for $D \rightarrow 0$

$$S_1 = e^{-D/{}_1D_0}$$ (where ${}_1D_0$ relates to single hit lethality)

and

$$S_n = 1 - \left(1 - e^{-D/{}_nD_0}\right)^n$$ (where ${}_nD_0$ relates to sub-lethality)

assuming the single hit, multi-target model,

or

$$S_n = e^{-\beta D^2}$$

assuming the two hit, single target model.

Two Component Model

So the survival curve is represented by

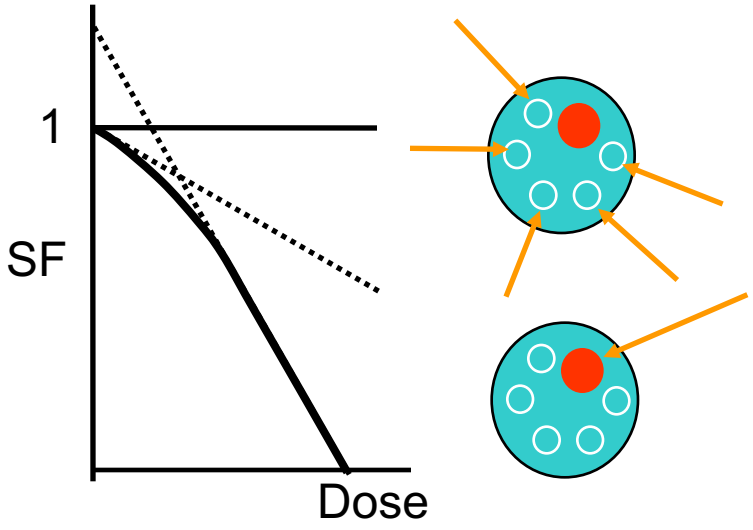
$$S = e^{-D/{}_1D_0}\left[1-\left(1-e^{-D/{}_nD_0}\right)^n\right]$$

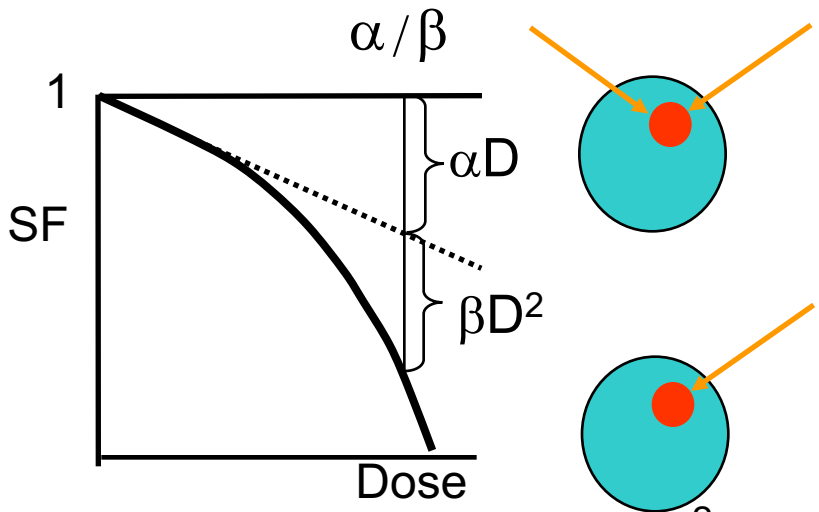
or

$$S = e^{-\left(\alpha D+\beta D^2\right)}$$

where $\alpha = 1/{}_1D_0$

Two Component Model





$$S = e^{-D/_1D_0}\left[1-(1-e^{-D/_nD_0})^n\right]$$

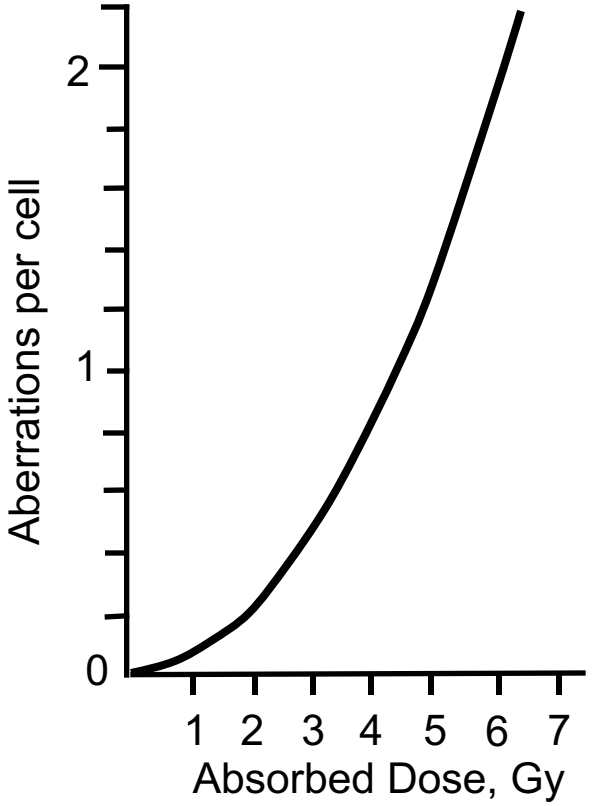
$D \rightarrow 0$, slope $\rightarrow -1/_1D_0$

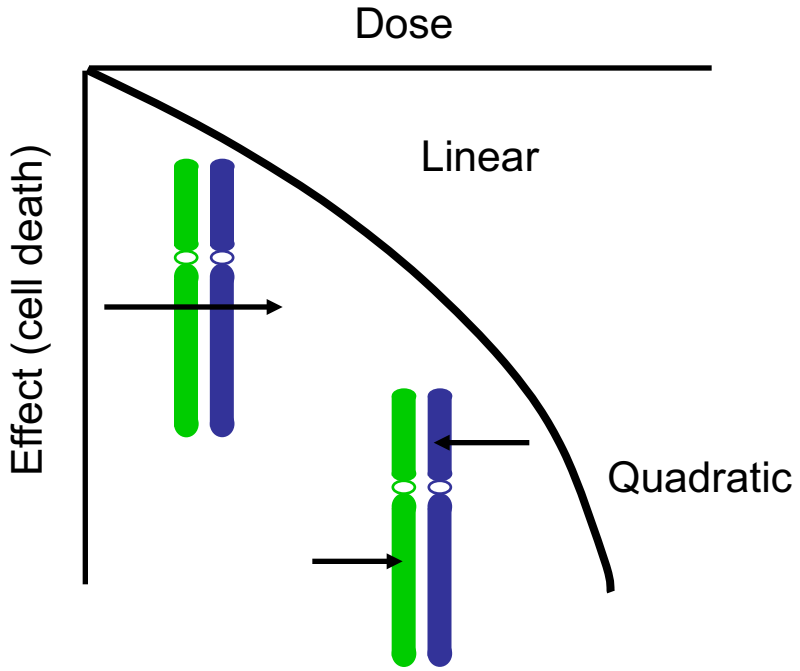
$$S = e^{-(\alpha D+\beta D^2)}$$

$D \rightarrow 0$, slope $\rightarrow -\alpha$

Statistical uncertainty of experimental data does not allow to distinguish between these two variations of two-component model

Chromosome Aberrations: Dicentrics & Rings

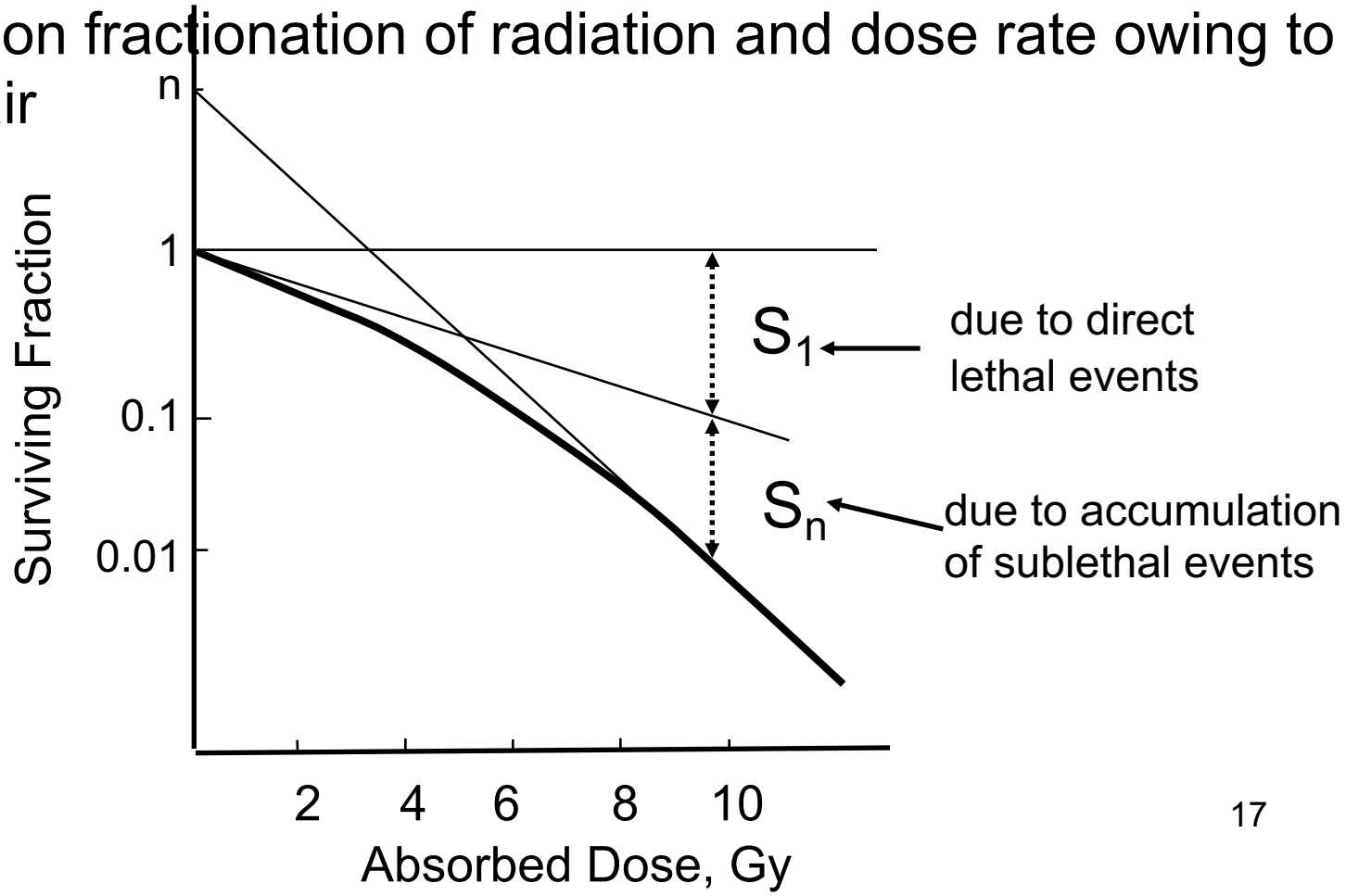




Data for human lymphocytes
irradiated by γ-rays

Survival Curve

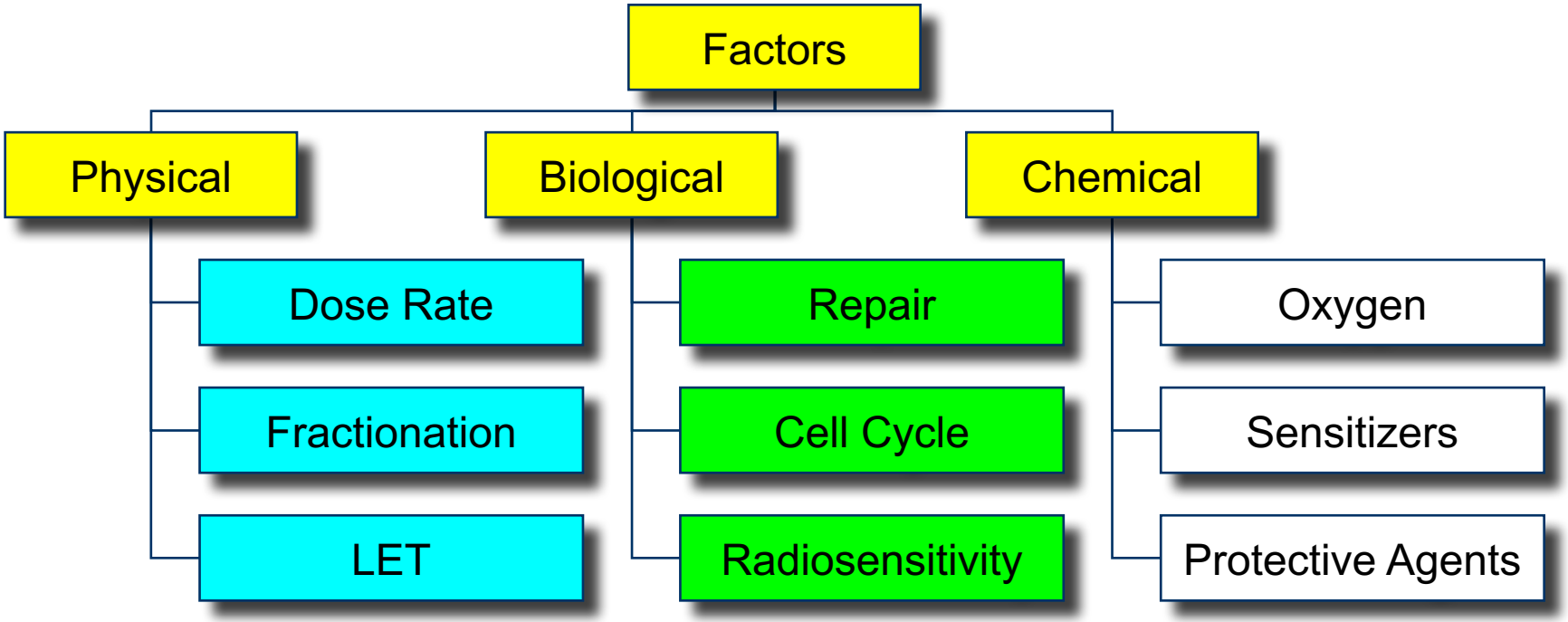
- For very small doses Surviving Fraction is approximately S_1
- For larger doses contribution of sublethal effects become increasingly important
- S_1 depends only on the total dose D
- S_n depends on fractionation of radiation and dose rate owing to cellular repair



Models Based on Intracellular Repair

- Two types of lesions: directly lethal and potentially lethal but susceptible to repair
- Repair requires action of enzymatic system and suitable time
- The shoulder on survival curve arises when the repair system is fully operative, only at low doses
- Repair enzyme might become saturated as the dose is increased
- When the repair system is completely saturated, the survival curve becomes exponential

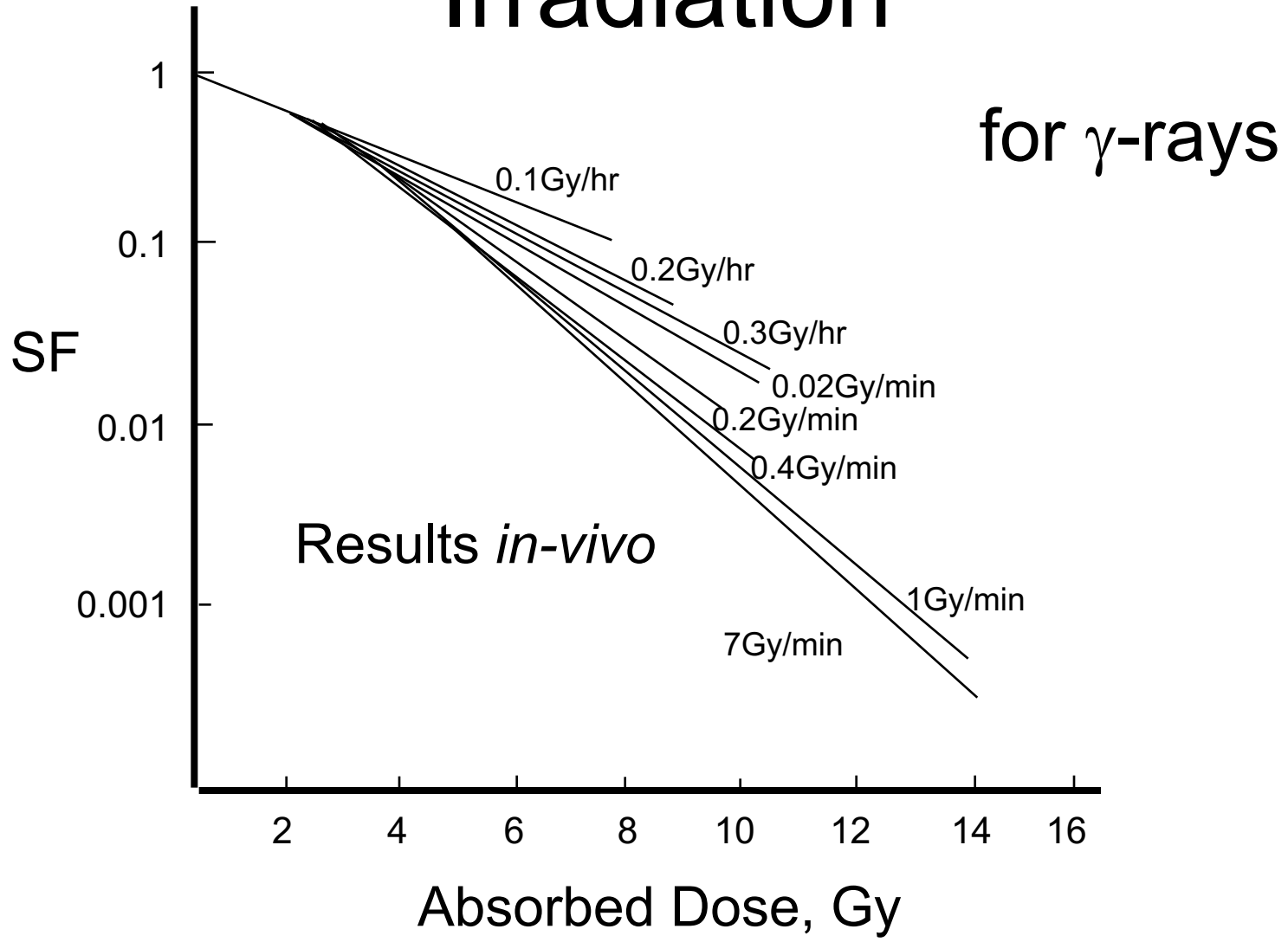
Factors Affecting Radiation Effects



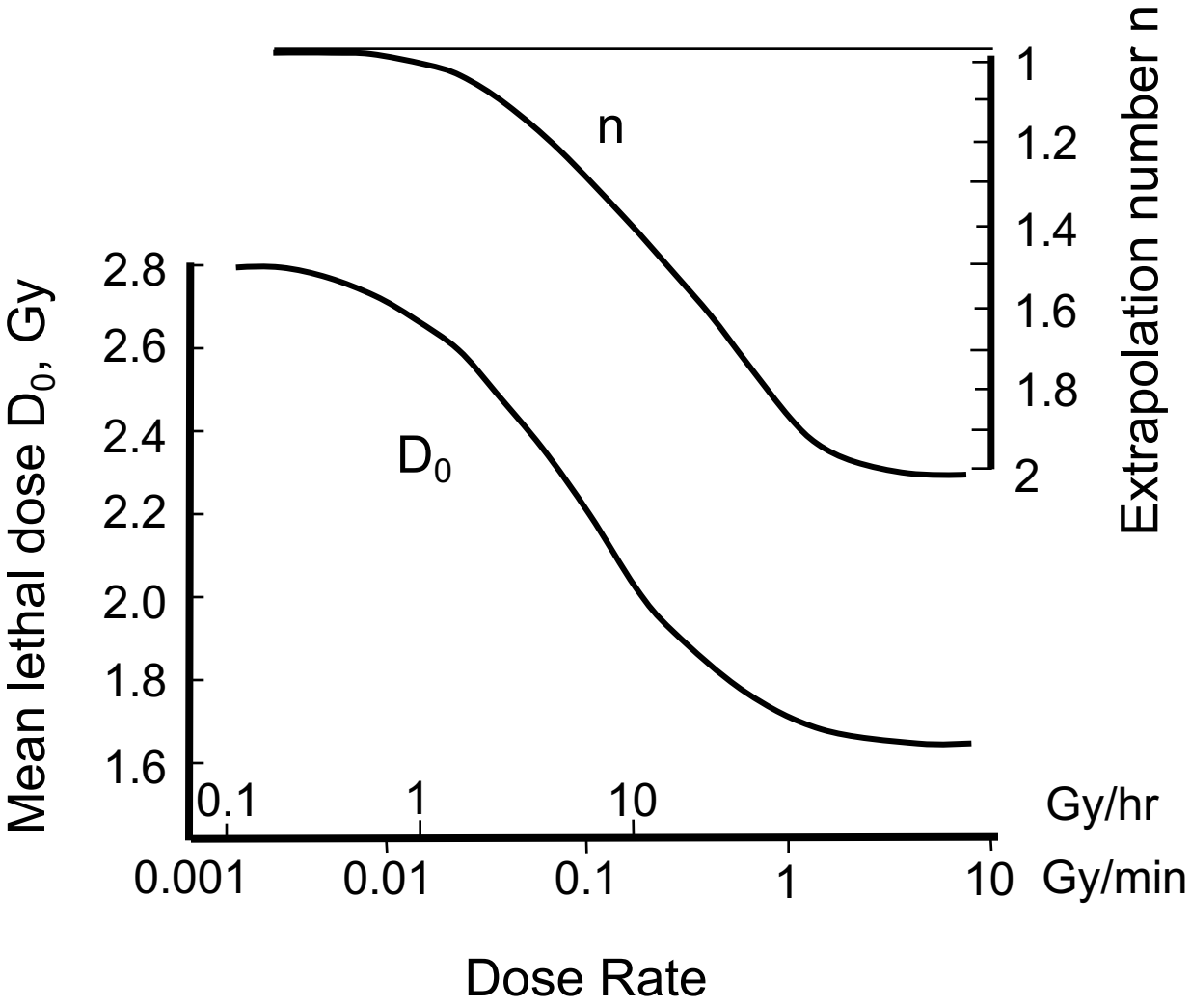
Dose Rate for Continuous Irradiation

- Survival fraction increases as dose rate is reduced over a range of 1.0 - 0.01Gy/min
- This is due to repair of sub-lethal lesions during irradiation
- For high dose rate (>1Gy/min) irradiation is too short to allow for repair
- For very low dose rate (<1Gy/hr) all sub-lesions are repaired. Killing is due to lethal lesions only and Surviving Fraction curve has no shoulder

Dose Rate for Continuous Irradiation



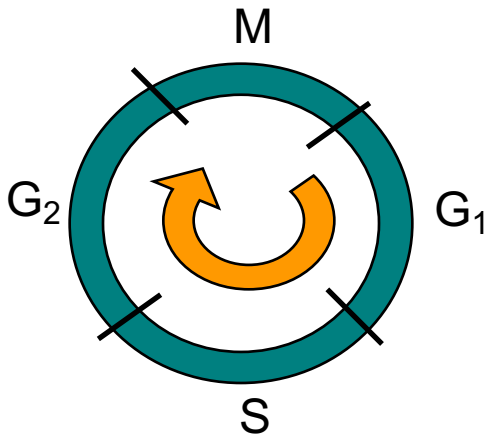
Dose Rate for Continuous Irradiation



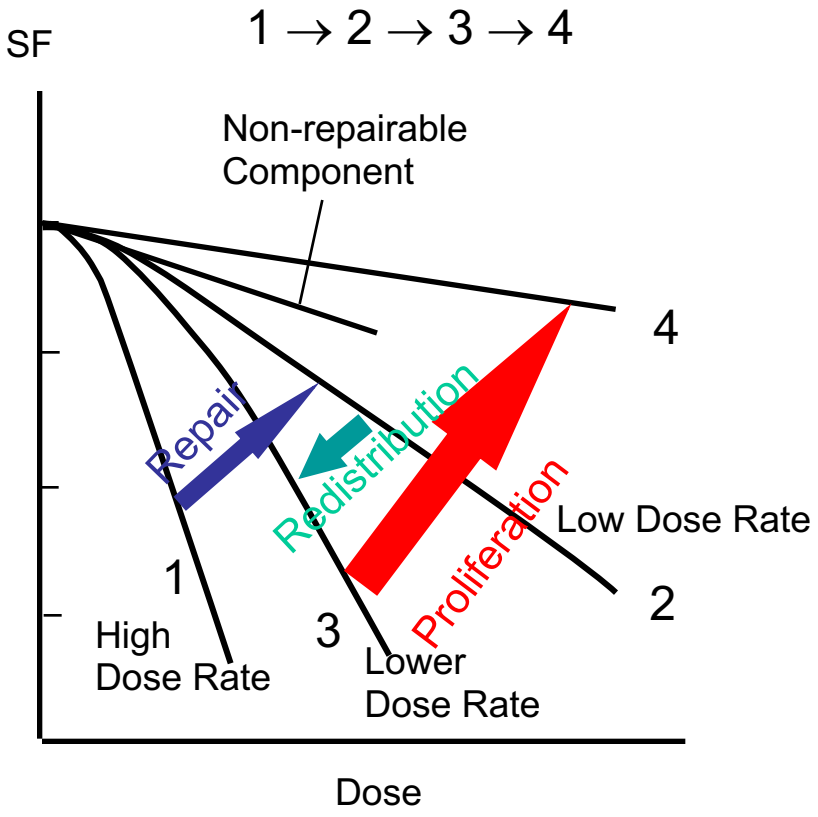
Dose Rate for Continuous Irradiation

- Dose rate is important for radiation effects when the fraction of cells killed is great so that sub-lethal damage contributes significantly to cell killing
- In brachytherapy continuous irradiation during hours/days/weeks is used. Dose rate is very important here
- For external beam radiotherapy with doses about 2Gy per fraction delivered within a minute the dose rate has little importance

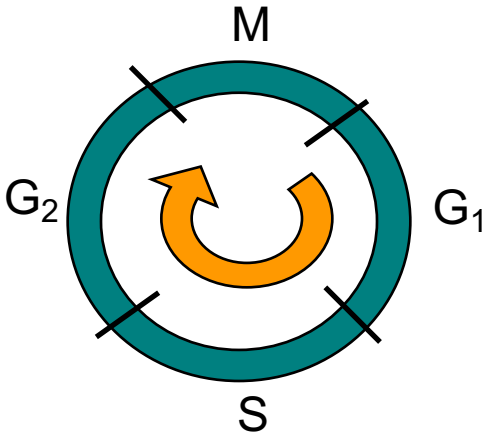
Inverse Dose-Rate Effect



- As the dose rate is reduced, more sub-lethal damage is repaired, but cells are “frozen” in their positions in the cell cycle and do not progress
- As the dose rate is further reduced, SF curve steepens again because cells can progress through the cycle to pile up at a block in radiosensitive phase G_2 and cannot divide (pre-mitotic block)
- The function of the G_2 pause in cell cycle is to allow a check of chromosome integrity before mitosis is attempted



Inverse Dose-Rate Effect

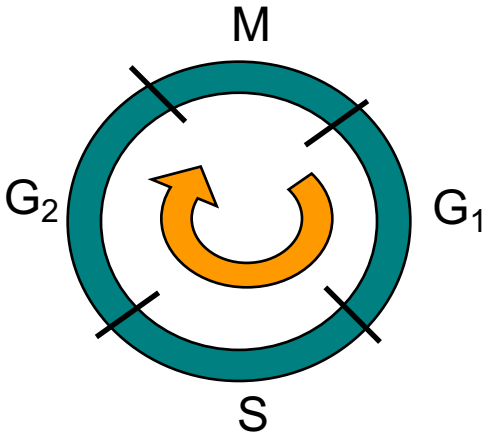


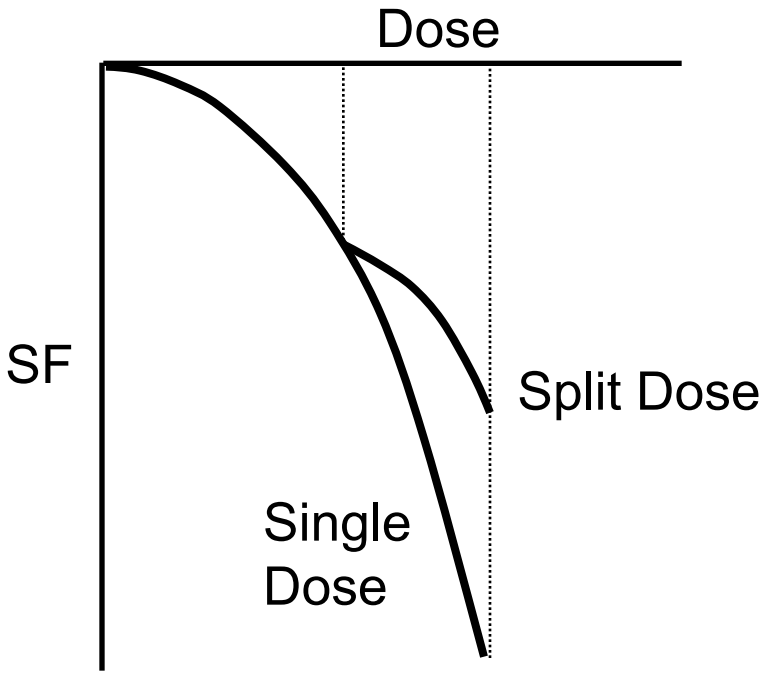
- Molecular checkpoint genes may block the cells in G_2 phase after cells are exposed to any DNA damaging agent
- This is inverse dose-rate effect: decreasing the dose rate results in increased cell killing (valid for dose rates around 0.3-0.4 Gy/h for some cell lines)
- A further lowering of dose rate allows cells to escape radiosensitive G_2 block and divide. Cell proliferation may occur and SF becomes shallower as cell birth from mitosis offsets cell killing from radiation
- Continuous irradiation is more damaging to cells with longer cycles, because a larger dose is absorbed per cell cycle

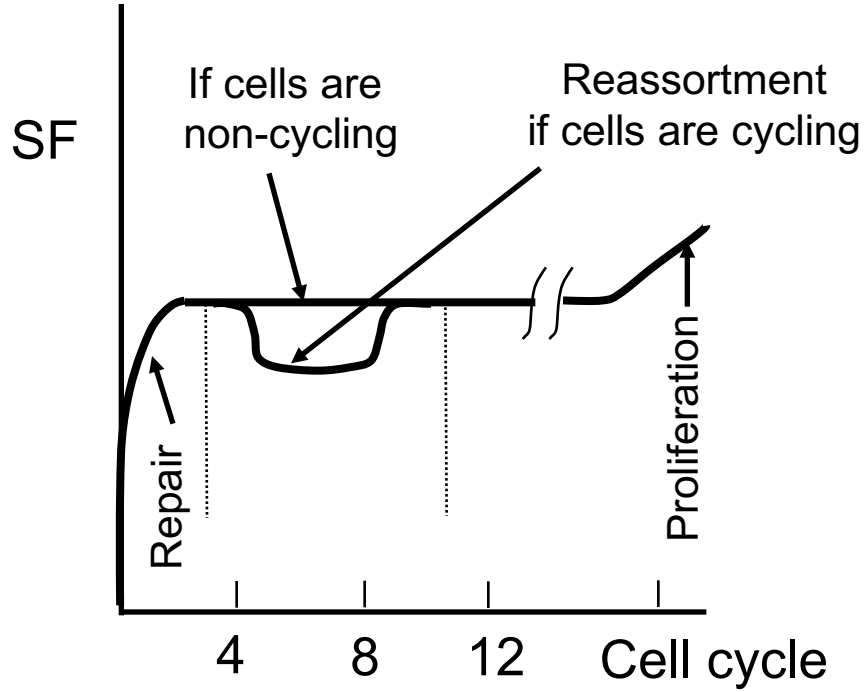
Fractionation

- Dose in fractions has a reduced effect
- SF curve for a fraction is superimposed on preceding fraction and has the same initial slope and the same shoulder $\Rightarrow$ shallow curve
- Sub-lethal damage is repaired between fractions
- Repair of sub-lethal damage takes place with a half-life of 0.5 - 1.5 hours, depending on cell type
- At least 6 hours separation between fractions should be used for allowing the repair of normal tissues surrounding the tumour (in practice 5 daily fractions of 2Gy are given during external beam therapy per week)

Fractionation

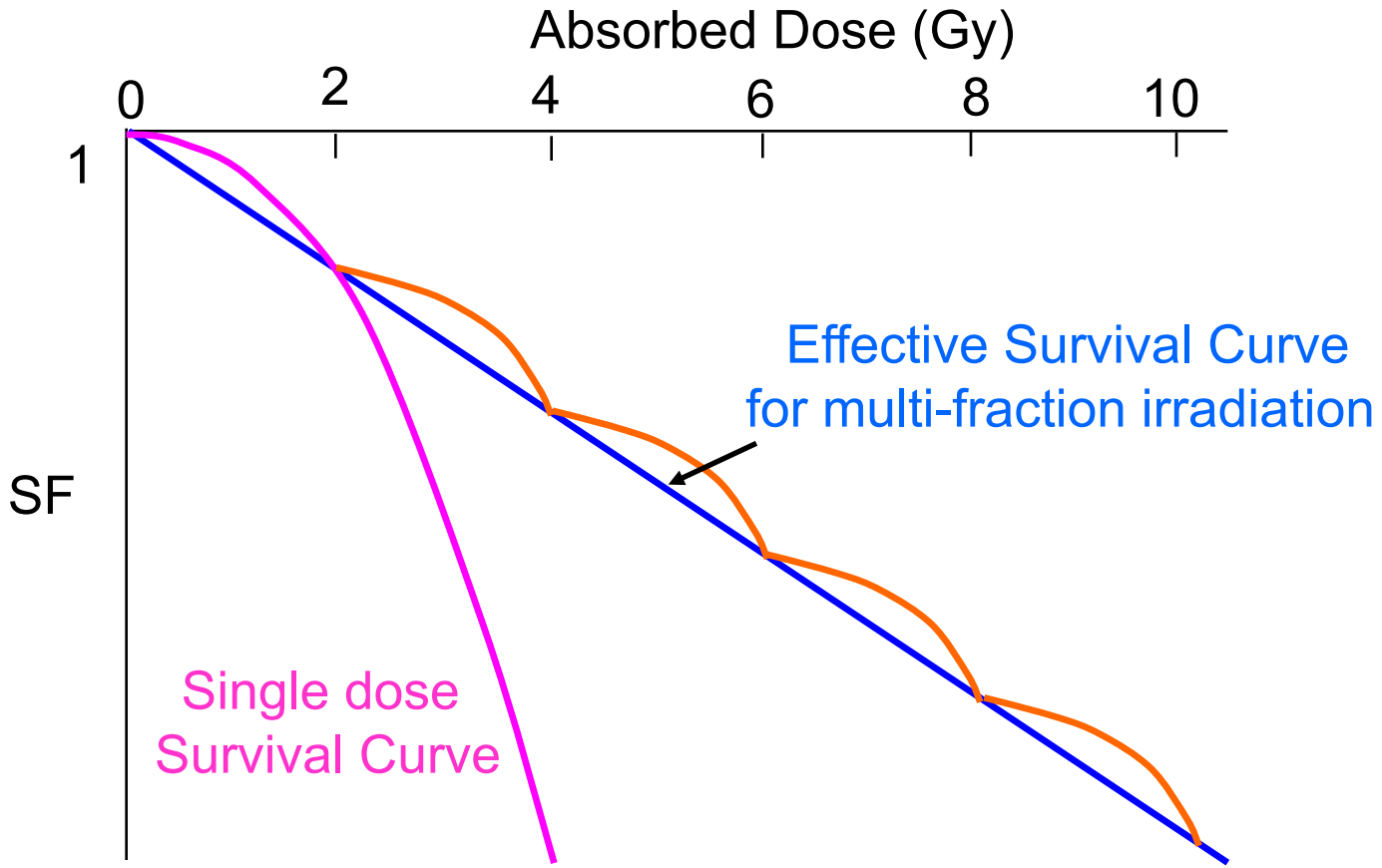






Time between two fractions of radiation (hours)

Fractionation



Fractionation

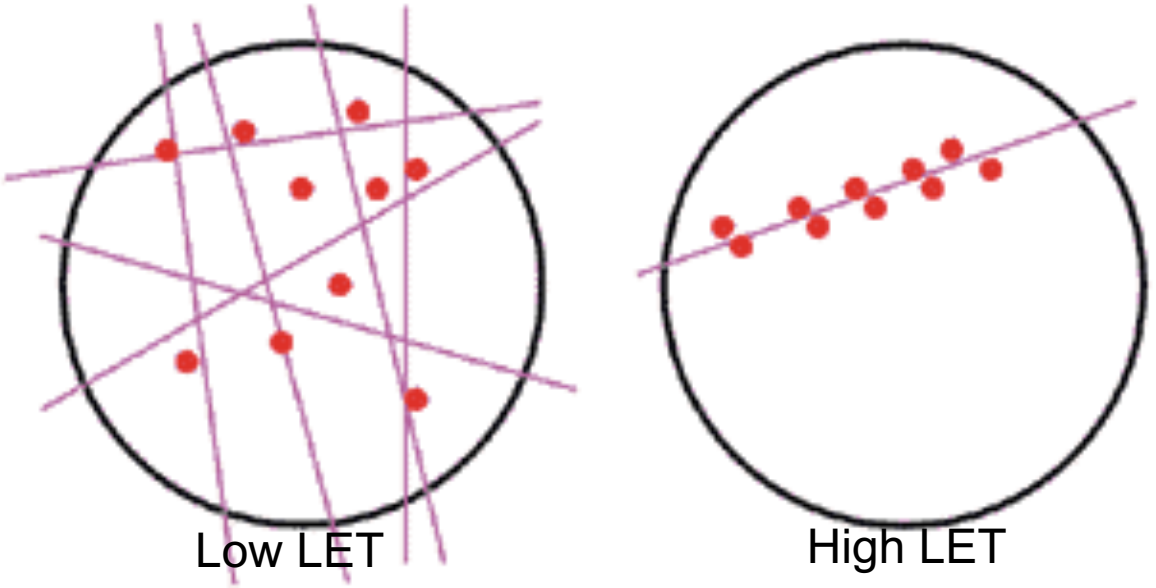
- If survival s is the same for each fraction, the total SF after N fractions is s^N
- For small dose per fraction, only get lethal lesions and sub-lethal damage is repaired between fractions
- The shoulder of the SF curve is repeated many times, so that the effective SF curve in semi-logarithmic scale is a straight line from the origin through a point on a single-dose SF curve corresponding to a daily dose fraction (e.g. 2 Gy)
- Resulting curve is exponential $S_{eff} = e^{-\alpha D}$
- The slope reduces as fractions become smaller

Linear Energy Transfer (LET)

LET is the <u>average</u> energy **dE** locally imparted to the medium by a particle of specified energy per unit distance **dl** (keV/μm):

LET = dE/dl

LET can be only an average quantity, because at microscopic level, the energy per unit length of track varies greatly

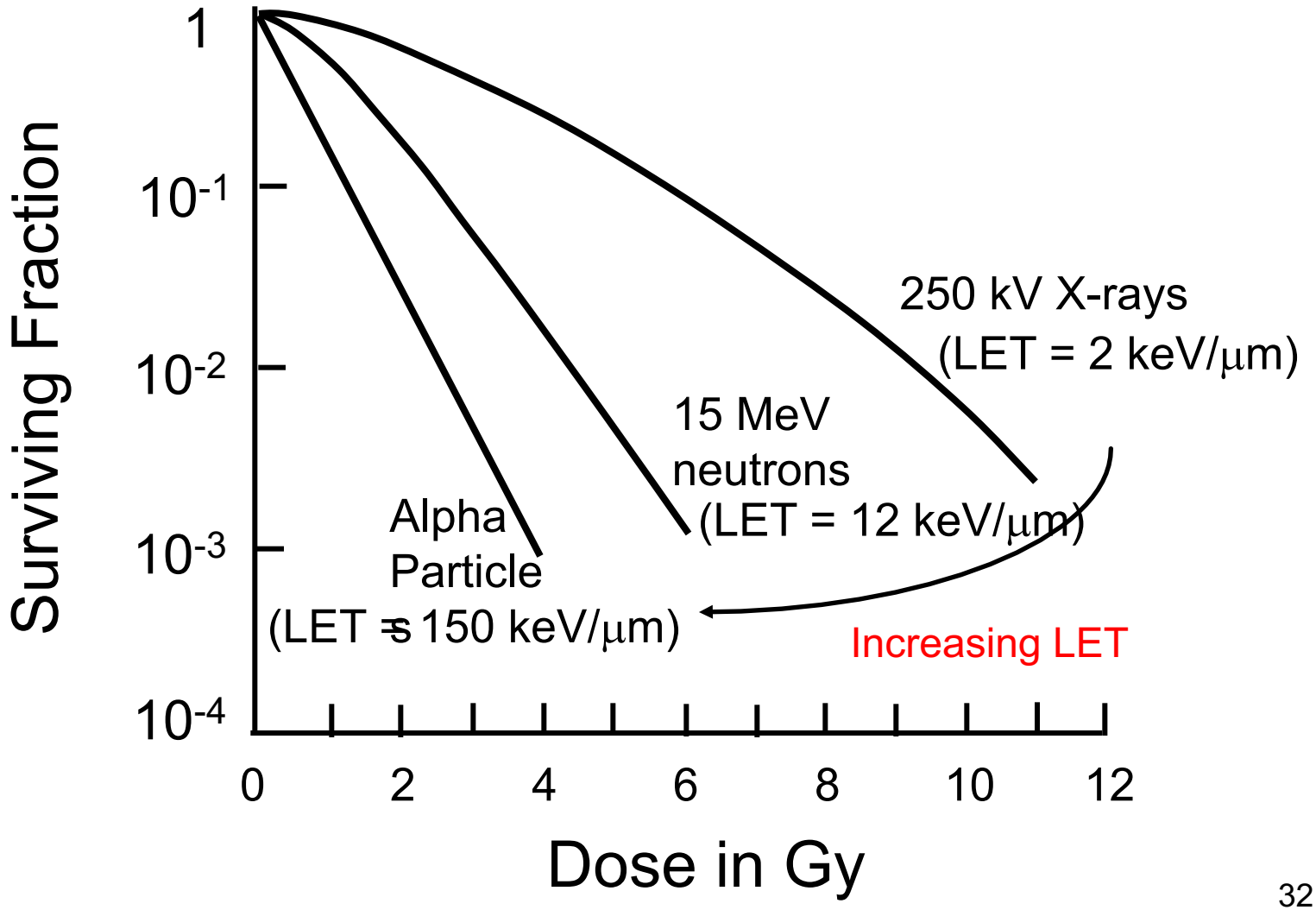


Linear Energy Transfer

Radiation	LET (keV/μm)
1 MeV γ– rays	0.2
250 kV X-rays	2.0
10 MeV protons	4.7
150 MeV protons	0.5
15 MeV neutrons	12
2.5 MeV α–particles	166
2 GeV Fe ions	1000

For a given type of particle, the higher the energy, the lower the LET

LET


Surviving Fraction
1
10-1
10-2
10-3
10-4
0
2
4
6
8
10
12
Dose in Gy
250 kV X-rays
(LET = 2 keV/μm)
15 MeV
neutrons
(LET = 12 keV/μm)
Alpha
Particle
(LET ≈ 150 keV/μm)
Increasing LET

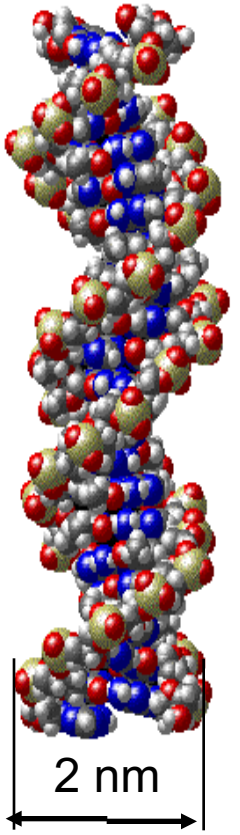
LET

$$S = e^{-(\alpha D + \beta D^2)}$$

- Survival curve becomes steeper with higher LET
- Shoulder at low LET disappears at high LET
- Implies high LET radiation inactivates cells by single hit whereas low LET radiation requires interaction of sub-lethal hits
- No reduced cell killing with fractionation at high LET
- In terms of linear-quadratic model, increase of LET represents a progressive increase in the linear component (αD) relative to quadratic component (βD^2)

Optimal LET

- Radiations with LET = 100 keV/μm are optimal for producing biological damage in mammalian cells
- At this optimal LET, the average separation between ionising events coincides with the diameter of mammalian DNA double helix (2nm) ⇒ the highest probability of causing double-strand break by passing a single particle
- Radiations having optimal LET = 100 keV/μm include
  - neutrons of few hundred keV
  - low-energy protons
  - high-energy α-particles
- For higher LET (e.g. 200 keV/μm) double-strand breaks are readily produced, but the energy is "wasted" because the ionising events are too close together



Relative Biological Effectiveness (RBE)

Biological effect of some radiation **r** is compared with biological effect of 250 kV x-rays that are used as a standard:

$$RBE = D_{250}/D_r,$$

where RBE is Relative Biological Effectiveness,

D_{250} and D_r are dose of 250 kV x-rays and dose of radiation **r** required for equal biological effect

Relative Biological Effectiveness versus LET

For LET = 100 keV/μm RBE for mammalian cells reaches maximum