TUTORIAL 1

Radiation Damage in Biological Systems

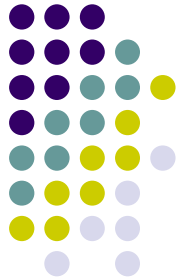
[WG1 – Radiation Damage in Physiological Environments]

DUBLIN INSTITUTE of TECHNOLOGY
Institiúid Teicneolaíochta Bhaile Átha Cliath

References

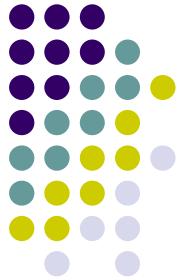
- J.T. Bushberg et al
  - “The Essential Physics of Medical Imaging”, Lippincott Williams and Wilkins, 2nd Edition (2000);
- E.J. Hall
  - “Radiobiology for the Radiologist”, Lippincott Williams and Wilkins, 6th Edition (2006);

Structure



1. Physics and Chemistry of Radiation Absorption;
2. Photon Interaction Cross-Sections;
3. DNA Strand Breaks and Chromosomal Aberrations;
4. Cell-Survival Curves;
5. Non-Targeted Effects;
6. Radiosensitivity and Cell Cycle;
7. Dose Rate Effects and Damage Repair;
8. Acute Effects and Radiocarcinogenesis;
9. Radiation Protection;

Structure



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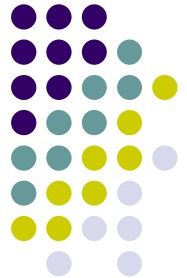
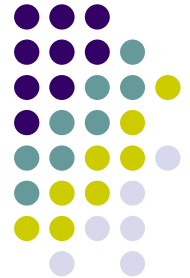
1. Physics and Chemistry of Radiation Absorption



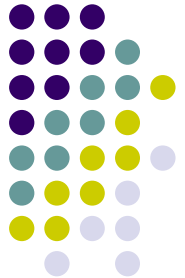
- Radiobiological damage occurs through the absorption of:
  - High energy photons of electromagnetic radiation;
  - Charged particles;
- Photons:
  - X-rays
  - γ-rays
- Charged Particles:
  - α-particles;
  - β-particles / electrons;
  - Protons;
  - Neutrons;
  - Heavy charged particles;

Physics and Chemistry of Radiation Absorption



- Absorption of photons occurs by a process of "**Attenuation**", the probability of occurrence of which depends on the photon energy and material properties;
- Photon Energy $E=h\nu$ (E~ 5 eV or above for molecular damage):
  - Packets (particles) of radiant energy;
  - Unit of energy = "electron-volt (eV)";
  - "1 eV = amount of energy required to move an electron through a potential difference of 1V";
  - h=Planck's Constant (6.626×10^{-34} Js);
  - ν=frequency of photon;

Structure



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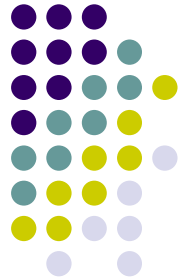
Photon Interaction Cross-sections



- **“Interaction Cross-Section” ≡ Probability of interaction;**
- Dependent on:
  - Photon energy;
  - Characteristics of material absorbing energy (Z, A);
- Interactions may be divided into:
  - Rayleigh Scatter;
  - Photoelectric Effect (τ);
  - Compton Scatter (σ);
  - Pair Production (κ);

Rayleigh Scatter

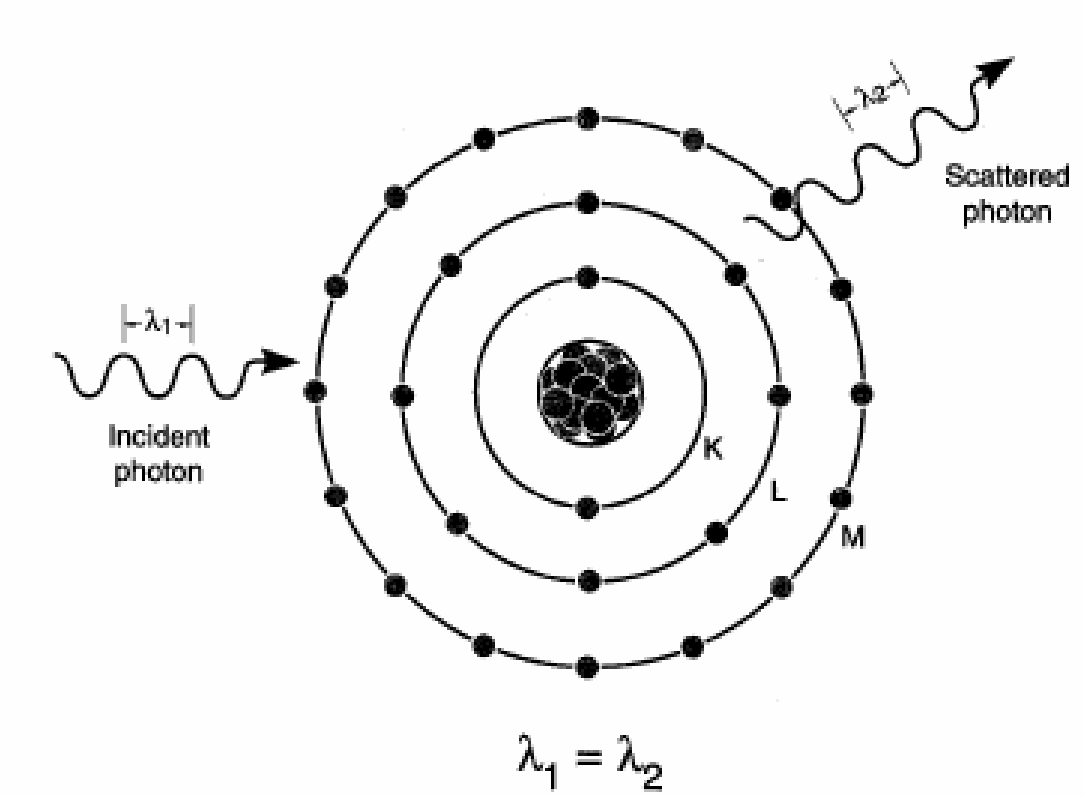


FIGURE 3-6. Rayleigh scattering. Diagram shows the incident photon λ_1 interacts with an atom and the scattered photon λ_2 is being emitted with approximately the same wavelength and energy. Rayleigh scattered photons are typically emitted in the forward direction fairly close to the trajectory of the incident photon. *K*, *L*, and *M* are electron shells.

Characteristics

1. Energy of Incident Photon = Energy of Scattered Photon;
2. Important @ photon energies<10 keV

Photoelectric Effect

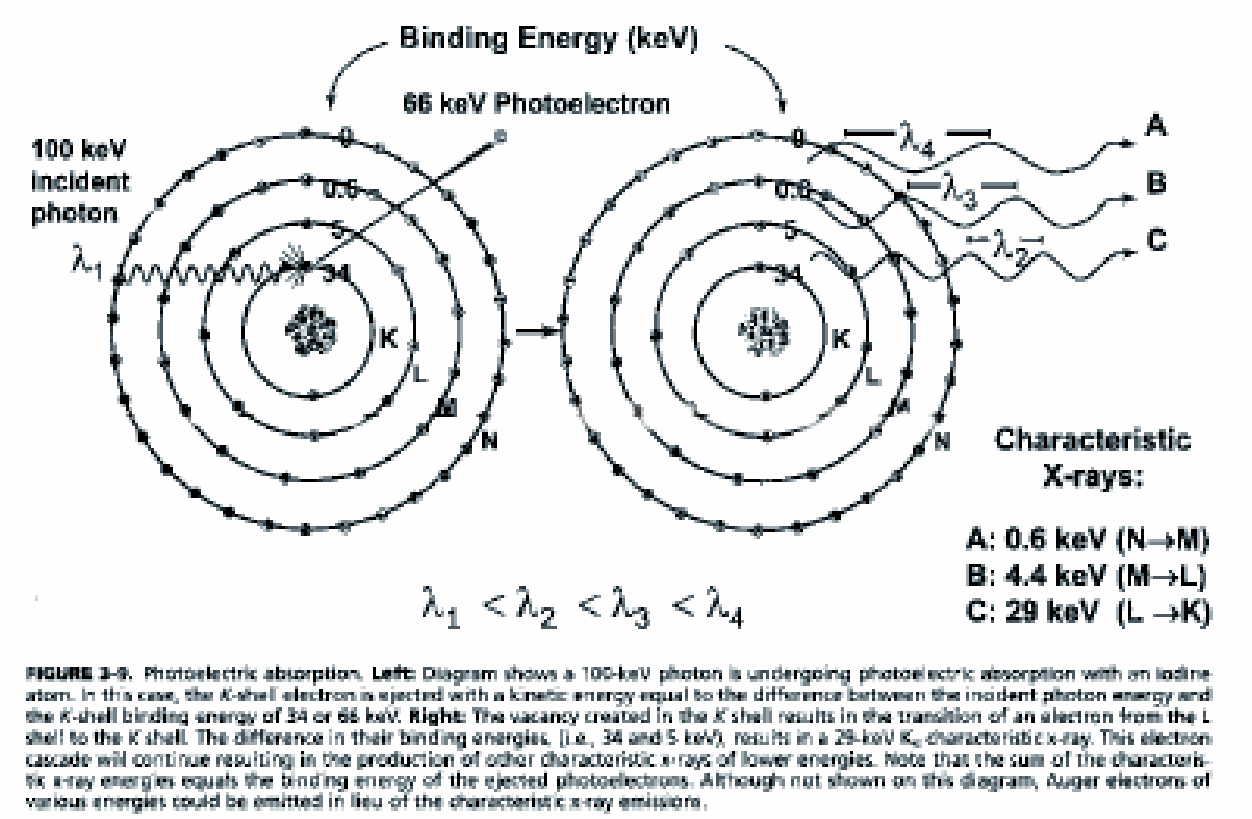


FIGURE 3-9. Photoelectric absorption. **Left:** Diagram shows a 100-keV photon is undergoing photoelectric absorption with an iodine atom. In this case, the *K*-shell electron is ejected with a kinetic energy equal to the difference between the incident photon energy and the *K*-shell binding energy of 34 or 66 keV. **Right:** The vacancy created in the *K* shell results in the transition of an electron from the L shell to the *K* shell. The difference in their binding energies, (i.e., 34 and 5 keV), results in a 29-keV K_α characteristic x-ray. This electron cascade will continue resulting in the production of other characteristic x-rays of lower energies. Note that the sum of the characteristic x-ray energies equals the binding energy of the ejected photoelectrons. Although not shown on this diagram, Auger electrons of various energies could be emitted in lieu of the characteristic x-ray emissions.

Characteristics

1. Energy of Incident photon totally absorbed;
2. Fast electron produced;
3. Cross section $\propto Z^3$ and $\propto 1/(h\nu)^3$

Compton Scatter

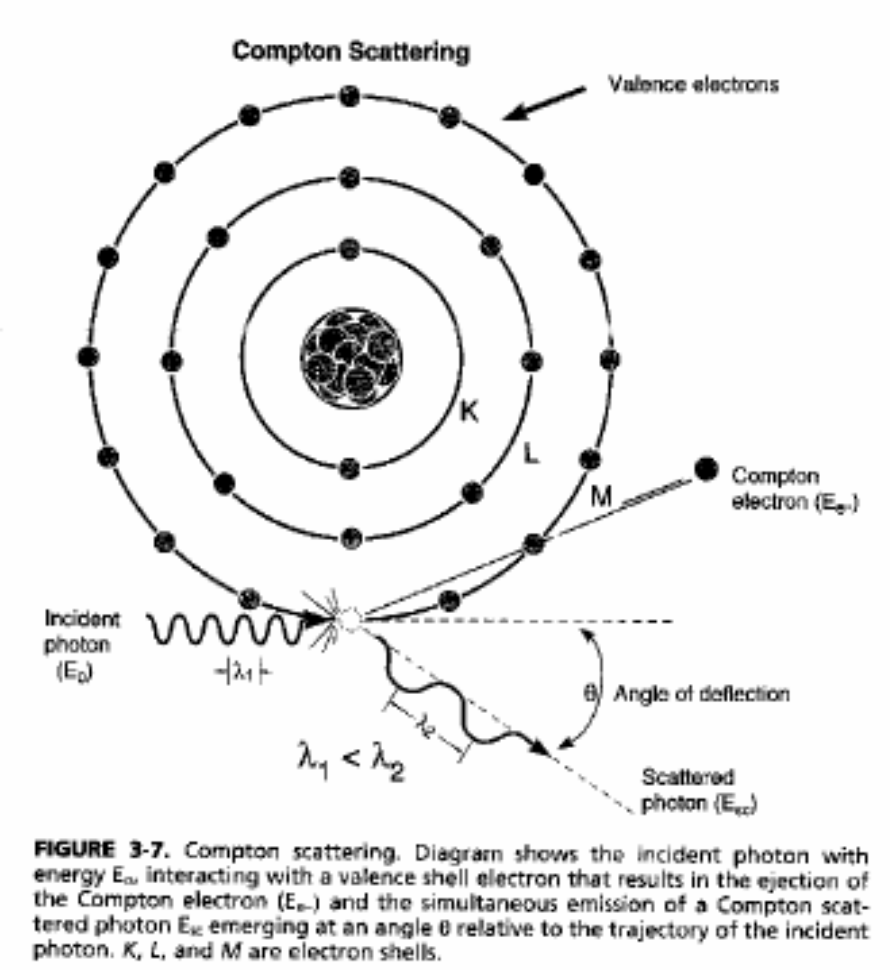


FIGURE 3-7. Compton scattering. Diagram shows the incident photon with energy E_0 interacting with a valence shell electron that results in the ejection of the Compton electron (E_{e-}) and the simultaneous emission of a Compton scattered photon E_{sc} emerging at an angle θ relative to the trajectory of the incident photon. *K*, *L*, and *M* are electron shells.

Characteristics

1. Energy of Incident photon not totally absorbed;
2. Fast electron and scattered photon produced;
3. Cross section $\propto$ Z/A and $\propto$ 1/(hν);

Pair Production

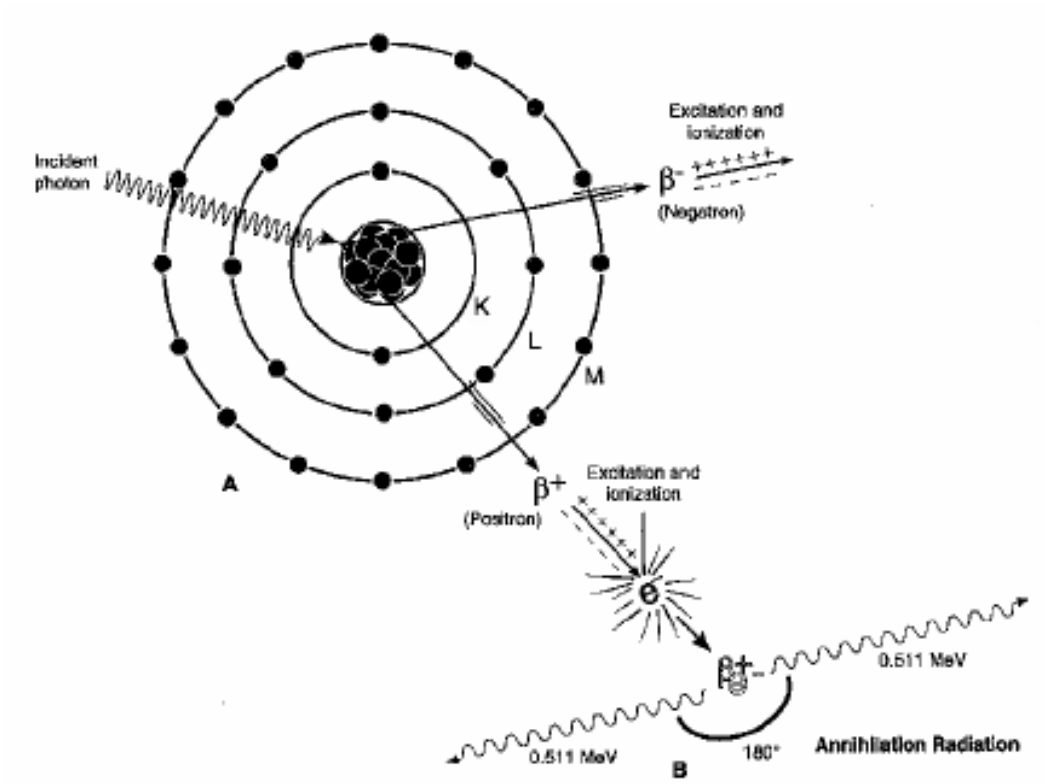


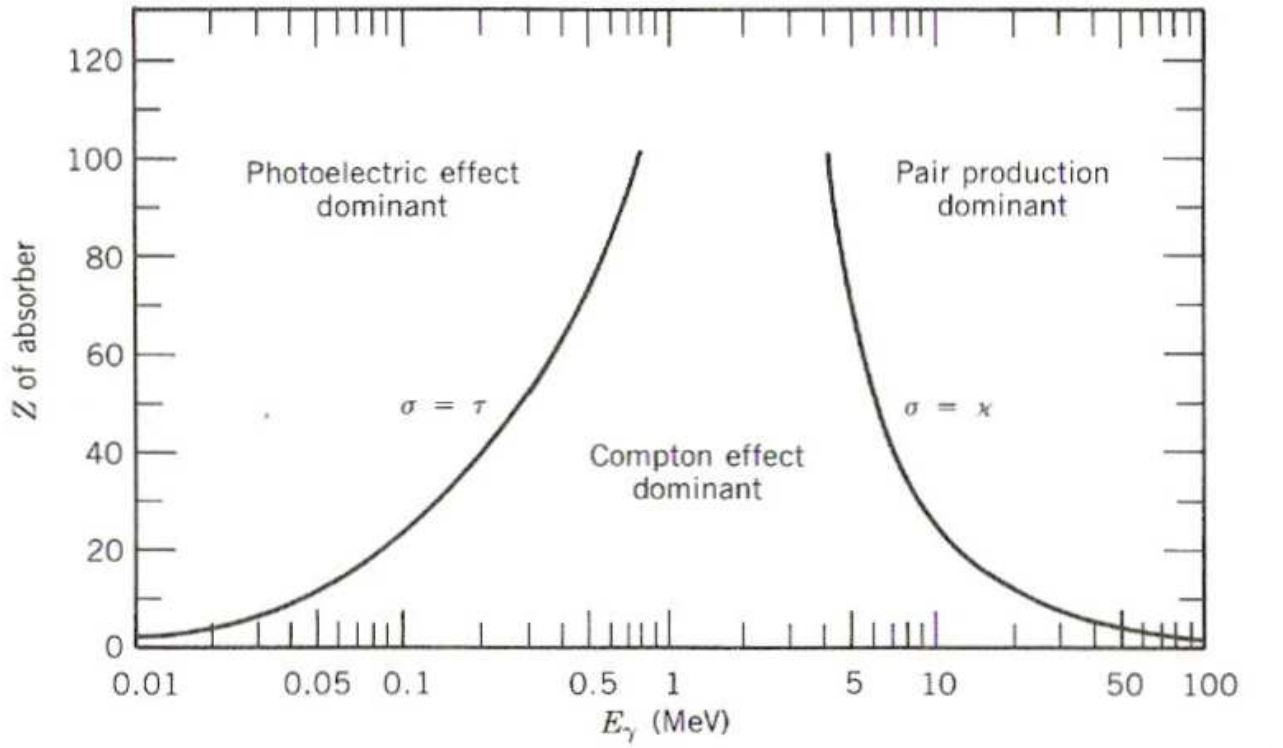
FIGURE 3-12. Pair production. **A:** Diagram illustrates the pair production process in which a high-energy incident photon, under the influence of the atomic nucleus, is converted to the matter and antimatter pair. Both electrons (positron and negatron) expend their kinetic energy by excitation and ionization in the matter they traverse. **B:** However, when the positron comes to rest, it combines with an electron producing the two 511-keV annihilation radiation photons. *K*, *L*, and *M* are electron shells.

Characteristics

1. Consequence of Relativity;
2. Photon converts to β^-(electron) and β^+(positron) in field of nucleus;
3. Only occurs when $h\nu \geq 1.022$ MeV;
4. Cross section $\propto Z$;

Combination of Effects

$\mu = \tau+\sigma+\kappa$ (total combined attenuation)



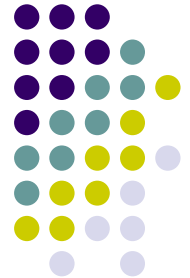
Characteristics

$$\tau \propto \left[\frac{Z}{h\upsilon}\right]^3$$

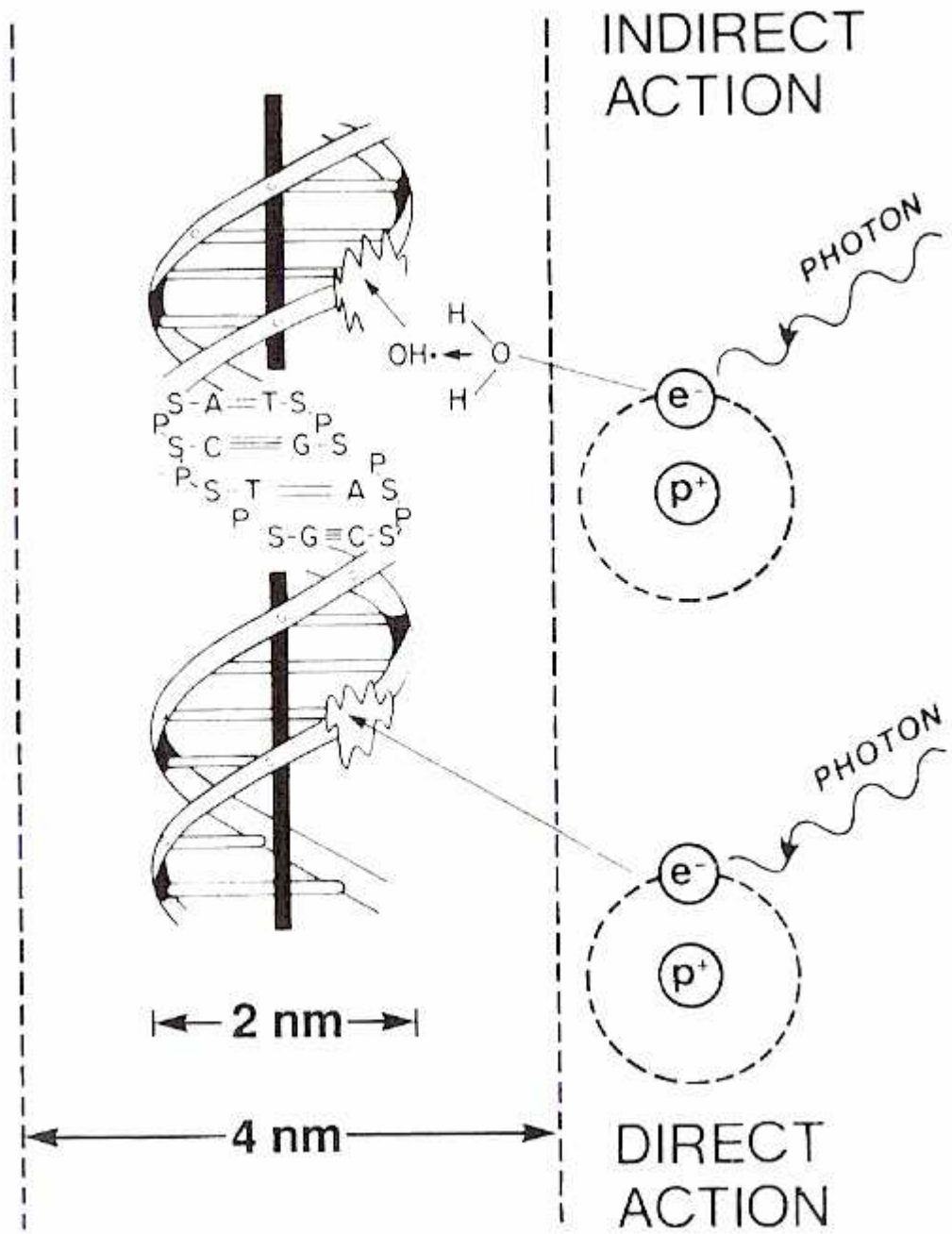
$$\sigma \propto \left[\frac{Z/A}{h\upsilon}\right]$$

$$\kappa \propto Z$$

Physics and Chemistry of Radiation Absorption



- Absorption of photons (X-rays or γ-rays) occurs by either:
  - Indirect interactions;
  - Direct interactions;
- Direct:
  - ‘Fast’ electrons / positrons produced by photon interactions collide directly with DNA;
- Indirect:
  - Photons interact with cellular material producing reactive species which subsequently collide and react with DNA;



•Cell is composed of 80% H_2O;

•Photons can interact with H_2O producing free radicals which can migrate and interact with critical targets;

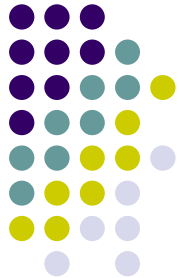
•$H_2O \rightarrow H_2O^+ + e^-$ (Short-lived ~10^{-10}s);

•$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^\bullet$;

•OH radical highly reactive (Long-lived ~10^{-5}s);

• ~66% of X-ray damage to DNA occurs by this process;

Structure



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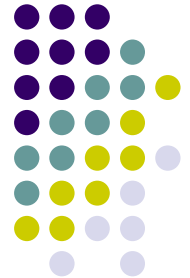
DNA Strand Breaks and Chromosomal Aberrations



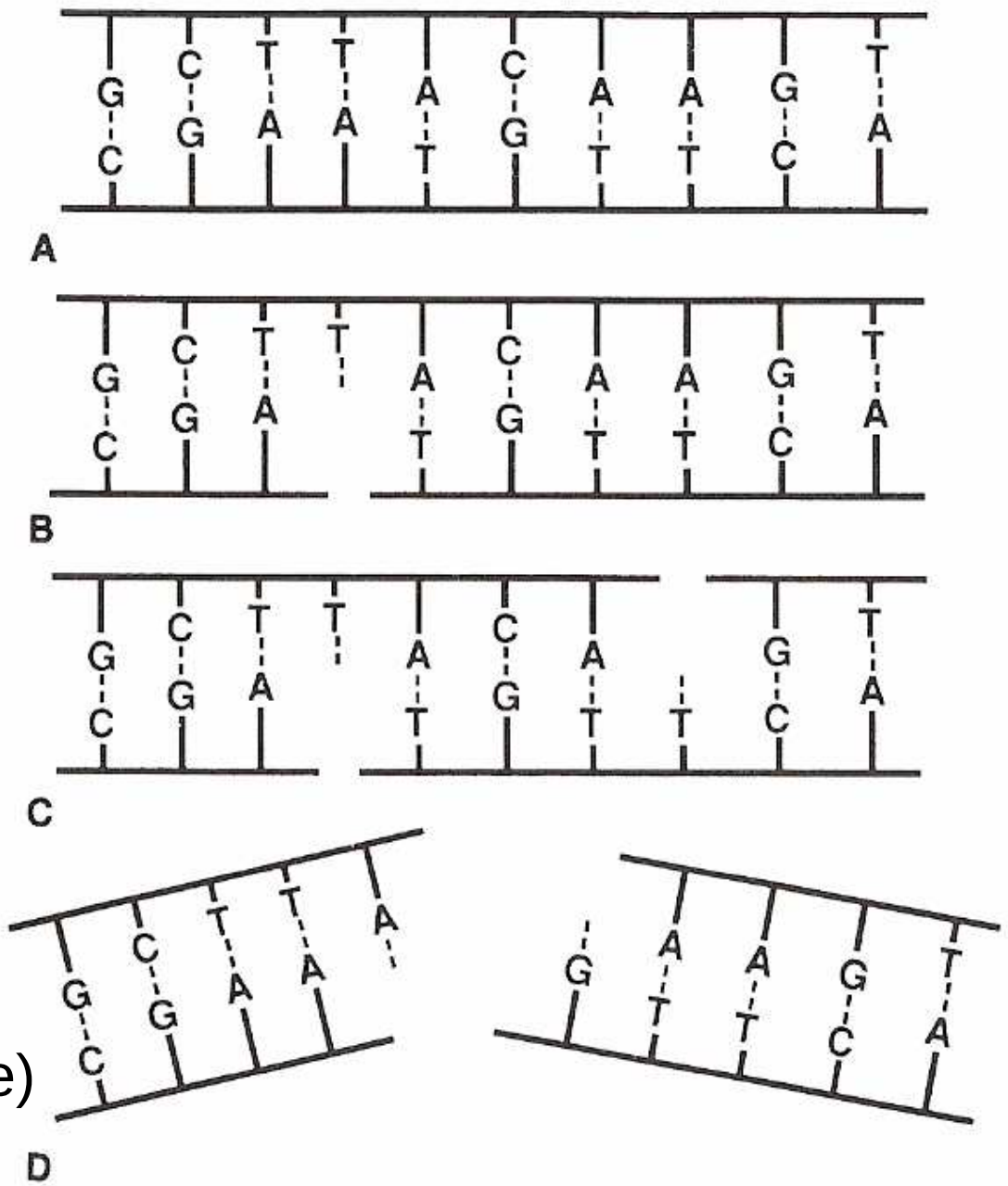
- Irradiation with X-rays induces many single strand breaks in DNA;
- This can be repaired using the other strand as a template;
- Incorrect repair can result in a mutation;
- If breaks are **not** opposite one another, this can be readily repaired;
- If breaks **are** opposite one another, a double strand break can occur;

Intact DNA

SSB

DSB
(reparable)

DSB
(irreparable)

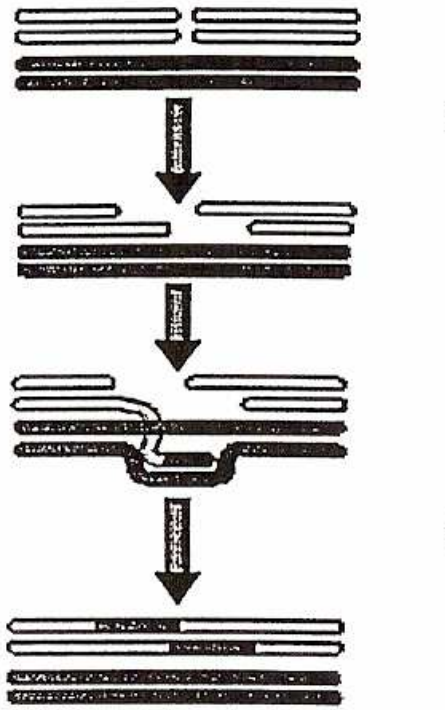


Can result in cell-killing, mutation or carcinogenesis

DNA DSB Repair Processes

Error Free

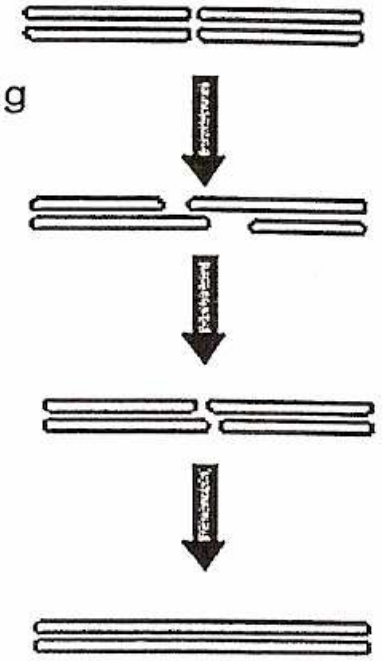
Rare in mammalian cells



Homologous Recombination

Processing

Rejoining



Illegitimate Recombination

Error Prone

Non-homologous end joining, BER;

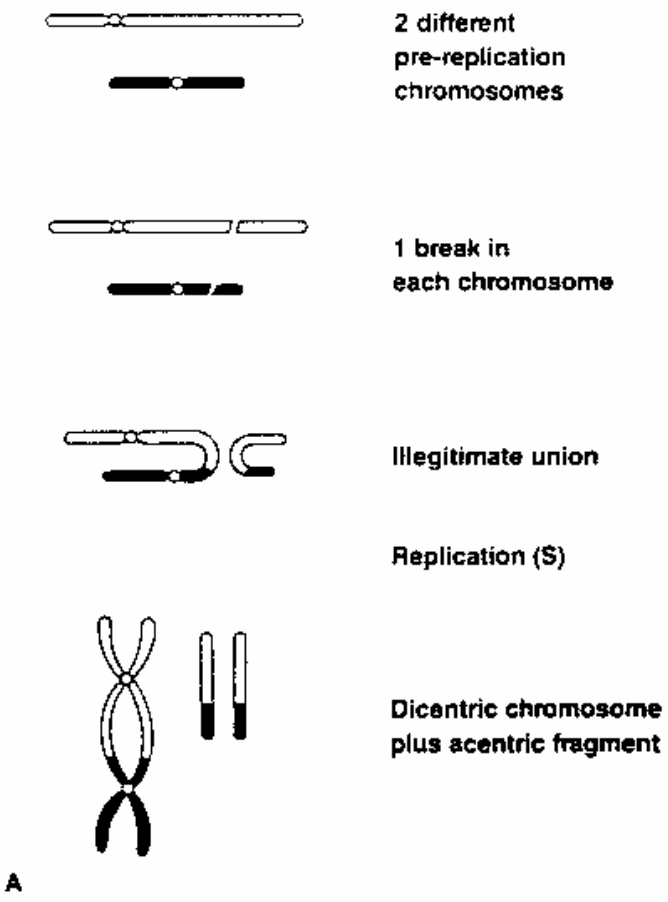
DNA dependant protein kinase, γH2AX, p95

D_0 for mammalian cells ~ 1 - 2Gy:

- Base Damage > 1000;
- SSB ~ 1000;
- DSB ~ 40;

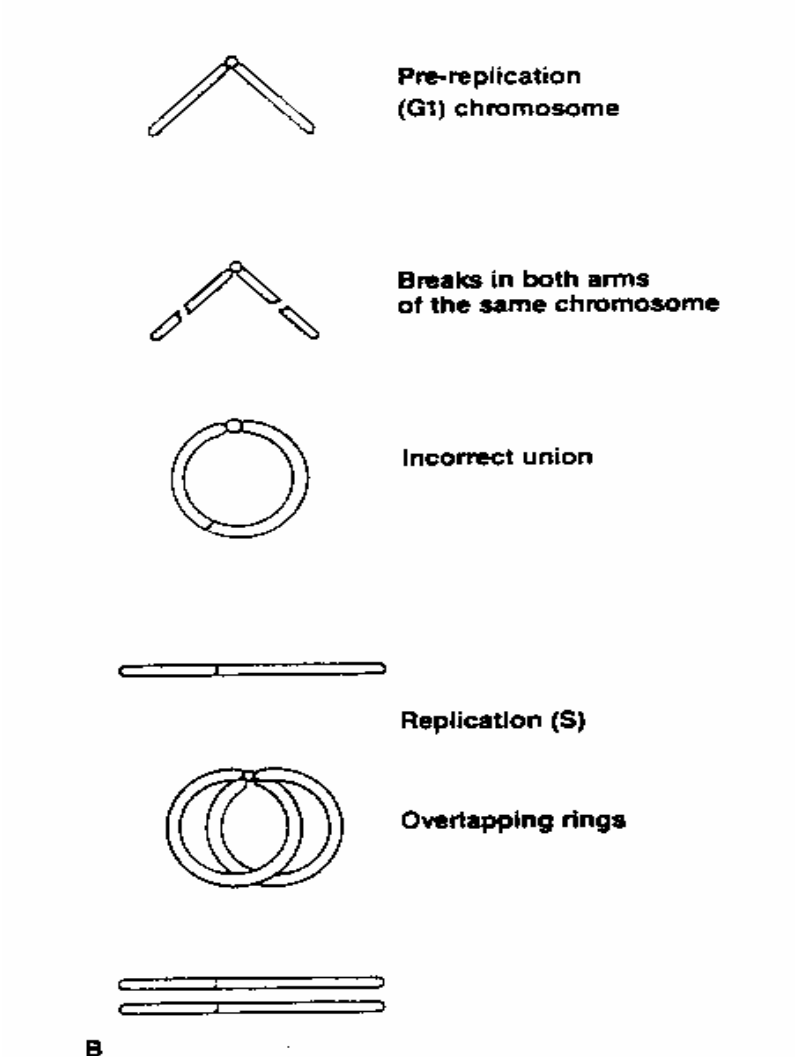
⇒ Cell Killing ∝ No. DSB's

Lethal Aberration 1: Dicentric Chromosome



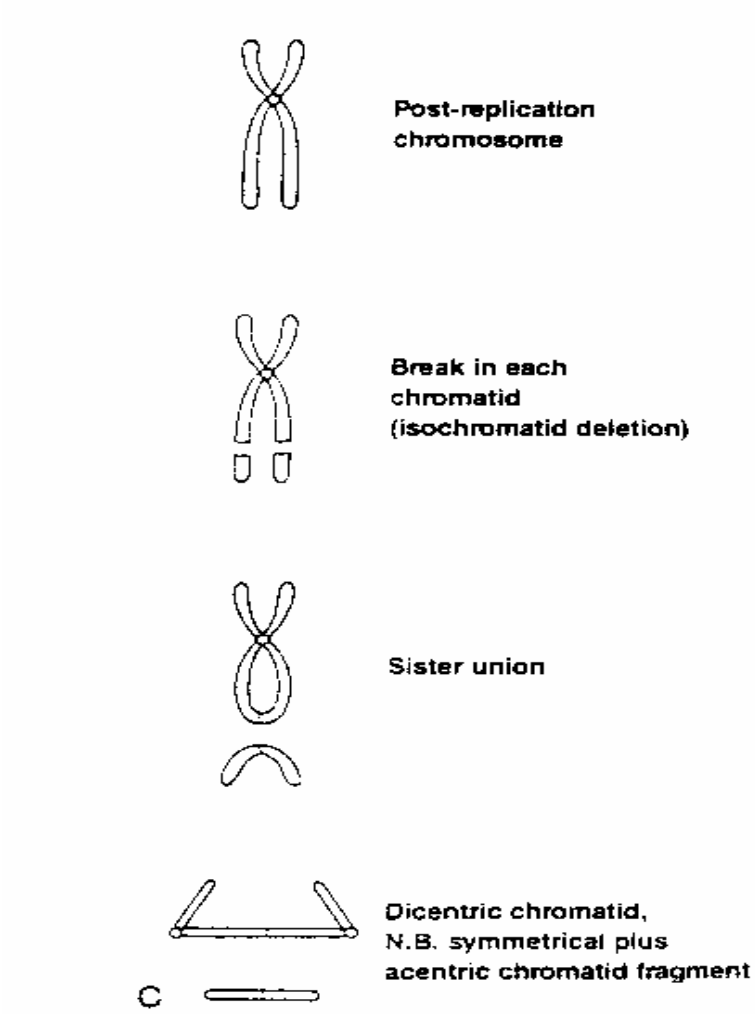
Interchange between two separate chromosomes

Lethal Aberration 2: Centric Ring



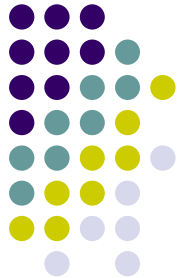
Break induced in each arm of single chromatid early in cycle.

Lethal Aberration 3: Anaphase Bridge



Breaks occur late in cycle in single chromatid

Structure



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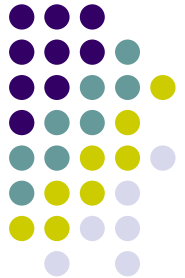
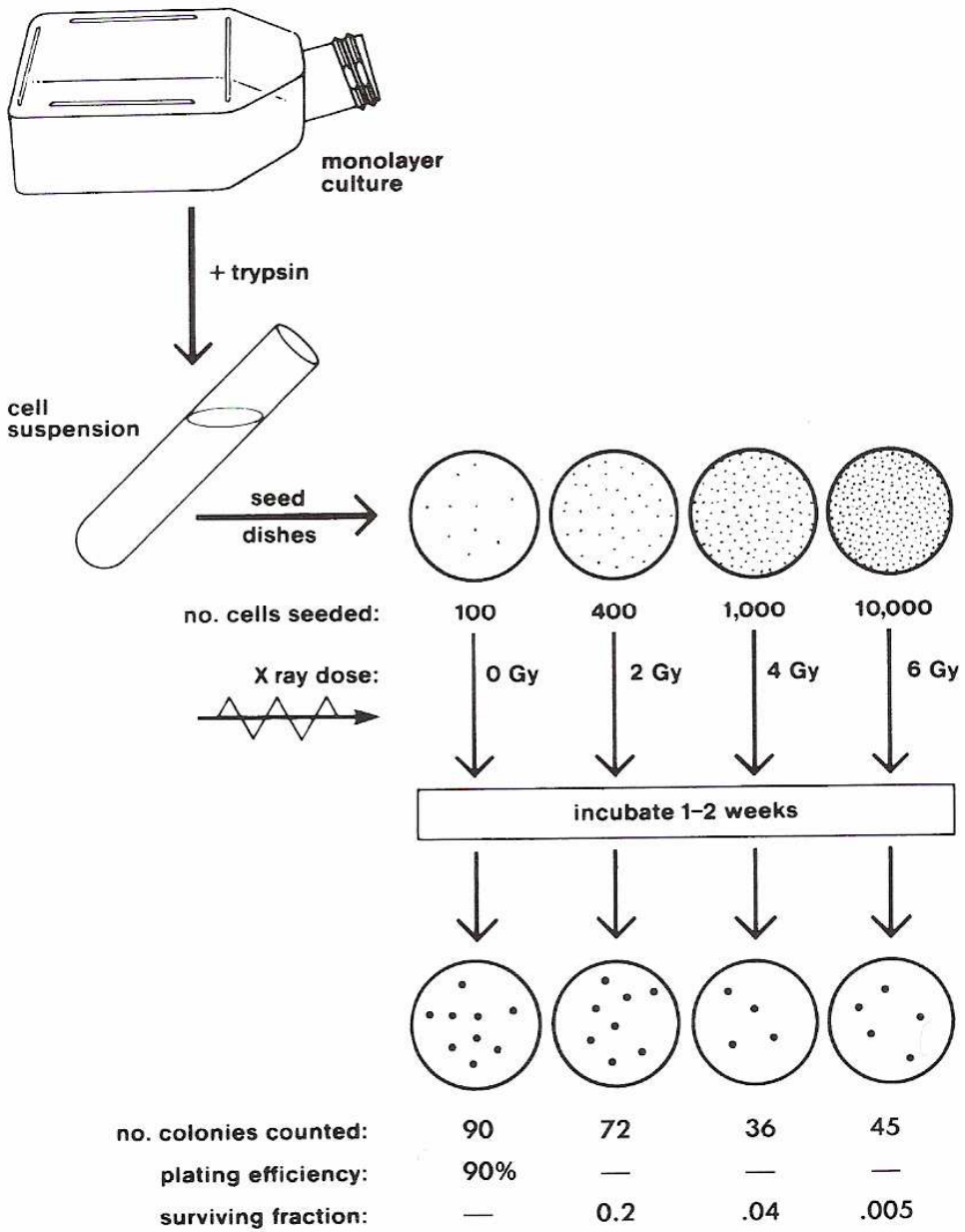
Cell Survival Curves



- Describes relationship between the number of surviving cells post-radiation exposure and the radiation dose;
- Measure of the *reproductive integrity* of the cell – ultimate measure of cell function post-irradiation;
- Commonly referred to as *Clonogenic Assay*;

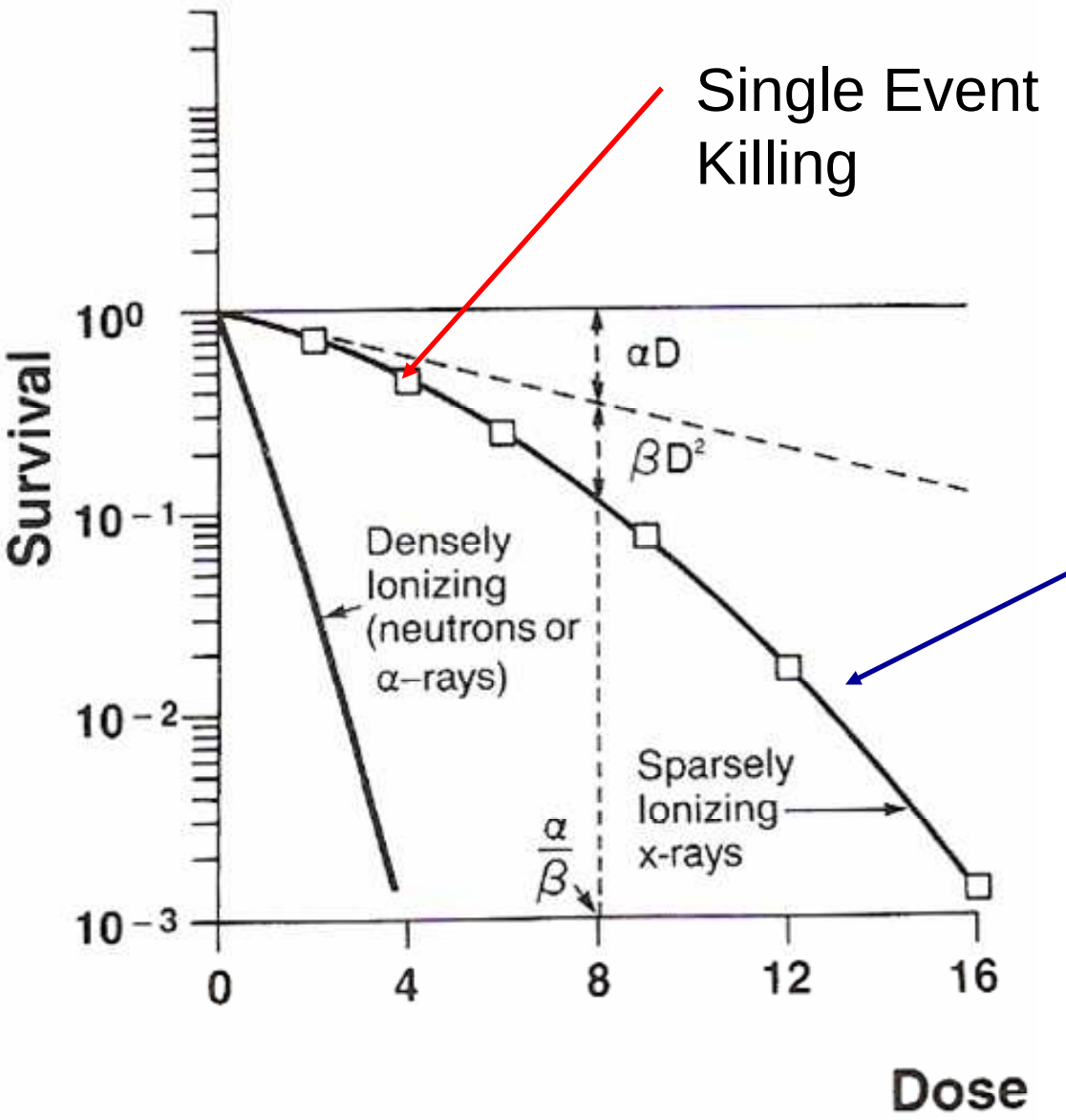


Surviving Fraction:

$$\frac{\text{Colonies Counted}}{\text{Cells Seeded} \times (\text{PE}/100)}$$

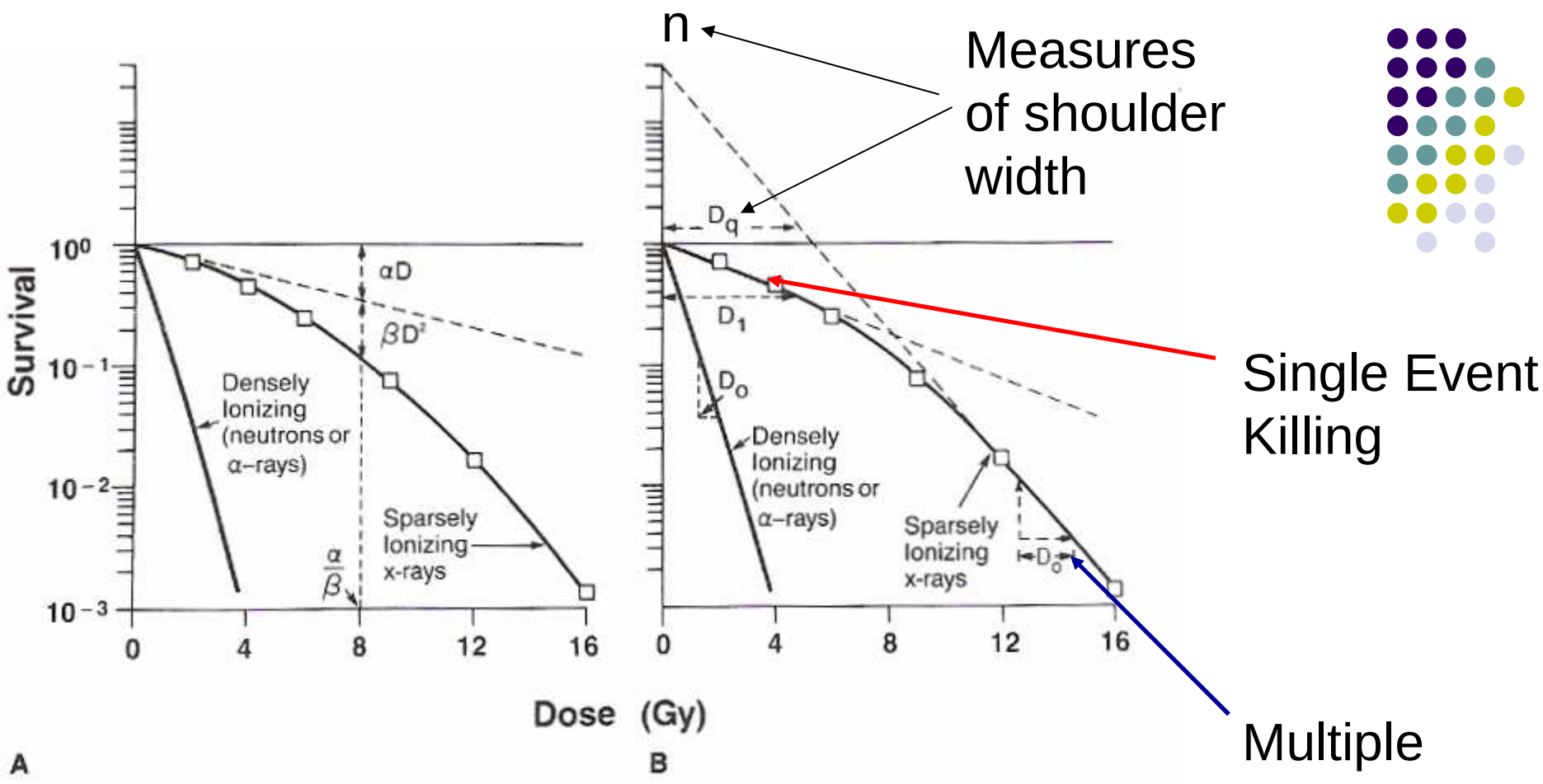
PE=Plating Efficiency:

$$\frac{\text{No. of surviving colonies in control}}{\text{Total number of seeded cells}}$$



A

Model: $S = e^{-\alpha D - \beta D^2}$ (Linear Quadratic Model)

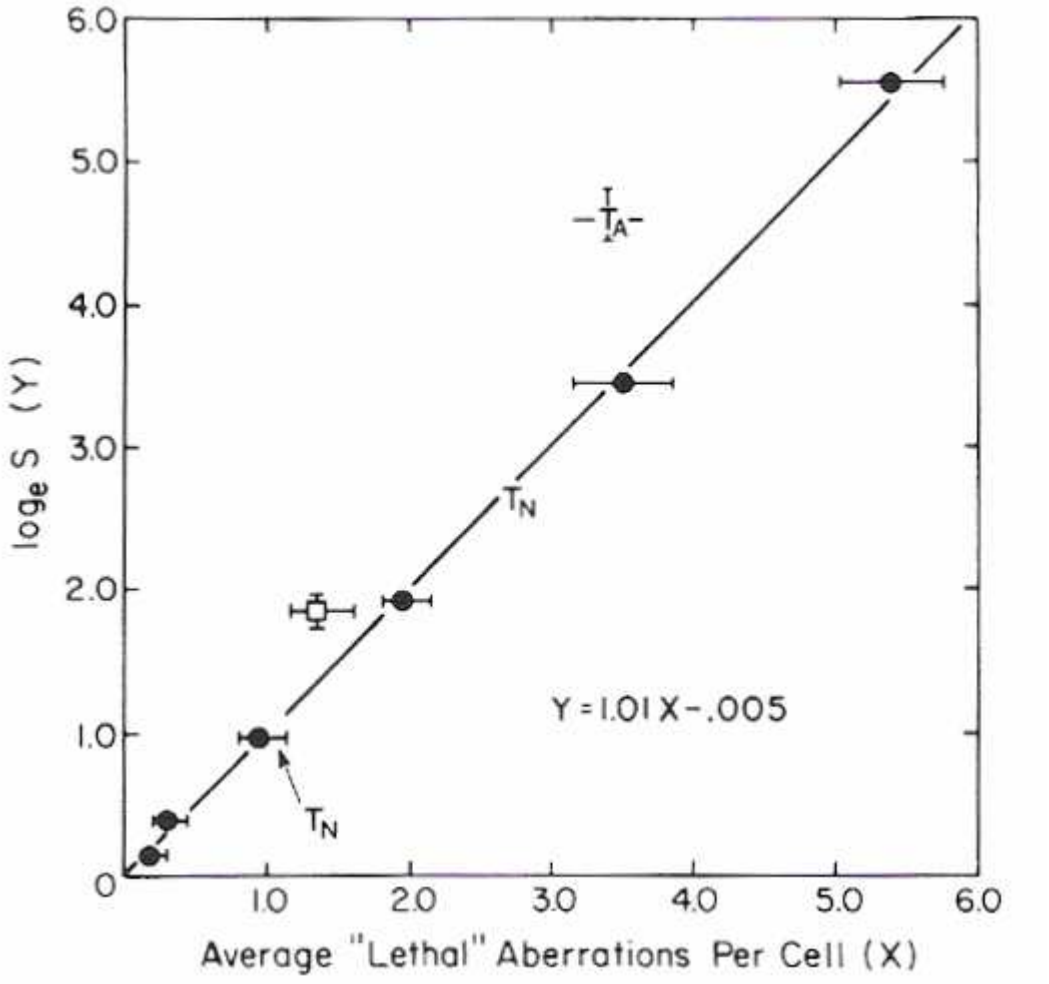


Model: $S = e^{-\alpha D - \beta D^2}$ (Log scale)

S = Survival; D = Dose; α, β are constants (linear and quadratic contributions)

D_1 and D_0 are reciprocals of initial and final slopes

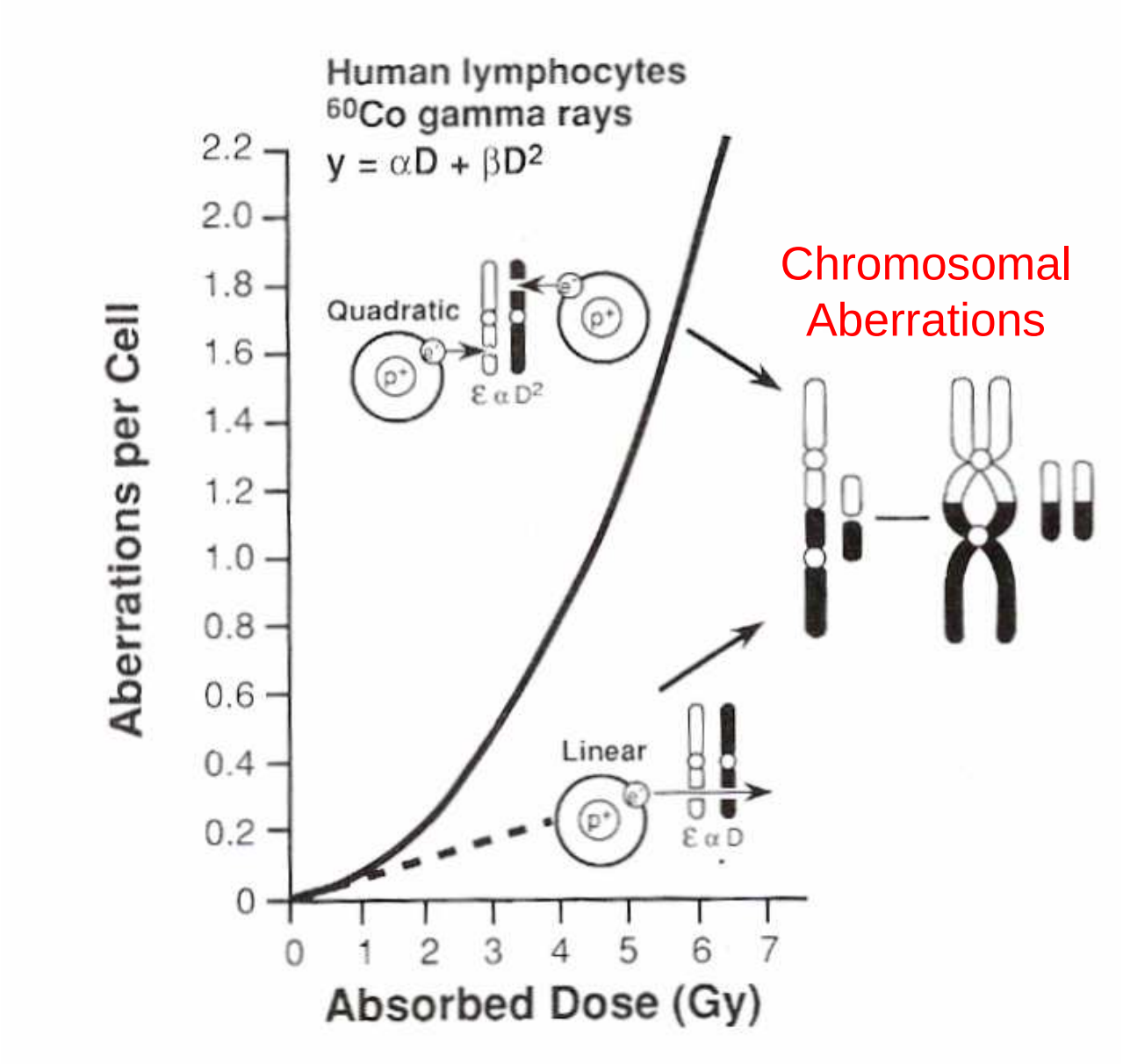
DNA IS THE TARGET



Conforth MN, Bedford JS, Radiat. Res., 111: 385-405, 1987

AC1522 normal human fibroblasts exposed to x-rays

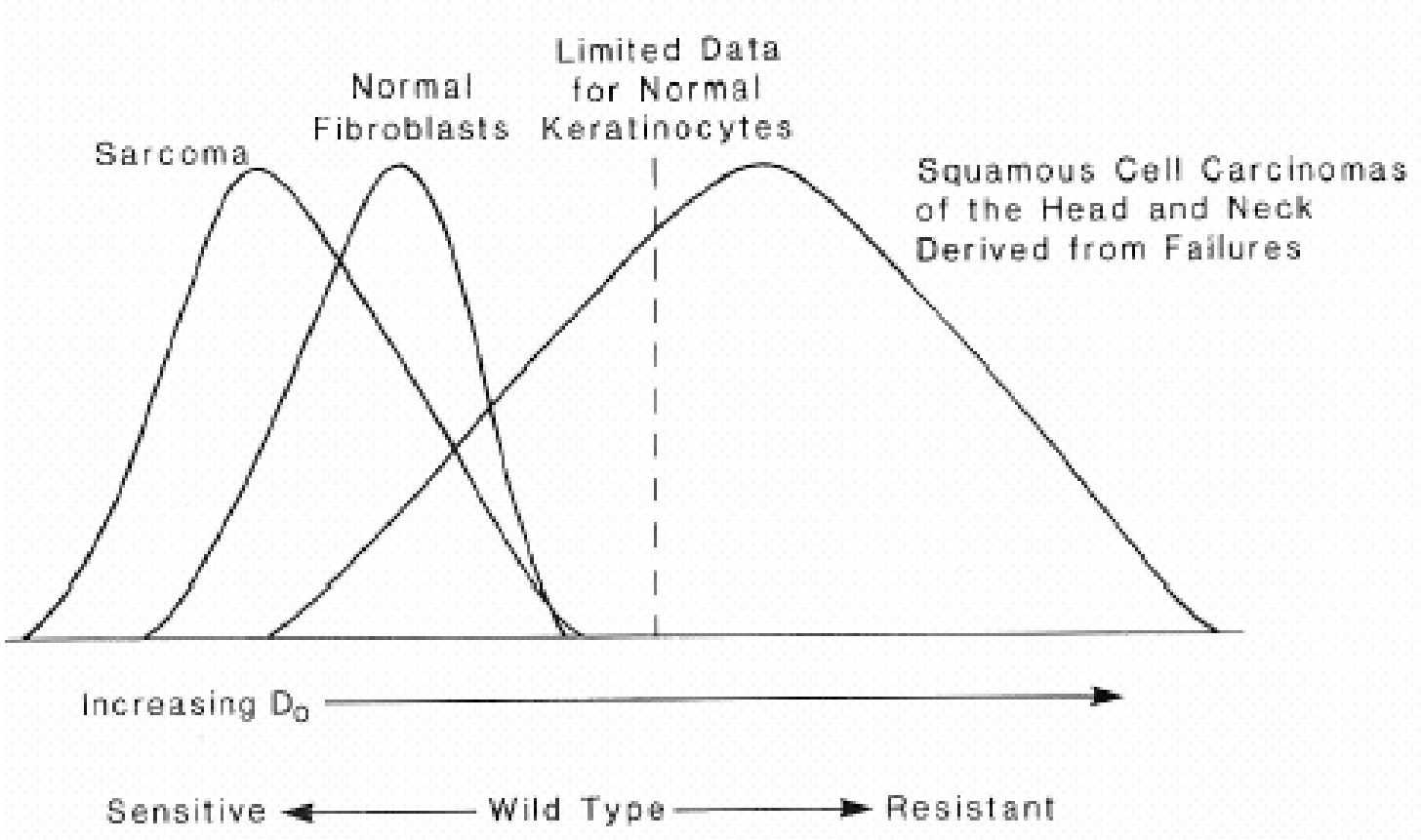
Linear Quadratic Response



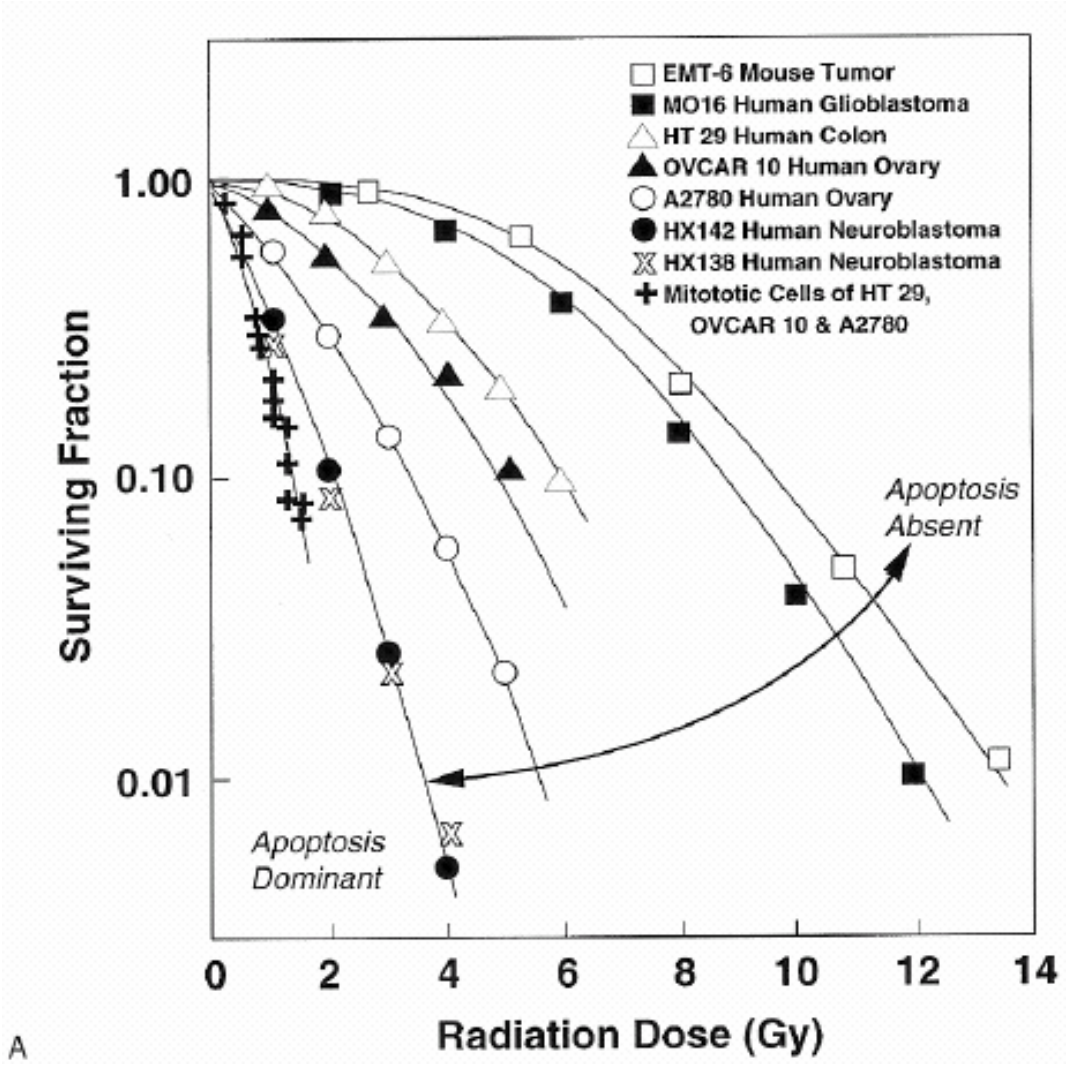
At low doses the two chromosomal breaks are the product of a single electron traversal.

At higher doses two electron events have a higher probability.

D_0 as Measure of Cellular Radiosensitivity



Radiosensitivity is Correlated with Apoptosis



Most radioresistive cell lines show no evidence of apoptosis, while the most radiosensitive show almost complete dominance of apoptosis

Apoptosis vs Necrosis

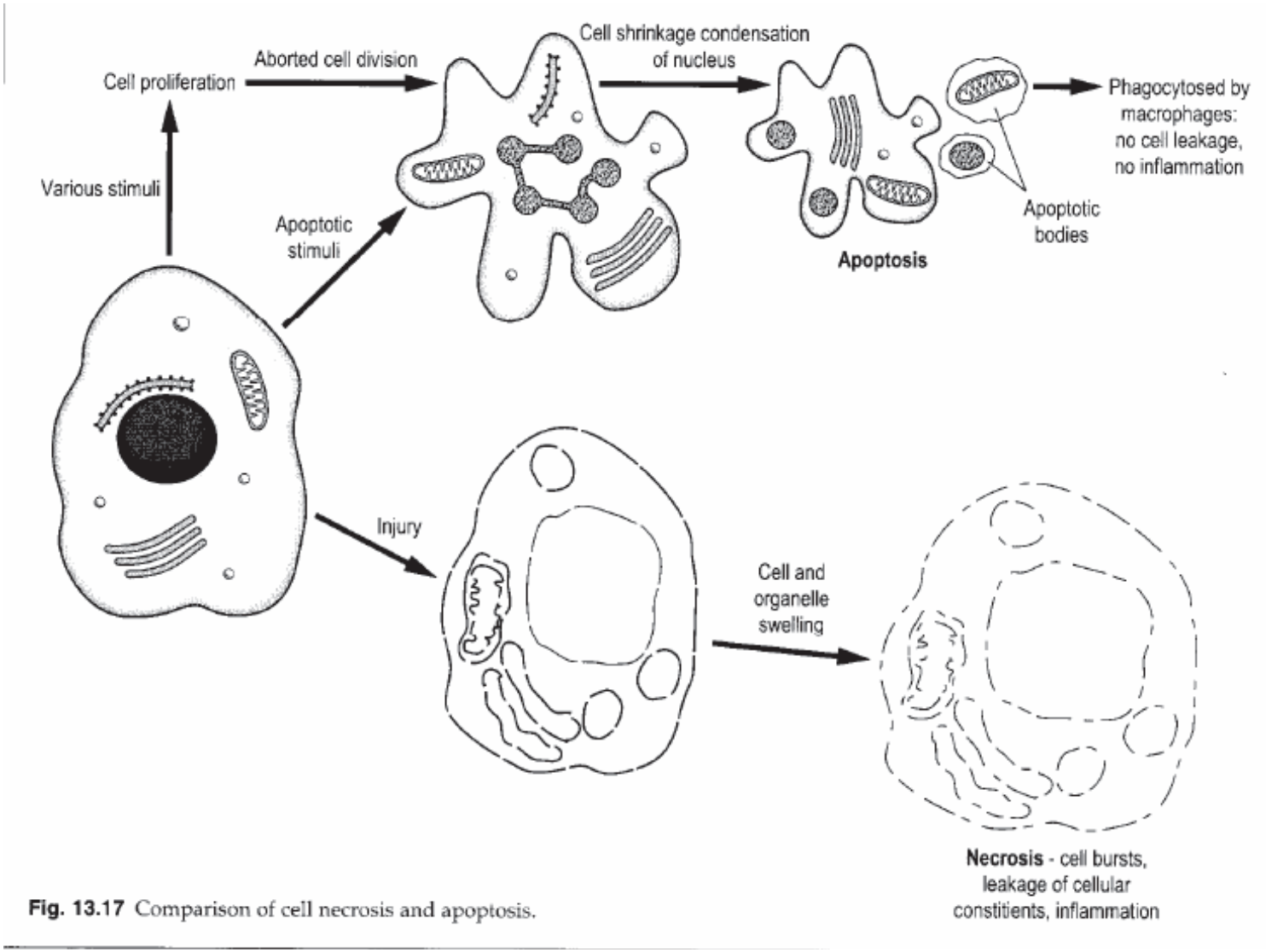
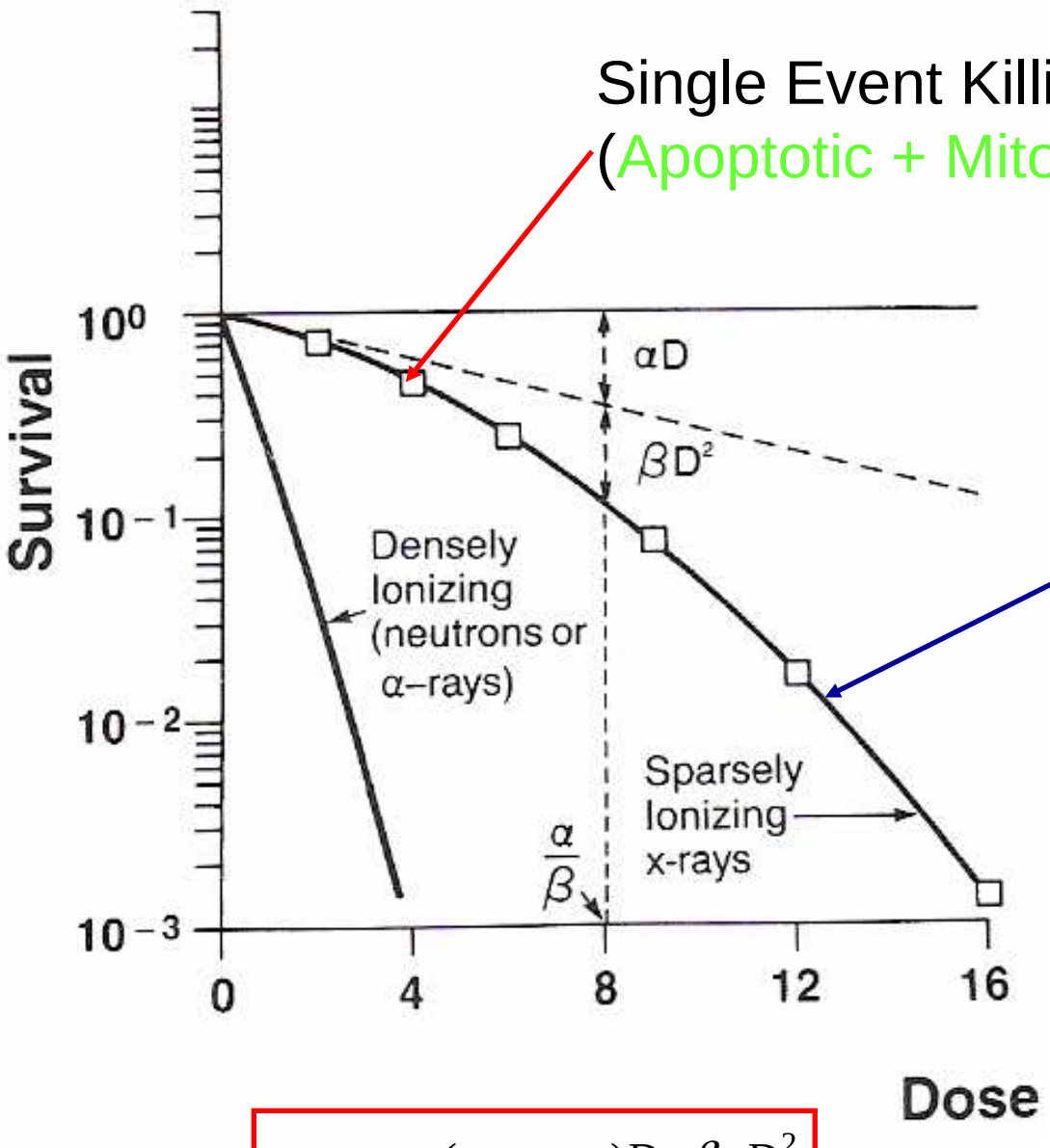


Fig. 13.17 Comparison of cell necrosis and apoptosis.

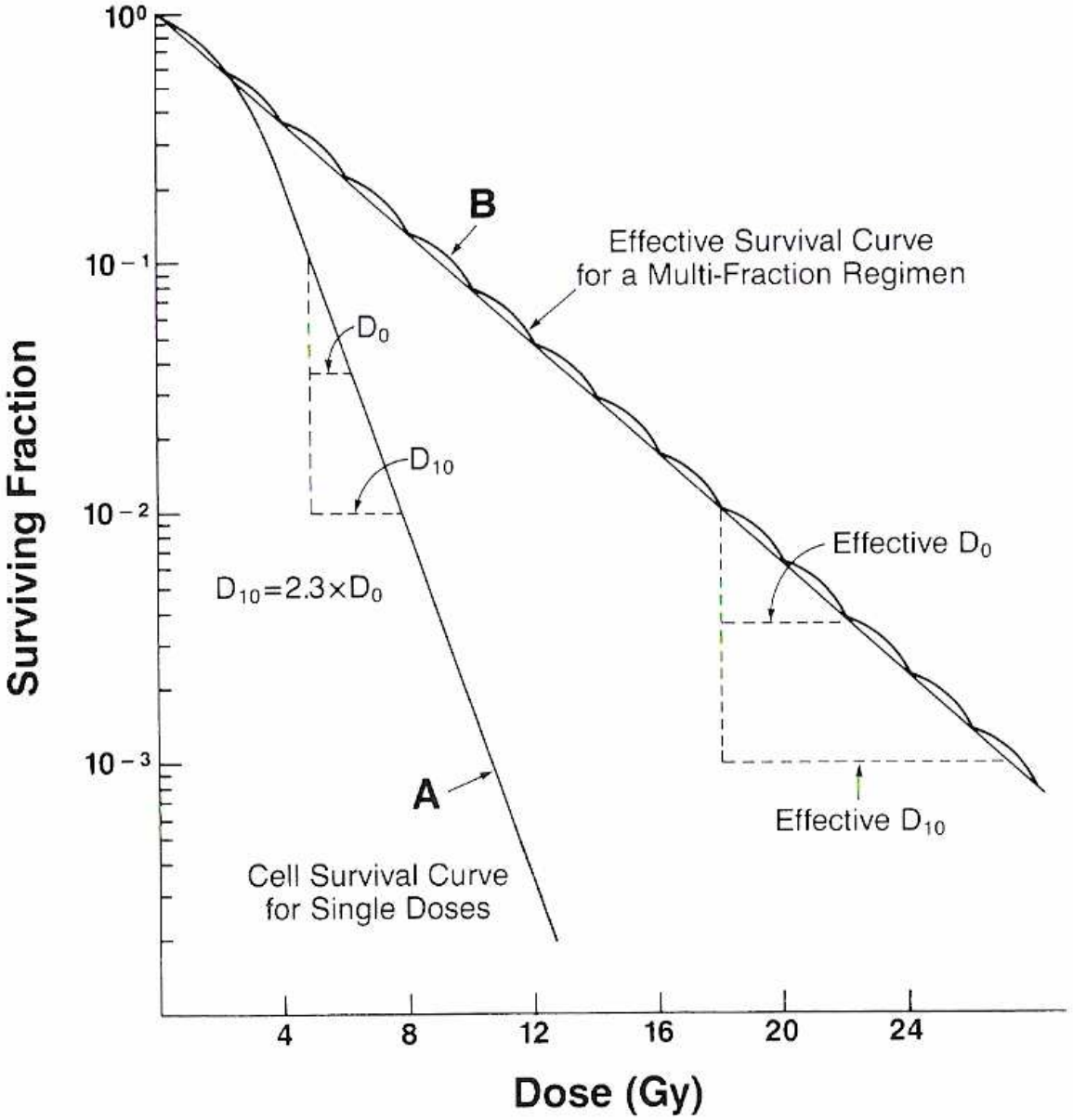


Model:

$$S = e^{-(\alpha_M + \alpha_A)D - \beta_M D^2}$$

(Linear Quadratic Model with compensation for cell killing mechanism)

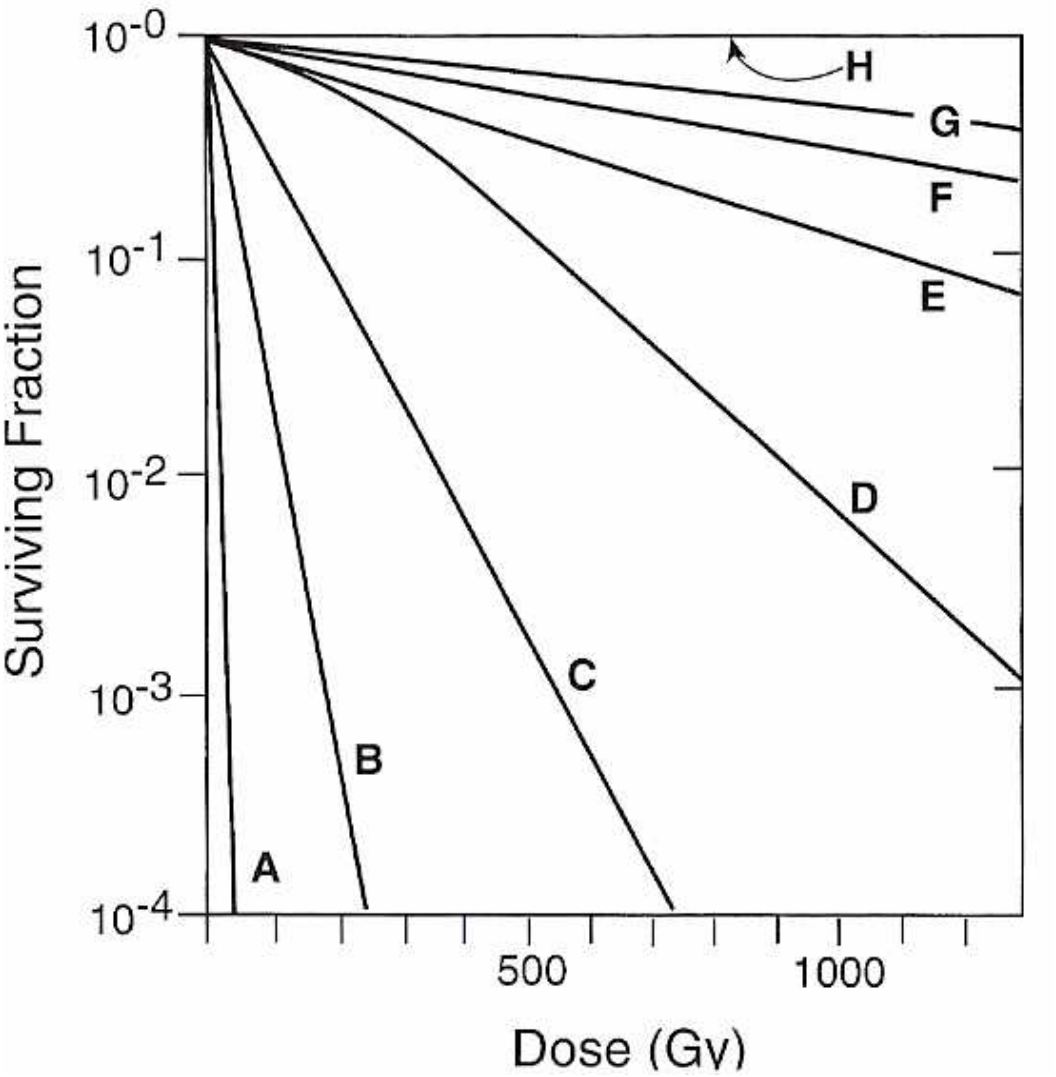
“Effective Cell Survival Curve” for Fractionated Doses



Shoulder of response is repeated a number of times such that response becomes practically linear.

Effective D_0 ~ 3 Gy for all human cells.

Radiosensitivity of Microorganisms



A: Mammalian Cells

B/C: E. Coli

D: Yeast

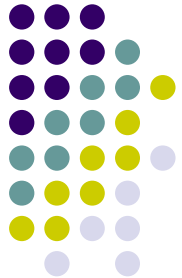
E: Phage Staph

F: B. Megatherium

G: Potato Virus

H: Deinococcus Radiodurans

Structure



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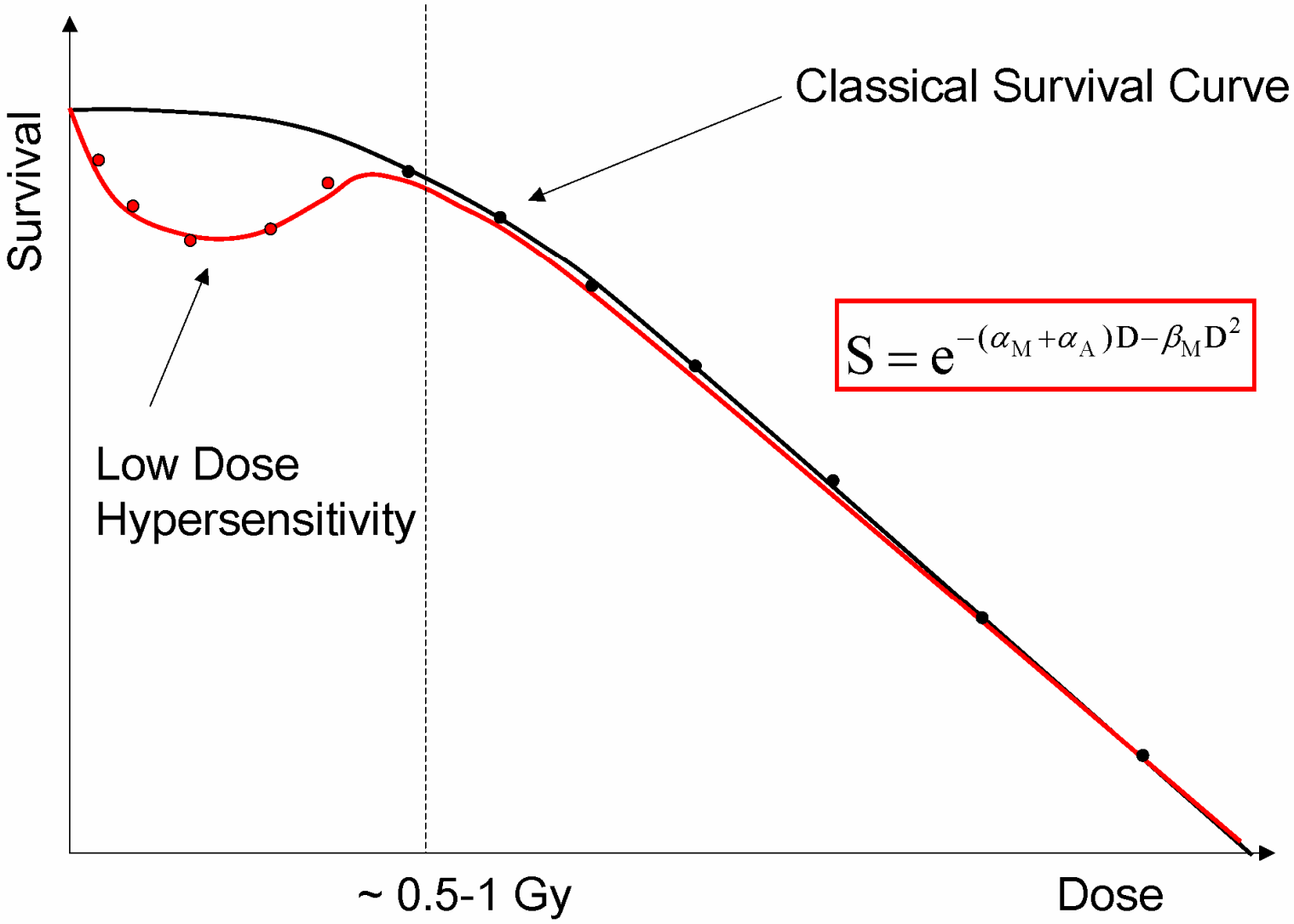
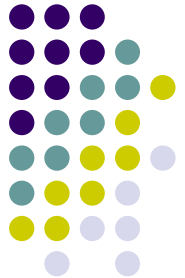
Low Dose Cell Survival



Non-targeted effects



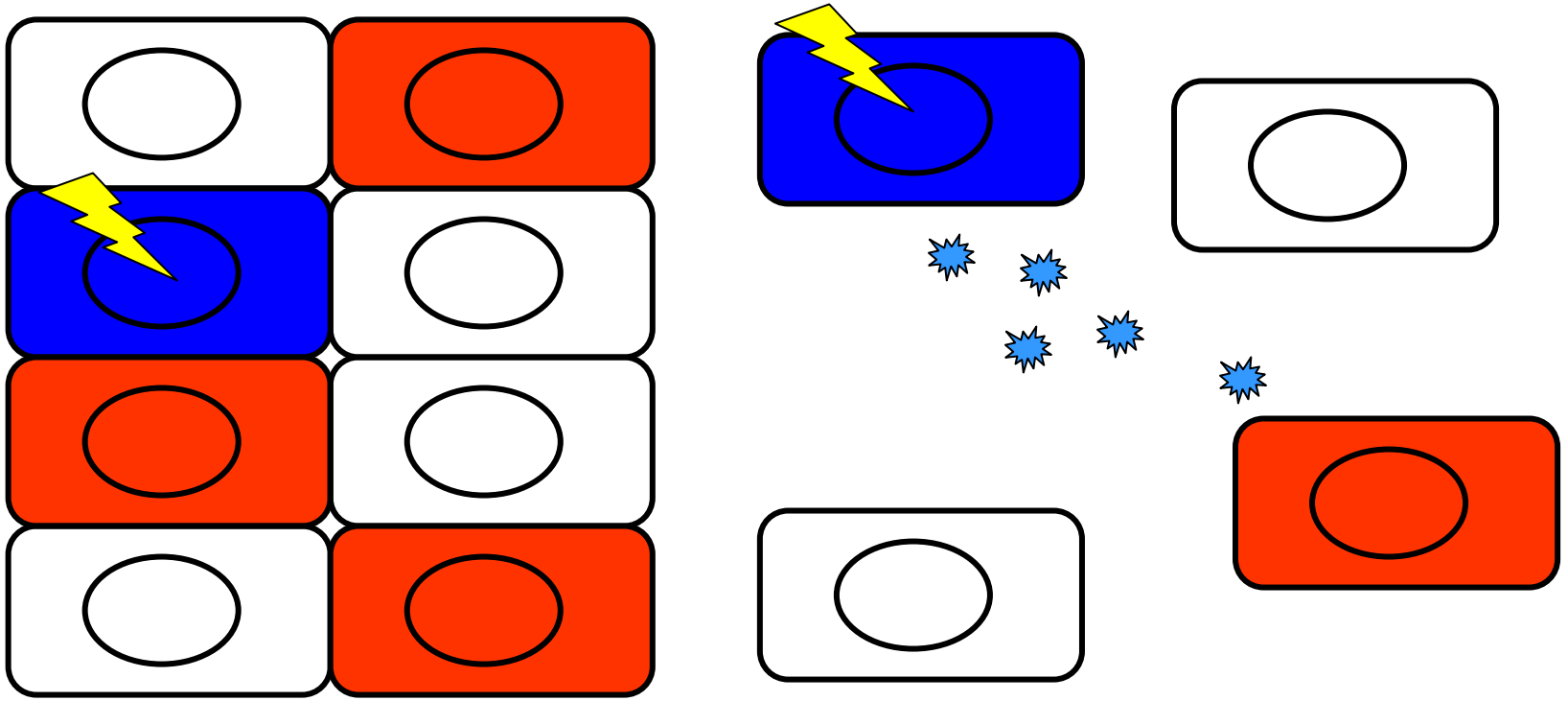
- Challenge classical paradigm
  - Direct deposition of energy in nucleus not required

eg.

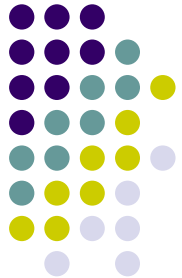
- Genomic instability
  - Effects in the progeny of irradiated cells
- Adaptive responses
  - Protective to further challenges of radiation
- Bystander effects
  - Effects in cells neighbouring irradiated cells

The Bystander Effect

Unirradiated cells can respond to signals produced by irradiated cells and exhibit responses similar to those of irradiated cells

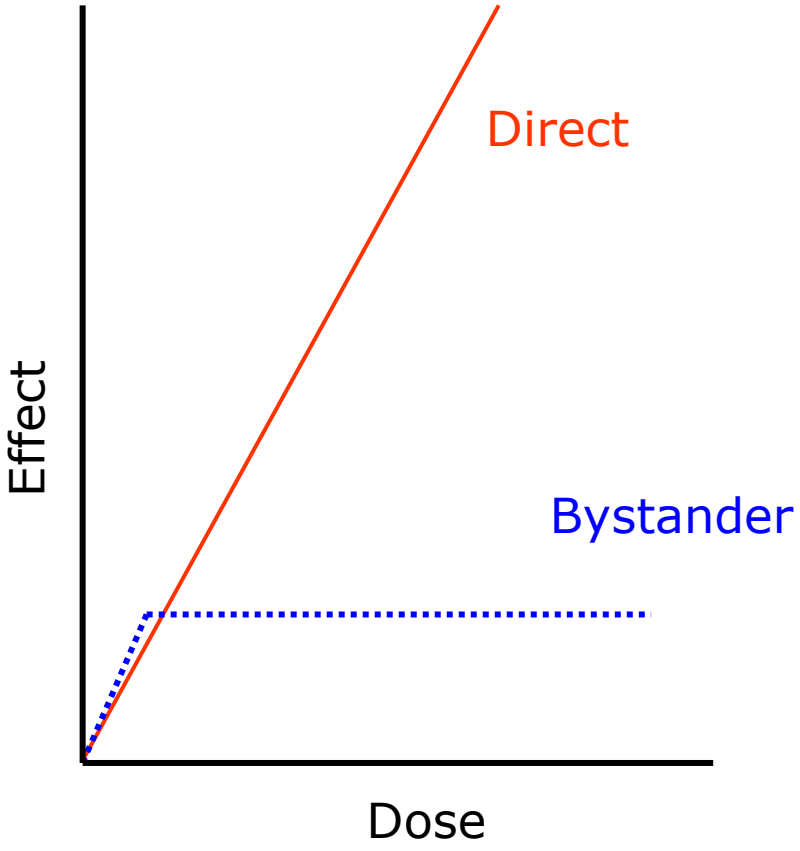


History



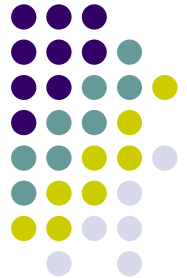
- 1954: First report of “clastogenic factors” in blood of exposed individuals
- 1968: Reports of persistent genetic factors in radiotherapy patients’ blood
- 1992: Sister chromatid exchanges shown to occur in more cells than would have been hit by low fluence α particles
- 1997: Low dose low LET radiation or low fluence α particles caused a medium borne cytotoxic factor to be produced
- To date: More than 400 papers describing bystander effects

In Vitro Studies of Bystander Effects



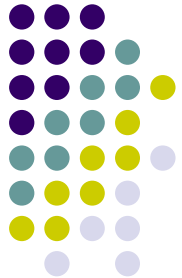
- Dominant at low doses (<1Gy)
- Different cell types
- A range of endpoints
  - cell killing, apoptosis, chromosomal damage, mutation
- Several mechanisms involved
  - GJIC, soluble factors
- Bystander factor still unknown
  - inhibited by heat treatment or protein synthesis inhibitors
- Bystander signals
  - ROS, RNS, cytokines, calcium

Experimental approaches for studying bystander effects



- Low fluence particle sources
  - α particles
- Microbeams
  - Charged particle, electron, soft X rays
- Shielding
  - Grids
- Medium transfer
  - From irradiated cells to unirradiated cells
- Co-culture
  - Membrane inserts, double Mylar dishes

Structure



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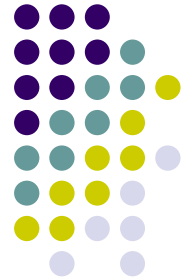
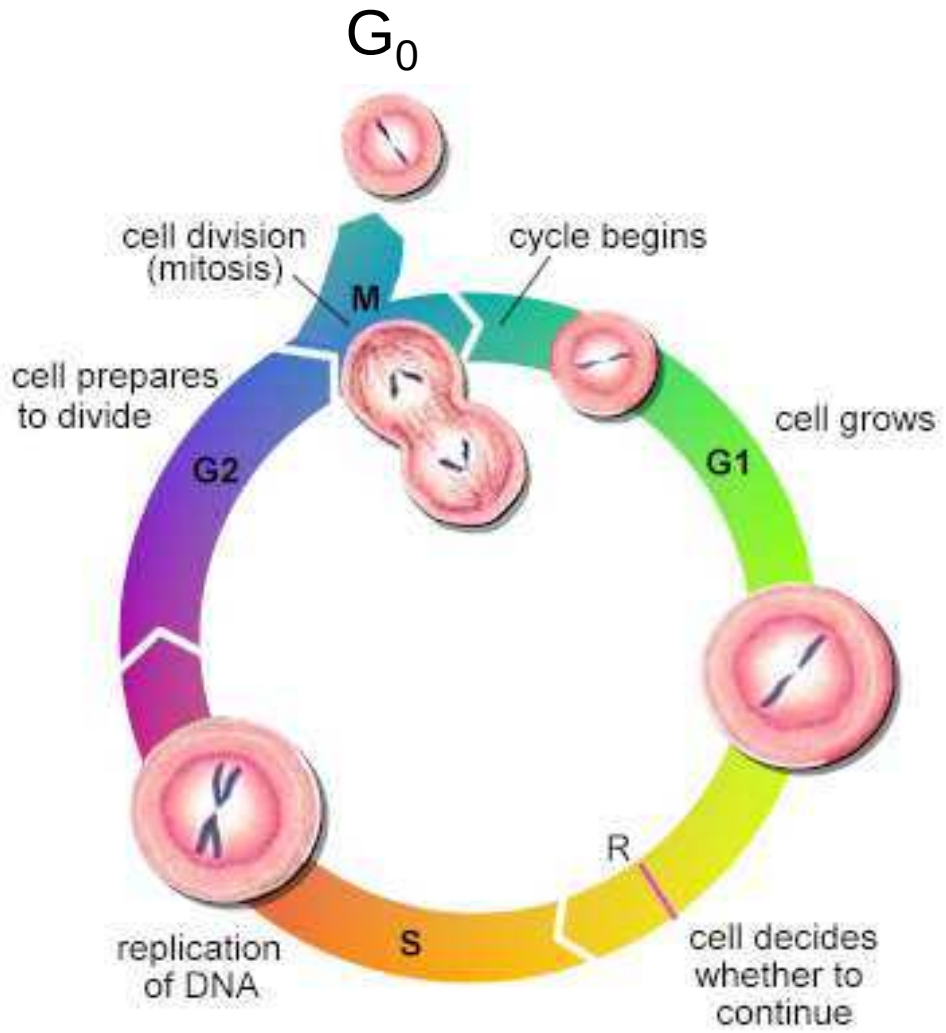
5. Non-Targeted Effects;
6. **Radiosensitivity and Cell Cycle;**
7. Dose Rate Effects and Damage Repair;
8. Acute Effects and Radiocarcinogenesis;
9. Radiation Protection;

Radiosensitivity and the Cell Cycle



- Cells propagate by mitosis, the result of mitosis being the production of two cells with identical chromosomal complements;
- The time between mitoses of a cell is called the mitotic cycle time or cell cycle time;
- Molecular checkpoints exist throughout the cell cycle which determine whether a cell is allowed to progress to the next phase of the cycle;



M-phase: Mitosis

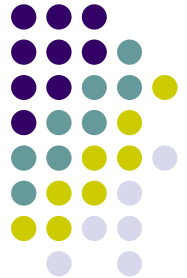
G_1-phase: Gap 1 (growth)

S-phase: Synthesis (DNA)

G_2-phase: Gap 2 (metabolism/differentation)

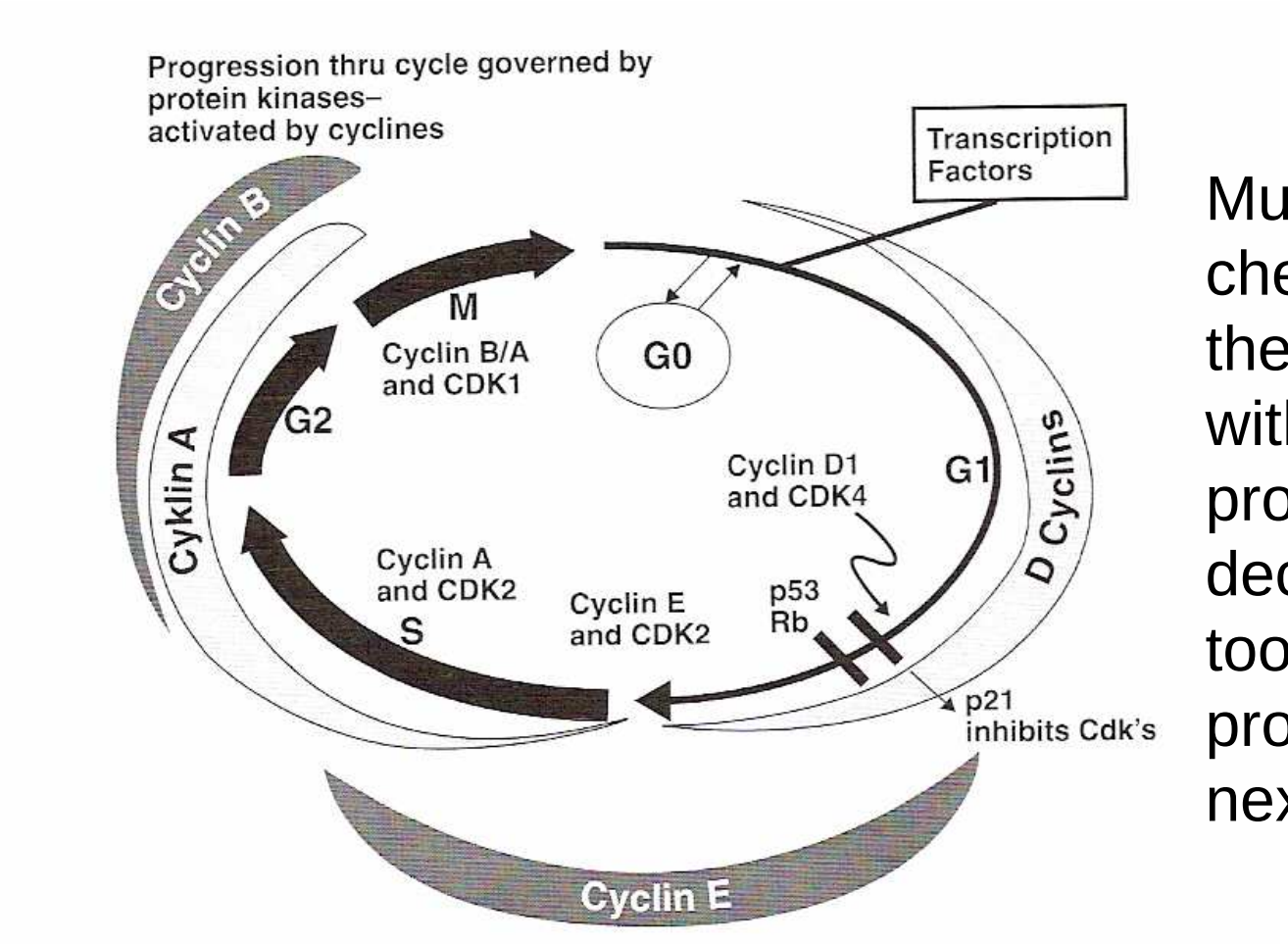
G_0-phase: Gap 0 (resting)

Regulation of Cell Cycle



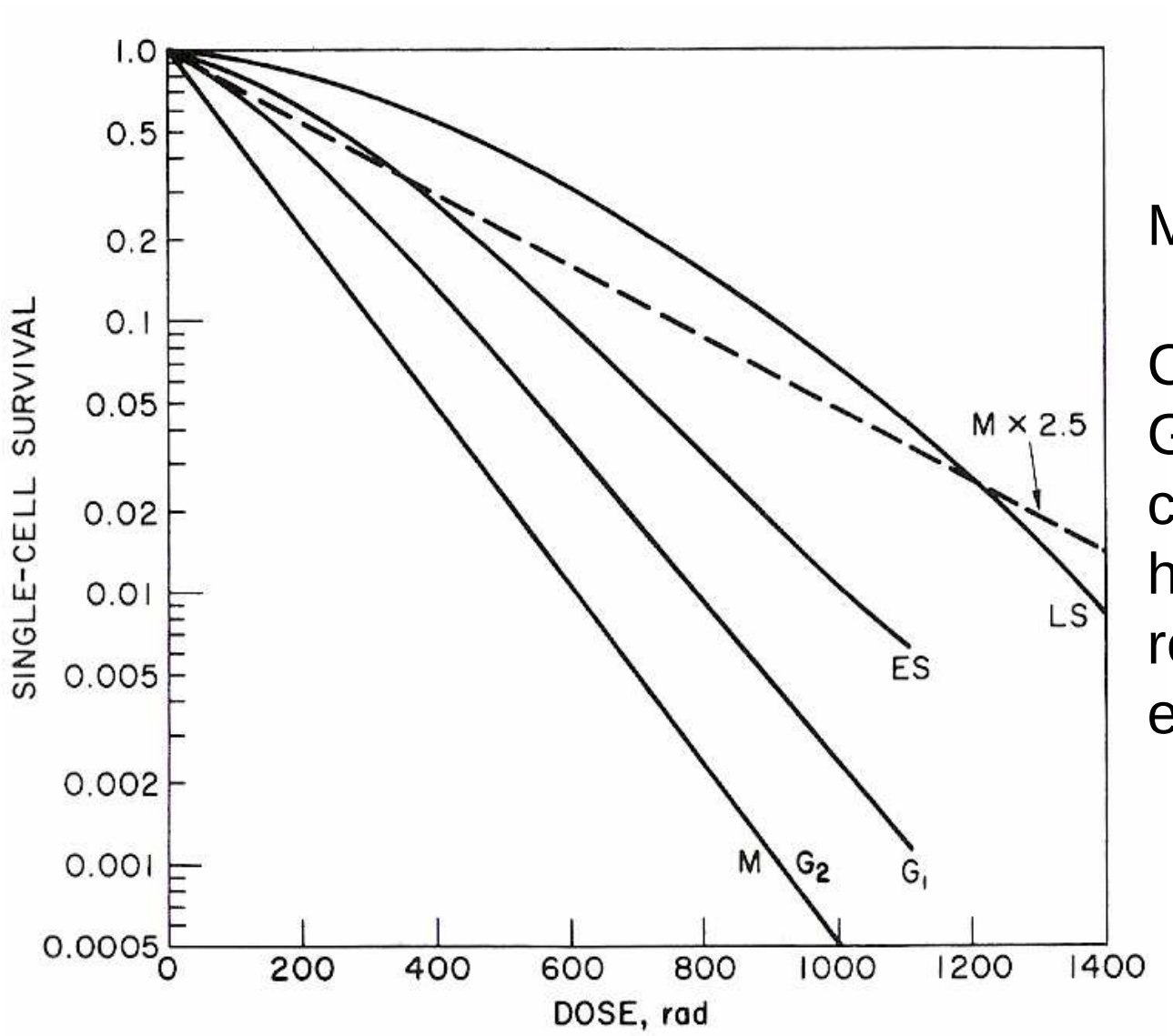
- The cyclin-dependent kinase (Cdk) family of proteins are activated periodically during the cell cycle, driving DNA replications, mitosis, etc.
- Entry into the cell cycle is determined by D Cyclins to begin with;
- Subsequently p53, p21, and the Cyclin A and B complexes determine whether a cell ultimately progresses beyond each phase of the cycle.

CdK's in the cell cycle



Multiple checkpoints within the cell cycle exist with specific proteins as decision making tools to decide on progression to the next phase.

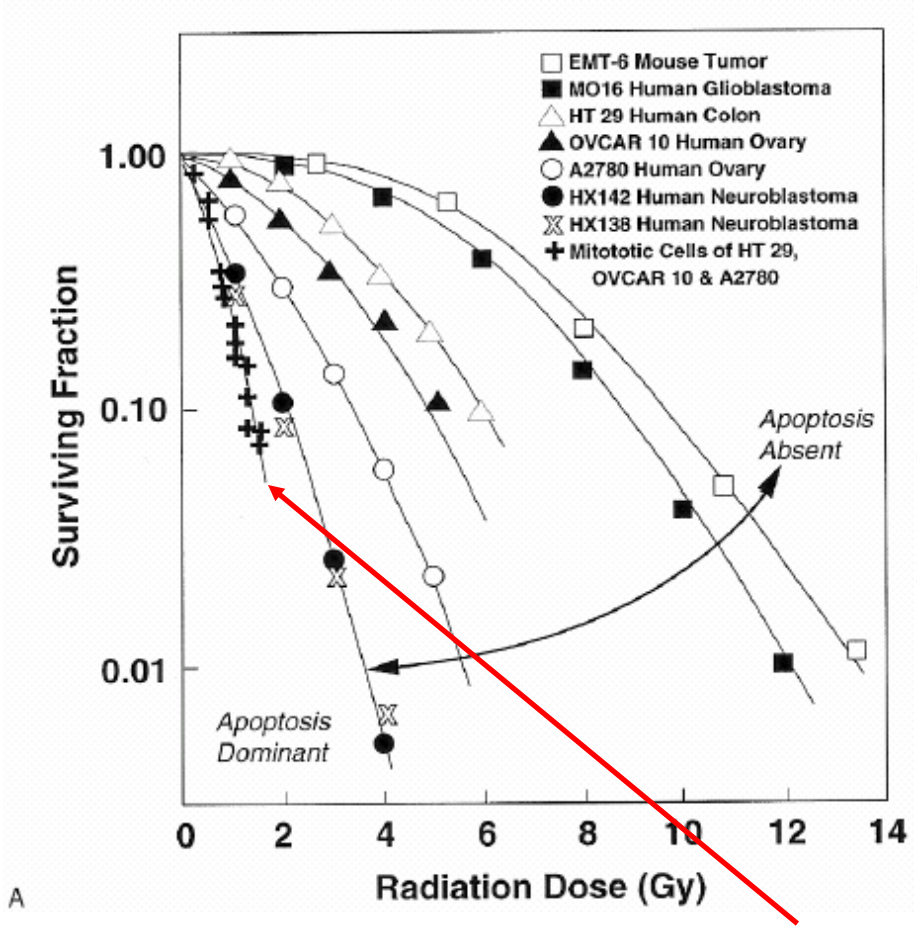
Radiosensitivity vs Cycle Phase

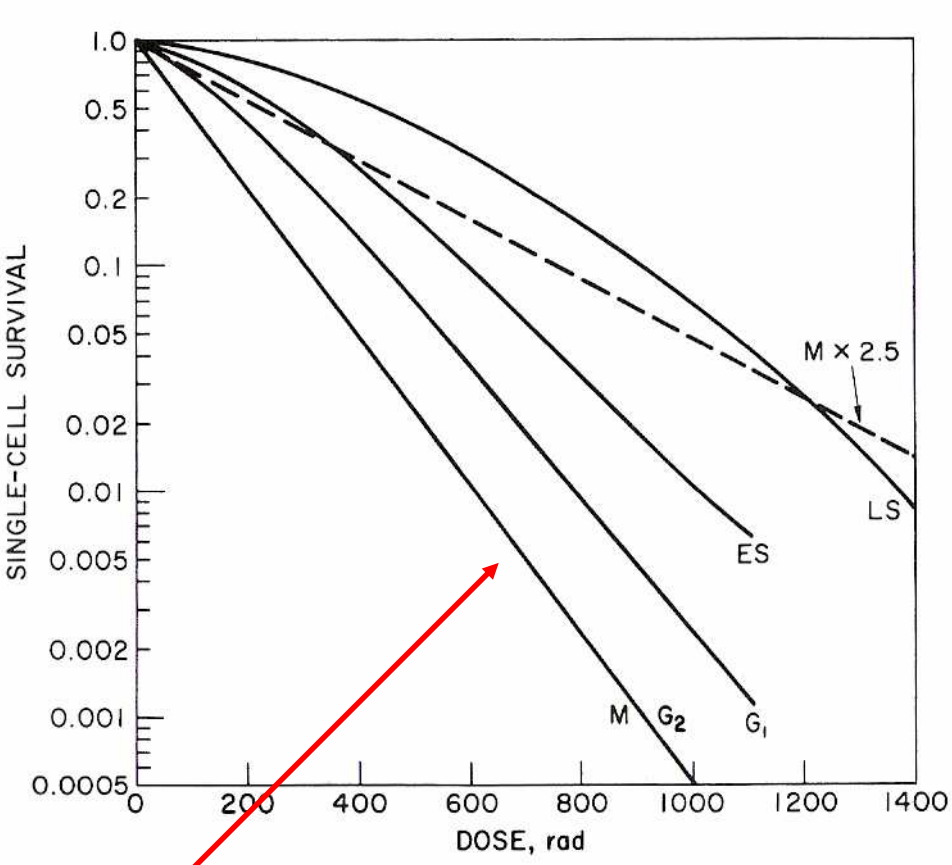


$M \geq G_2 > G_1 > S$

Cells with long G_1 and long cycle phases will have a peak of resistance in early G_1

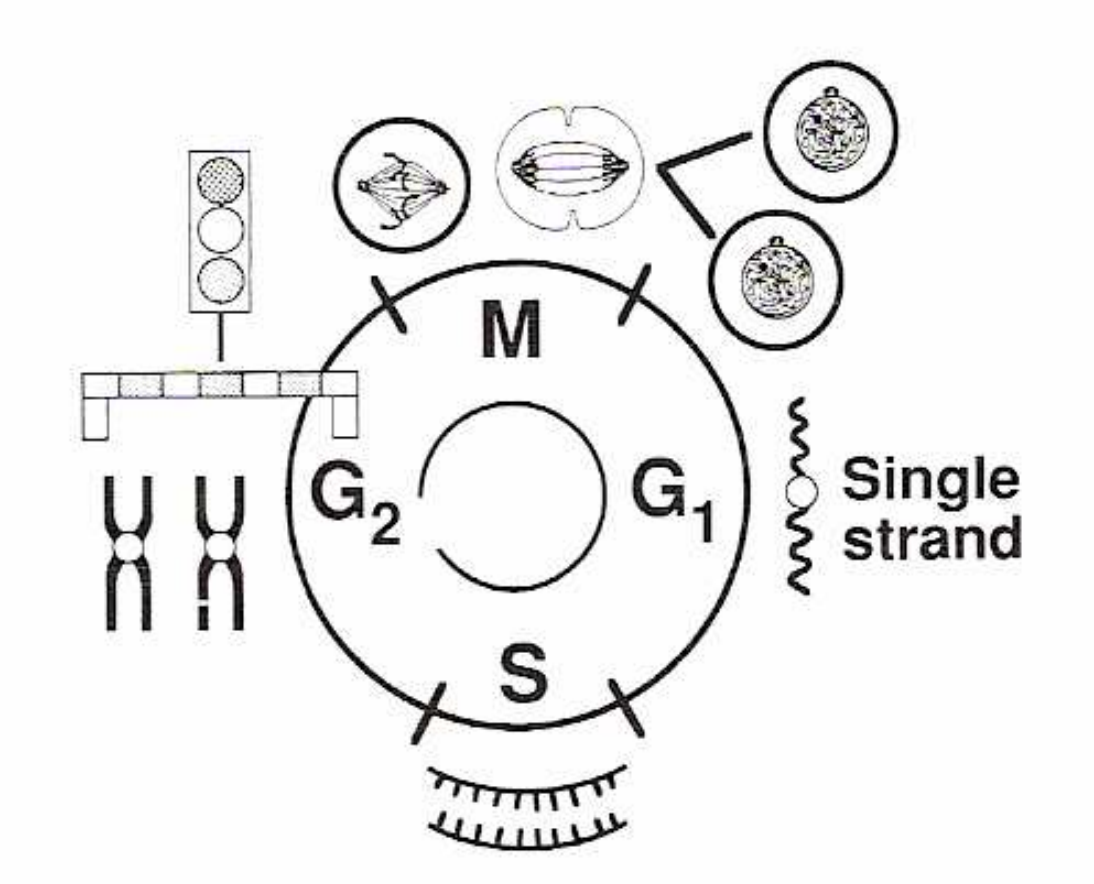
Radiosensitivity is Correlated with Apoptosis





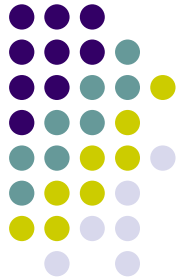
Apoptosis Dominant in M/G_2 phase

G_2 Phase Arrest



Genes involved in radiation damage response halt the cell in G_2 phase to prevent mitosis occurring and to allow repair.

Structure



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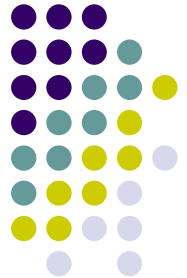
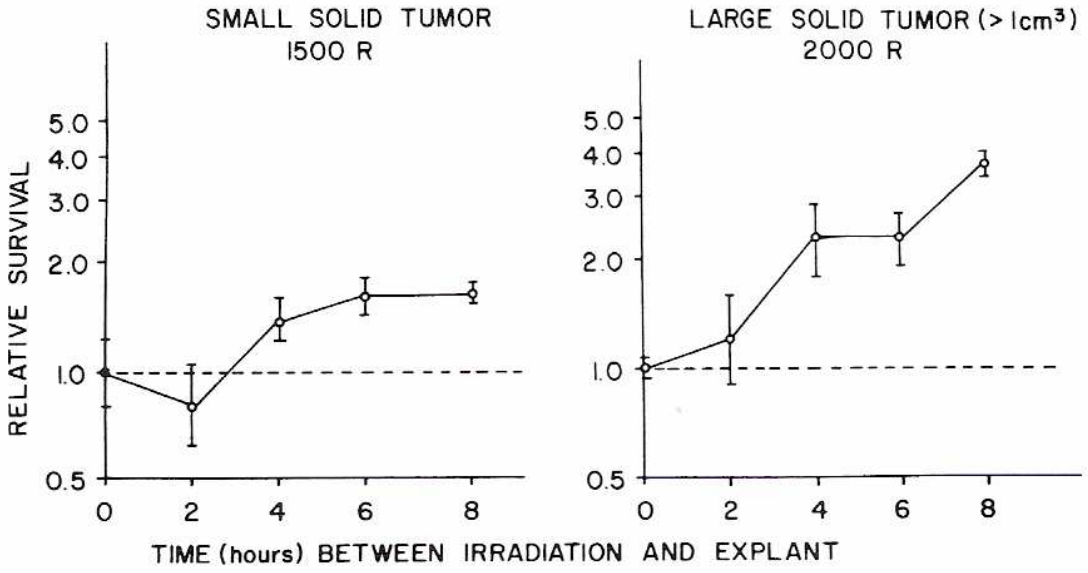
Repair of Radiation Damage – Dose Rate Effects



- Radiation damage may be divided into three categories:
  - Lethal damage – irreparable – cell death;
  - Sub-lethal damage – can be repaired in hours unless further sub-lethal damage occurs;
  - Potentially lethal damage – radiation damage that can be modified by external environmental conditions;

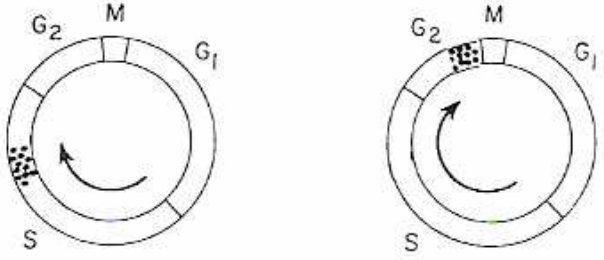
Repair of Potentially Lethal Damage

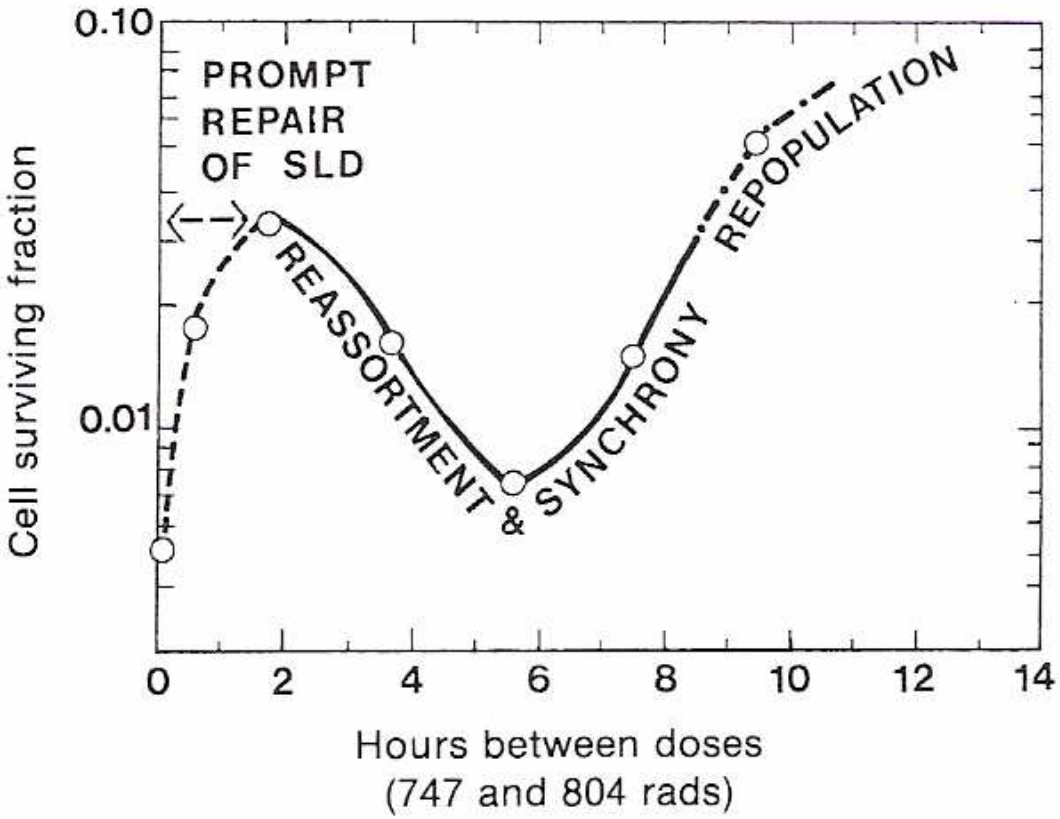


Little JB, et al, Radiology, 106: 689-694 (1973)

- Tumors irradiated and explanted to form single cell cultures.
- Cells incubated in balanced salt solution for several hours to allow repair to occur.
- i.e. if post-irradiation conditions are sub-optimal for growth, PLD can be repaired.

Sublethal Damage Repair





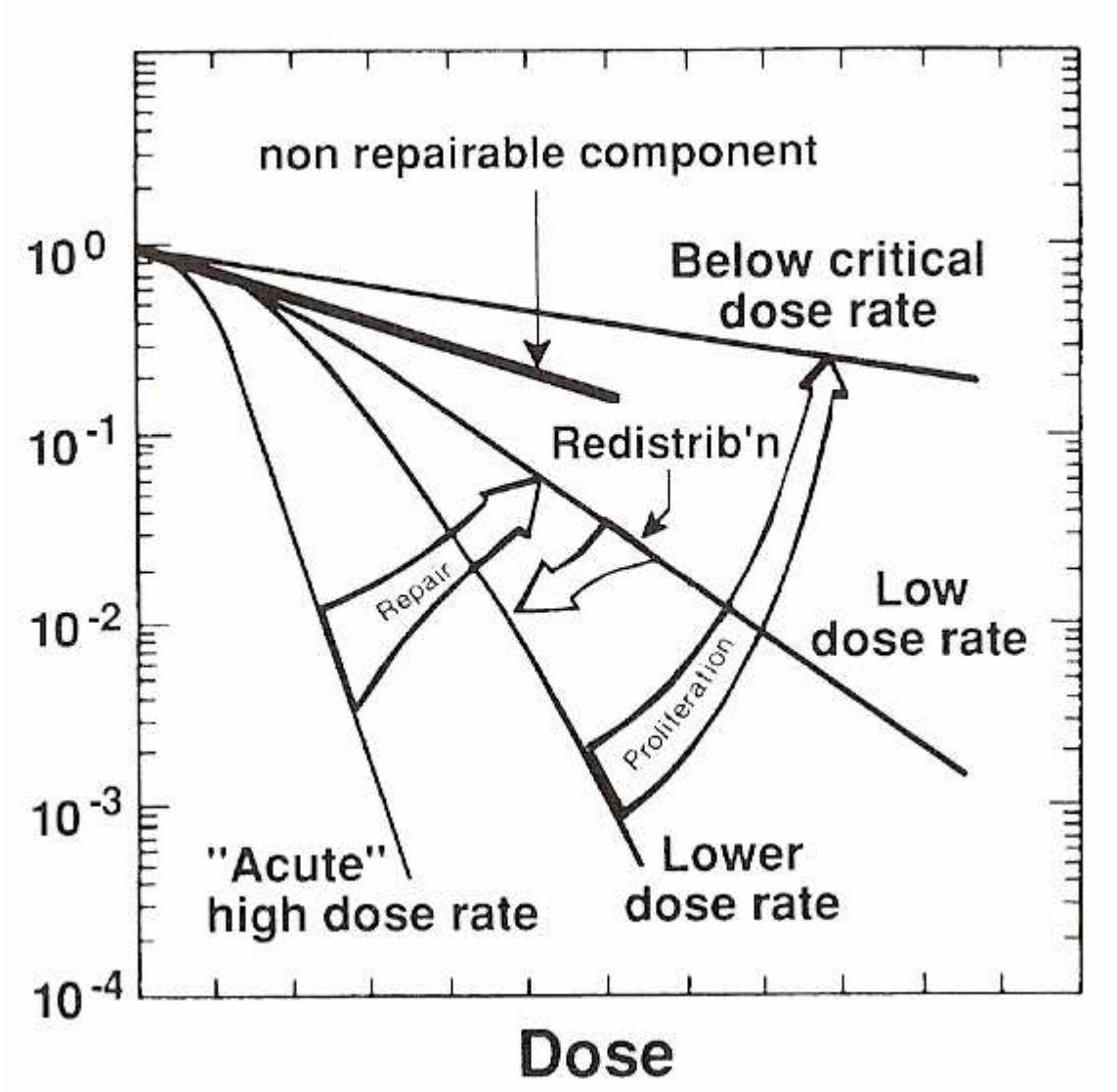
- Cells irradiated with two sublethal doses of radiation with variation in time interval between doses.

- Survivors of first dose are generally in S phase; Repair is prompt; Cells progress (Reassortment) through cell cycle.

- Subsequent Repopulation;

Elkind MM, et al, Radiat. Res. 25:359-376 (1965)

Dose Rate Effects

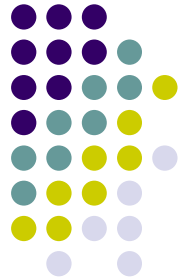


Generally, as dose-rate is reduced effects are ameliorated (as repair mechanisms are given time to establish).

However even at very low dose rates cells "pile up" at G_2 phase as a result of this checkpoint and cannot progress.

"Inverse Dose Rate Effect"

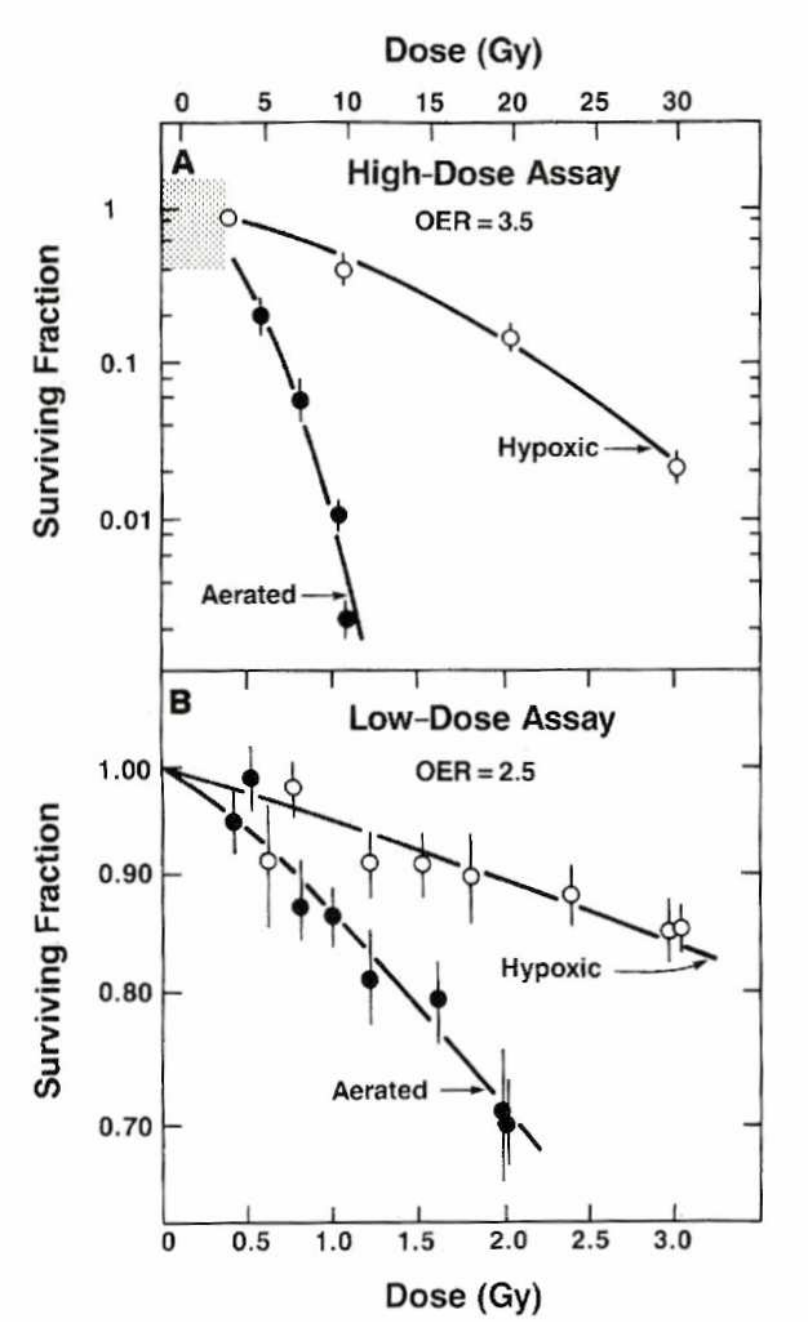
The Oxygen Effect and Reoxygenation



- Oxygenation of mammalian cells during irradiation enhances cell killing.
- For the oxygen effect to be observed, O_2 must be present during, or within microseconds, of the administration of the radiation exposure.

HDR

LDR

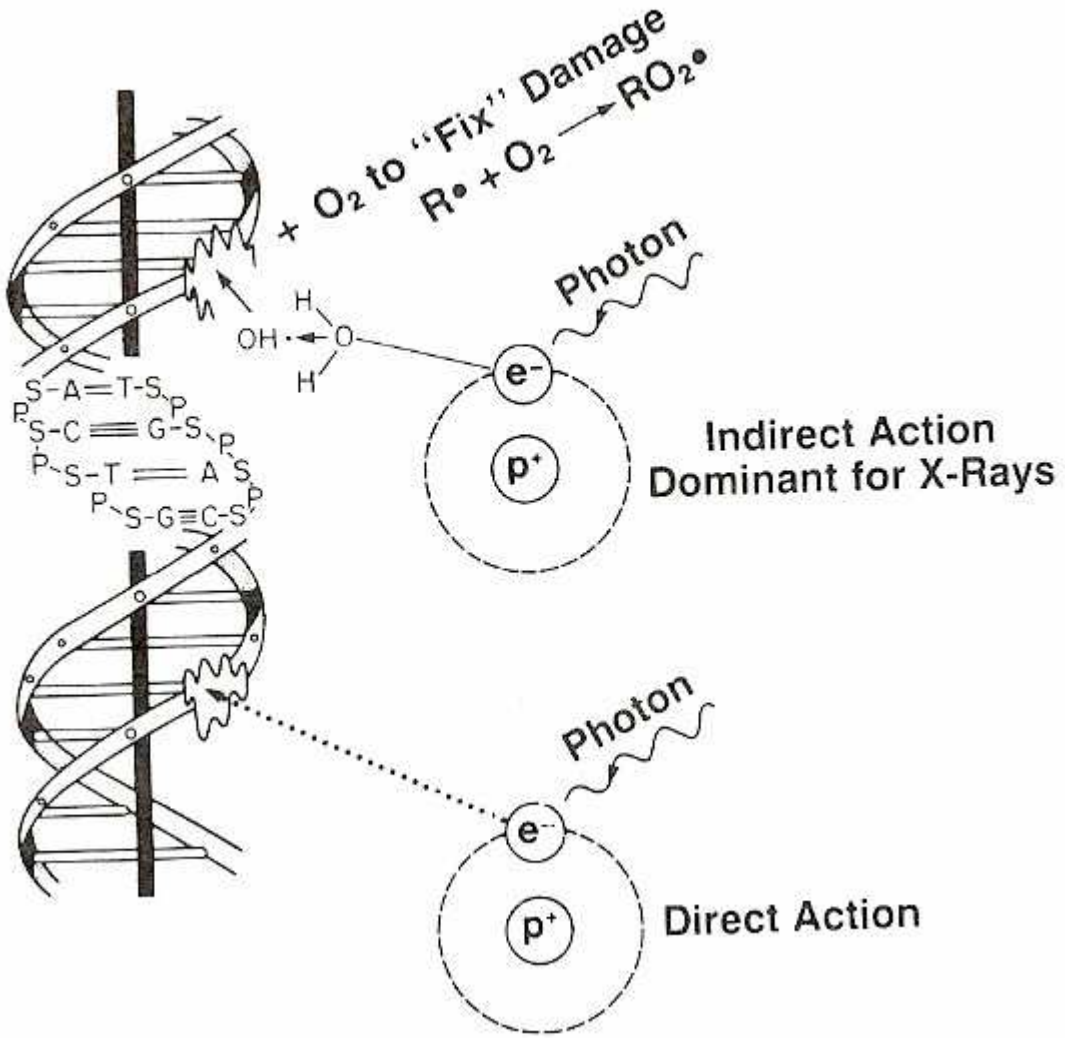


OER=Oxygen Enhancement Ratio (2.5-3.5 for x- and γ-rays).

Similar effects seen with other ionising radiations.

Palcic B, et al, Radiat. Res. 100:328-339 (1984).

Mechanism of Action



Oxidation of DNA by OH• may generally be reduced.

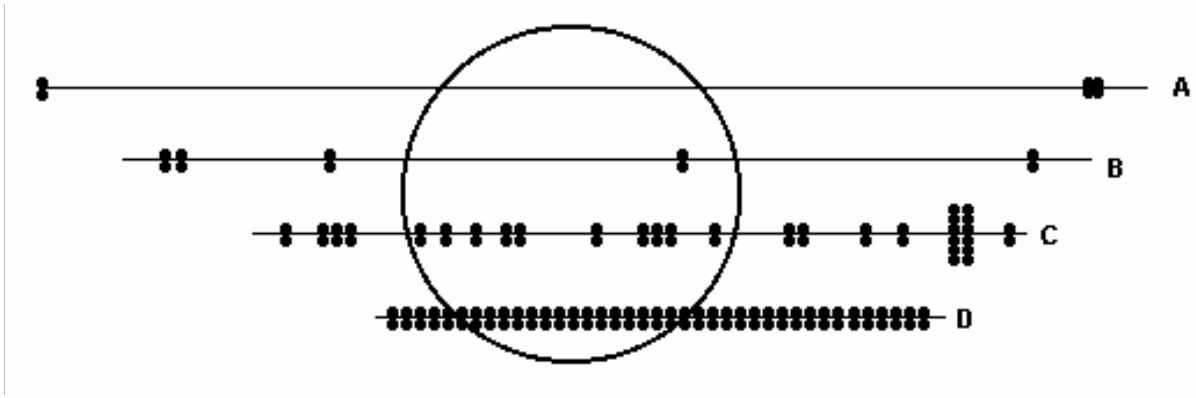
In presence of O_2 an organic peroxide of DNA is formed which “fixes” the damage.

LET

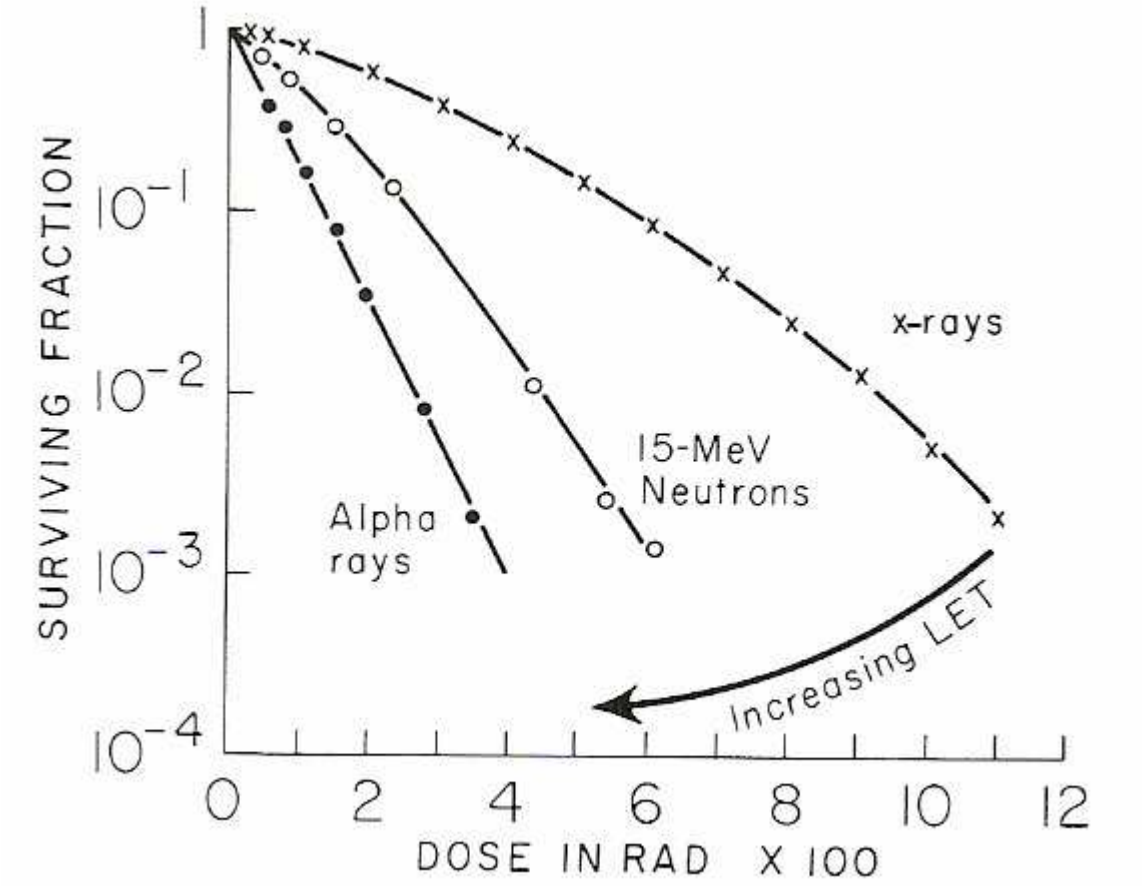
- The “Linear Energy Transfer”, or LET (L_Δ), defined the amount of energy an ionising radiation deposits per unit length in an absorbing medium.

$$L_\Delta = \left(\frac{dE}{dl} \right)$$

- This determines the lethality of the radiation per unit dose.

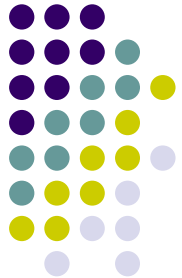


A: γ-rays;
B: 6 MeV x-rays
C: ‘Soft’ X-rays
D: α-particle



At higher LET’s, more lethal damage is accumulated per unit dose than for lower LET radiations.

Structure



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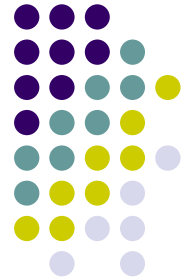
Acute (Deterministic) effects of TBI

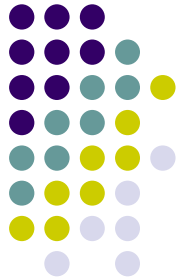


- Acute Radiation Syndrome (ARS) is quite well defined in humans (Chernobyl, A-bomb, accidents etc.):
  - Early symptoms – prodromal radiation syndrome;
  - >100 Gy: Cerebrovascular syndrome (24-48 hours);
  - 5-12 Gy: Gastrointestinal syndrome;
  - 2.5-5Gy: Hematopoietic syndrome;

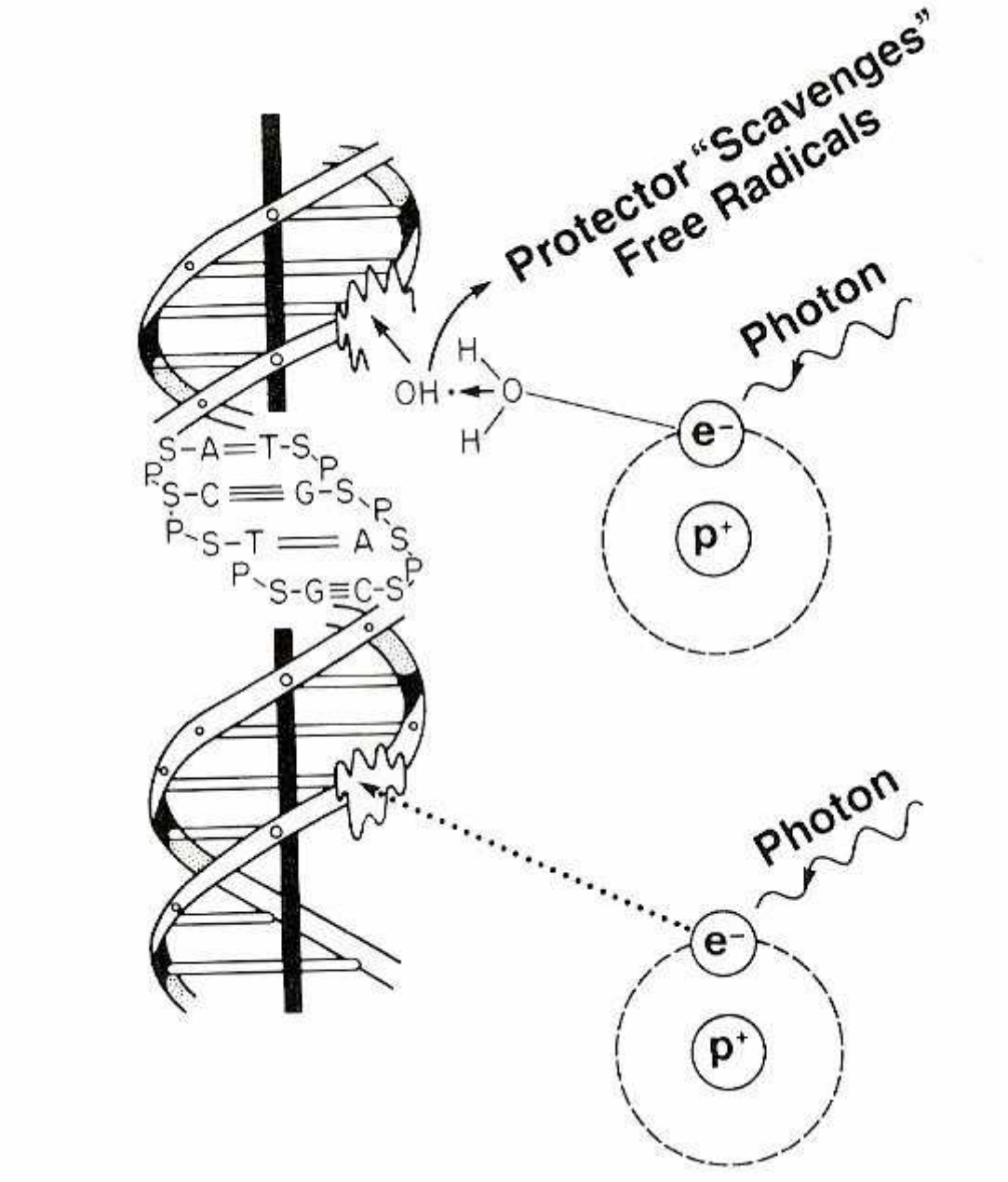
Critical Phase of Acute Radiation Syndrome (ARS)

	Degree of ARS and Approximate Dose of Acute WBE (Gy)				
	Mild (1–2 Gy)	**Moderate (2–4 Gy)**	**Severe (4–6 Gy)**	**Very Severe (6–8 Gy)**	**Lethal (>8 Gy)**
Onset of symptoms	>30 days	18–28 days	8–18 days	<7 days	<3 days
Lymphocytes (G/L)	0.8–1.5	0.5–0.8	0.3–0.5	0.1–0.3	0–0.1
Platelets (G/L)	60–100	30–60	25–35	15–25	<20
	10–25%	25–40%	40–80%	60–80%	80–100%[a]
Clinical manifestations	Fatigue, weakness	Fever, infections, bleeding, weakness, epilation	High fever, infections, bleeding, epilation	High fever, diarrhea, vomiting, dizziness and disorientation, hypotension	High fever, diarrhea, unconsciousness
Lethality (%)	0	0–50	20–70	50–100	100
		Onset 6–8 weeks	Onset 4–8 weeks	Onset 1–2 weeks	1–2 weeks
Medical response	Prophylactic	Special prophylactic treatment from days 14–20; isolation from days 10–20	Special prophylactic treatment from days 7–10; isolation from the beginning	Special treatment from day 1; isolation from the beginning	Symptomatic only

Radioprotectors



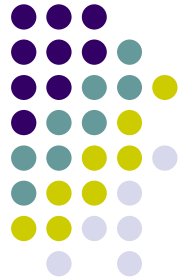
- Radioprotectors have been found to prevent the effects of ionising radiation (by reducing the amount of oxygen in critical organs):
  - Cysteine;
  - Cysteamine;
  - Amifostine;



Radioprotectors containing a sulfhydryl group scavenge free radicals created by indirect interactions of ionising radiation with H_2O.

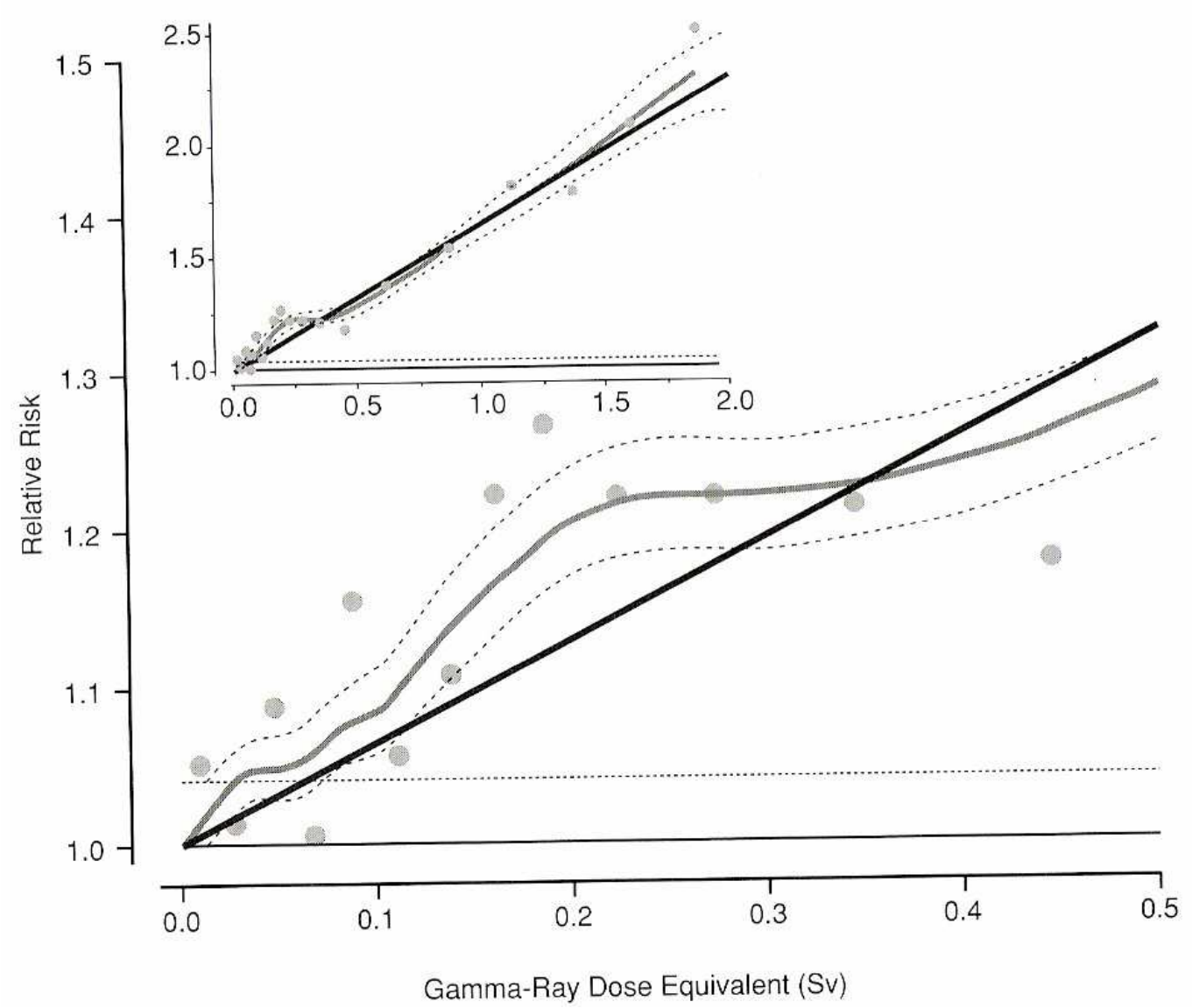
Most effective for low LET radiations.

Radiocarcinogenesis (Stochastic Effects)



- Evidence for radiocarcinogenesis began to accumulate almost immediately post the discovery of ionising radiation (Marie Curie);
- Risk estimates are currently available from large irradiated cohorts, eg:
  - A-bomb survivors (Japan – 120,000);
  - Ankylosing spondylitis patients (UK – 14,000);
  - Tinea capitis patients (Israel – 10,834);

Dose Response in A-Bomb Survivor Data

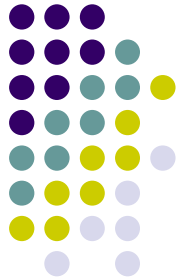


Although response is non-linear at low doses it is not statistically significantly different from a linear response

LNT Model

Pierce DA, et al, Radiat. Res., 154:178-196 (2000)

Structure



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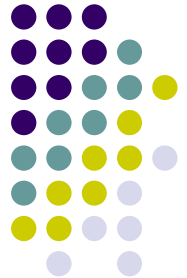
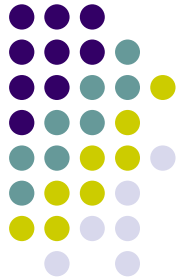
Radiation Protection



- System of Radiation Protection based on LNT hypothesis giving:
  - "ALARA Principle";
  - Dose Limits for Workers;
- Constantly reviewed by ICRP and NCRP;
- Designed to protect against risks of:
  - Deterministic Effects;
  - Stochastic Effects;
- Limit expressed as quantity of Effective Dose (Sievert, Sv):
  - Organ radiosensitivity-weighted cumulative whole body dose;
  - Risk estimate ~ 5-7 % per Sv Effective Dose;

Risk Estimates in Context



- For a typical Chest X-ray the Effective Dose is 0.02 mSv (0.00002 Sv)[7]
- Therefore risk = approx. 1.5 per million
- Risk of fatality in car accidents = approx 1 in 10,000 (1.3% of all deaths) *(CSO Vital Statistics, 1998)*
- Risk of natural Cancer Death in Ireland almost 1 in 500 (23% of all deaths) *(DOHC Vital Statistics, 1999)*

Acknowledgements

DIT

- Dr. Fiona Lyng;
- Dr. Orla Howe;
- Dr. James Murphy;

- Many previous colleagues!