PAM2001
Molecular & Cellular Radiobiology

In this Lecture

- Radiation Interaction
- Irradiation of Macromolecules
- Radiolysis of Water
- Direct & Indirect Effects
- Target Theory
- Cell Survival Kinetics
- Single-Target, Single-Hit Model
- Multitarget, Single-Hit Model
- Recovery

Radiation Interaction

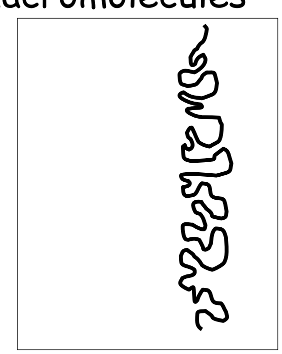
- Initial interaction between radiation & tissue occurs at electron level
- Observable human injury results from changes at molecular level
- Separated into effects on;
  1. Macromolecules
  2. Water

Radiation Interaction

- Radiation exposure of tissue is uniform because tissue is large on an X-ray scale
- Radiation interacts RANDOMLY with tissues

Irradiation of Macromolecules

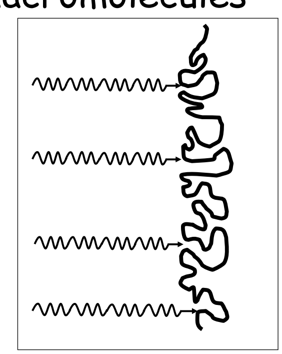
- In-Vitro Irradiation
  - Macromolecules irradiated outside of body or cells
- In-Vivo Irradiation
  - Macromolecules irradiated inside a living cell



Irradiation of Macromolecules

- When irradiated in-vitro, the following occur:

  1.Main-chain scission
  2.Cross-linking
  3.Point lesions



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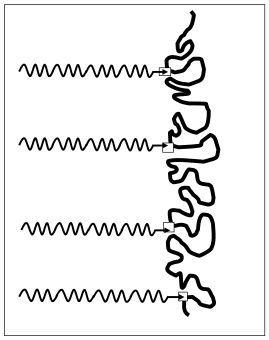

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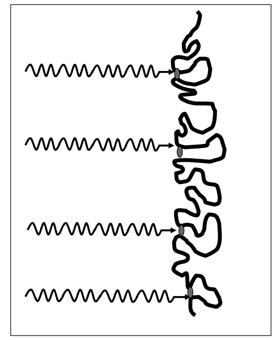

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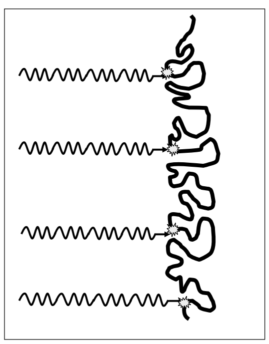
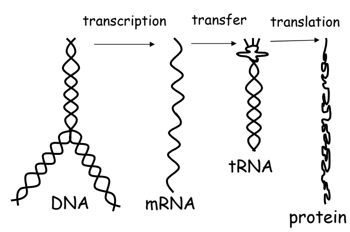


At low dose; point lesions are the cellular damage resulting in the late effects observed at whole body level

Irradiation of Macromolecules

Macromolecular Synthesis

- Function of a normal cell:
  - Catabolism, Anabolism, & Synthesis
- Synthesis of protein & nucleic acid is critical for cell survival

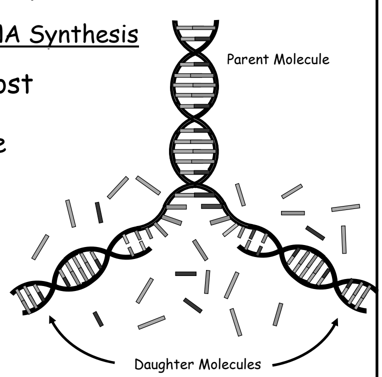


- DNA is the most radiosensitive macromolecule

Irradiation of Macromolecules

DNA Synthesis

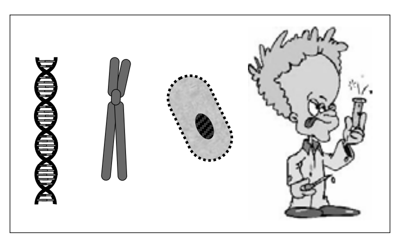
- DNA is the most radiosensitive macromolecule



Irradiation of Macromolecules

Radiation Effects on DNA

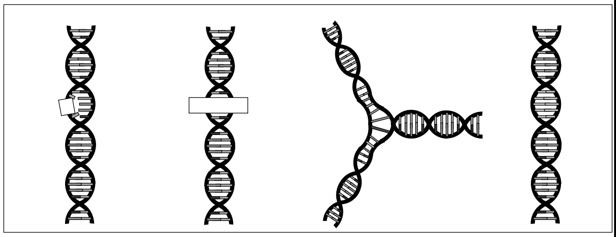
- DNA is the most important molecule in the human body



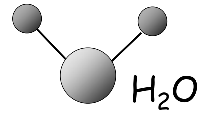
Irradiation of Macromolecules

Radiation Response of DNA

1	2	3	4
Main-chain scission with one side rail severed	Main-chain scission with both sides rail severed	Main-chain scission and subsequent cross-linkage	Change or loss of base: Molecular Lesion (*Point Mutation*)



Radiolysis of Water

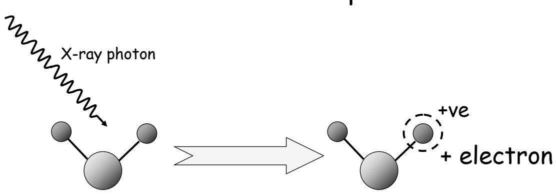


H_2O

- Human body is 80% water
- Irradiation of water represents principle radiation interaction
- Dissociation into other molecules
  - Radiolysis of water

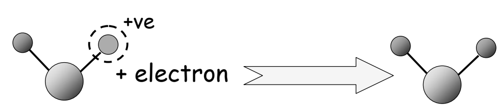
Radiolysis of Water

- Irradiation causes ionization
- Dissociates into two ion pairs



Radiolysis of Water

- After initial ionization
- May rejoin into stabilize water molecule
- May attach to another water molecule



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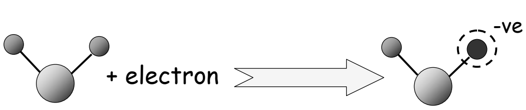
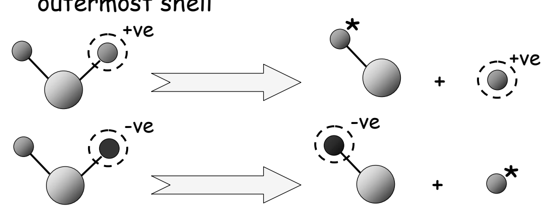


Radiolysis of Water

- HOH^+ & HOH^- are unstable
- Dissociate into smaller molecules
- Free-radicals:
  - Uncharged, contain single unpaired electron in outermost shell

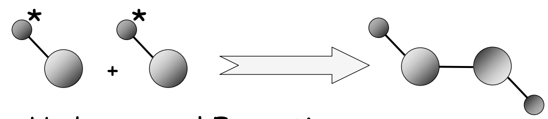


Free Radicals

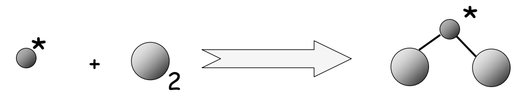
- Highly Active
- Unstable (~1ms)
- Diffuse through cell & interact at distance site
- Excess energy transferred to other molecules, disrupting bonds, causing point lesions
- Can also produces toxins

Free Radicals

- Hydrogen Peroxide Formation



- Hydroperoxyl Formation



Direct & Indirect Effects

<u>Direct Effect</u>

•Initial ionizing event occurs on the most radiosensitive molecule, DNA

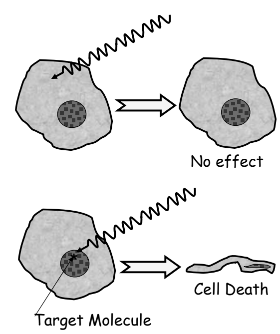
<u>Indirect Effect</u>

•Initial ionizing event occurs on any other molecule (usually water) which transfers energy to the DNA

Principle effect in humans is indirect

Target Theory

- Some abundant molecules
  - Damage causes no noticeable effect
- Some molecules are vital & scarce
  - Damage causes severe effects
- Target theory
  - For a cell to die, target molecule must be inactivated
- Radiation interaction that inactivates target molecule is called a 'Hit'

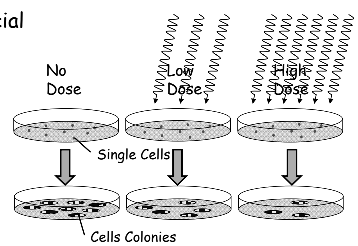


Hit may result from direct or indirect interaction

Cell Survival Kinetics

<u>Measuring lethal effects of radiation on cells</u>

- Cells cultured on artificial growth medium
- Plant individual cells
- Incubate for 10 -14 days
- Each cell divides many times
- Producing visible colonies of 1000s cells

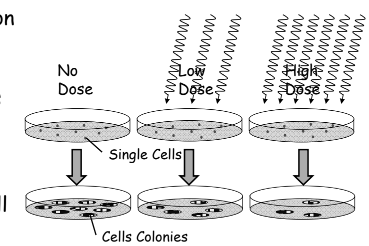


- After irradiation: Some cells die -> Fewer colonies formed

Cell Survival Kinetics

<u>Measuring lethal effects of radiation on cells</u>

- Mathematical extension of target theory
- Produces TWO models of cell survival
- Radiation dose-response for single cell

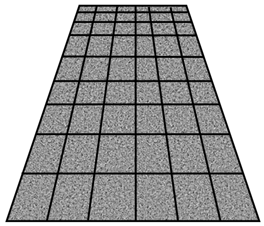


1. Single-Target, Single-Single Hit Model
2. Multitarget, Single-Hit Model

Single-Target, Single-Hit Model

Simple Analogy

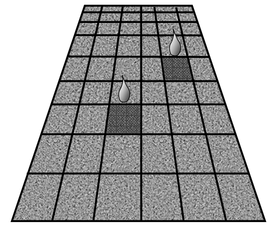
- 100 paving slabs
- Each represents a cell
- It Starts to Rain !
- A slab is considered wet when ONE raindrop has hit it



Single-Target, Single-Hit Model

Simple Analogy

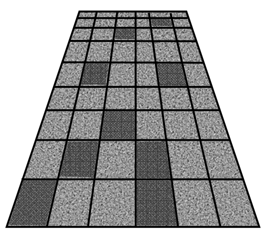
- First raindrop falls
- One Slab WILL be wet
- 1/100 of slabs are wet
- Second raindrop falls
- It will probably hit a dry Slab
- Two slabs will be wet
- 2/100 of slabs are wet



Single-Target, Single-Hit Model

Simple Analogy

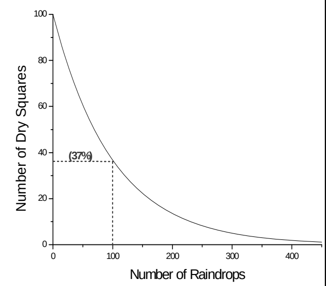
- As the number of raindrops increases it becomes more probably that a square will be hi by more than one drop
- Raindrops are falling RANDOMLY
- Probability that a given slab will become wet governed by *Poisson Statistics*



Single-Target, Single-Hit Model

Poisson Statistics

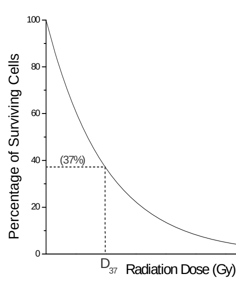
- When 100 raindrops have fallen:
  - 63% slabs will be wet; 37% slabs dry
  - If rain fell uniformly; 100% wet
- 200 raindrops fallen:
  - 0.37x0.37 (14%) dry slabs
- 300 raindrops fallen:
  - 0.37x0.37x0.37 (5%) dry slabs
- And So On…



Single-Target, Single-Hit Model

Extend Analogy to Cells

- A large sample of cells
- Each cell contains ONE target molecule
- D_{37} : Dose required to kill 63% of cells
- If radiation interacted uniformly (no wasted hits)
  - D_{37} would kill 100% cells



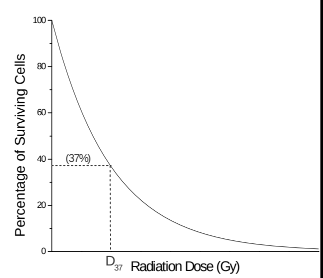
- Lower D_{37}, higher radiosensitivity

Single-Target, Single-Hit Model

Single-Target, Single-Hit Model of Radiation Induced Lethality

$$S = N / N_o = e^{-D/D_{37}}$$

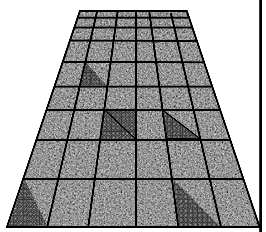
S	=	Surviving fraction
N	=	Number of surviving cells
N_0	=	Initial number of cells
D_{37}	=	Constant dose related to radiosensitivity
D	=	Dose



Multitarget, Single-Hit Model

Simple Analogy

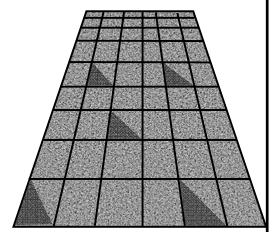
- 100 paving slabs
- Slabs divided into TWO
- I.e. cells with TWO target molecules
- It Starts to Rain !
- A slab is only considered wet when both halves are wet
- Many drop must fall before a square is wet
- Threshold



Multitarget, Single-Hit Model

Simple Analogy

- First few raindrops
  - Only one half of any slab is wet
  - All considered dry
- More raindrops fall
  - Some slabs will have both halves wet
  - Considered wet
- Many rain drops have fallen
  - Most slabs have one wet half
  - Each additional hit causes a wet slab



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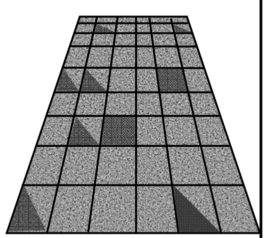

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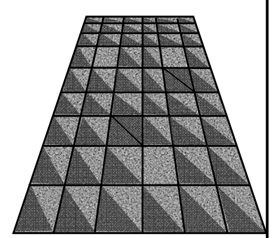
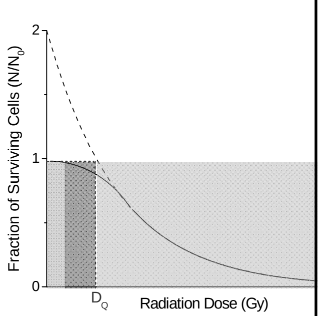


Multitarget, Single-Hit Model

Extend Analogy to cells

- Low Dose – Below Threshold (D_Q)
  - Only one target molecule hit
  - All cells alive
- Intermediate Dose
  - Some cells have both target molecules hit
  - Some dead cells
- High Dose
  - Most cells have received one hit
  - Each additional hit causes death
- High D_Q indicates cell can readily recover

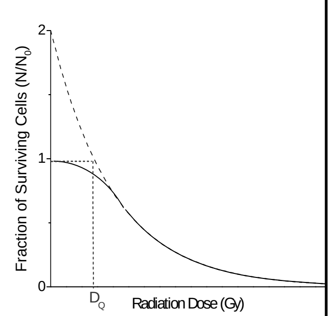


Multitarget, Single-Hit Model

Multitarget, Single-Hit Model of Radiation Induced Lethality

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

- S = Surviving fraction
- N = Number of surviving cells
- N_0 = Initial number of cells
- D_0 = *Mean Lethal Dose* Dose required to reduce survival by 37% after threshold region
- D = Dose
- n = Extrapolation number (N° of target molecules)



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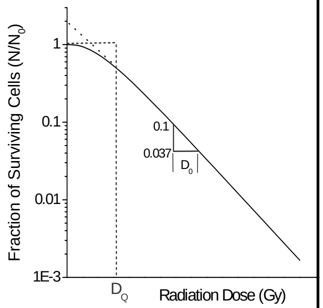


Example

- Blood cells have a radiation response that follows the **multi-target single-hit model** with 3 target molecules.
- An average dose of 5 mGy is required to kill 63% of the cell population beyond the threshold dose.
- What fraction of the cell population would survive a dose of 10 mGy?

Recovery

- Shoulder of the graph show that some damage must accumulate before a cell dies
  - *Sublethal Damage*
- Wider shoulder, more subleathal damage can be sustained

Recovery

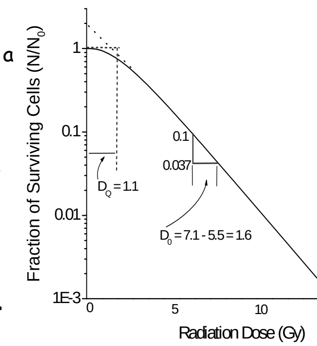
Split Dose Radiation Measuring the Capacity of a Cell to Recover from a Sublethal Dose

Example:

Typical human cell survival curve
D_0 = 1.6 Gy
D_Q = 1.1 Gy
n = 2

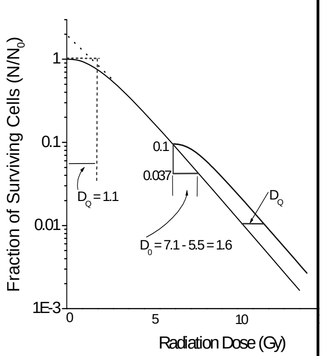
Large Dose: 5.5 Gy

Reincubated in growth media for at least one cell generation



Recovery

- New population used for 2nd cell-survival experiment
- Surviving cells subjected to another dose
- Generate another response curve
- Precisely the same shape as the original curve
- Same extrapolation number
- Separated along dose axis by D_Q
- D_Q is not only a measure of a cells capacity to accumulate sublethal dose
- Also a measure of cell ability to recover



Summary

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- Irradiation of Macromolecules
- Radiolysis of Water
- Direct & Indirect Effects
- Target Theory
- Cell Survival Kinetics
- Single-Target, Single-Hit Model
- Multitarget, Single-Hit Model
- Recovery