- Radiation
  - EM Waves (x-ray, γ ray)
  - particles (β rays / e^-, neutrons, ions)
  - For medical uses:
    - Non-ionizing ⇒ infrared, lasers, ultrasound, microwaves
    - ionizing ⇒ EM (x, γ, lasers), particulate radiation
- Radiation for therapeutic purposes:
  - Teletherapy
    - photon
    - x-rays (high Energy) ⇒ 35 MV on electron gun
      - Co diagnostic: 30 - 150 kV_p
    - γ rays from ^{60}Co, ^{137}Cs
    - electrons (4-35 MeV)
    - photons, pions, heavy charged particles (MeV)
    - Neutrons (14 MeV)
  - Brachytherapy
    - Source: ^{60}Co, ^{137}Cs, ^{192}Ir, ^{125}I
- UV radiation
  - UV A: 315 - 380 nm ⇒ least damaging
  - UV B: 280 - 315 nm ⇒ can damage DNA ⇒ high absorption for UV B of DNA
  - UV C: 200 - 280 nm ⇒ close to ionizing radiation, most damaging

- Deposition of Energy
  - Heat Radiation: Uniformly absorbed
    - ↳ need higher quantity to produce damage
  - EM radiation: each photon can create interactions
    - ↳ potency is of E of each individual photon
    - ↳ Biologically ionizing: ≥ 124 eV

- Ionizing Radiation
  - Generation:
    1. accelerate charged particles → interact w/ medium → 2° radiation
    2. Radioactive nuclei (γ ray from Co-60)
  - Type:
    1. EM ⇒ indirect interaction with matter (2° particle)
       1. X-ray:
          - e^- → matter
            - → knocking off e^- → falling → characteristic X-ray
            - → slow down near nucleus → Bremsstrahlung Radiation
          - Max of emission energy (in eV) = ½ × tube voltage
       2. γ-ray: (very penetrating, a few cm)
          - nuclear disintegration ⇒ well defined energy
          - ^{60}Co / ^{137}Cs / ^{125}I / ^{131}I → daughter nuclei + γ rays
       3. 2 photon ionization with UV lasers

② Particulate Radiation ⇒ direct interaction with material

① e⁻ (β-ray) ⇒ short range, interact strongly w/ matter, (μm to a few nm)

↳ small & can be accelerated to high E

↳ Cancer therapy

② p^+

↳ heavier, more complex experiment to accelerate

↳ Cancer therapy due to favorable dose distribution

③ α particles (He nuclei = $2p^+ + 2n^0$)

↳ produced by radioactive decay (uranium or radium)

↳ useful in studying cellular radiation biology

↳ Small range ⇒ high Ionization ability (nm to 1/10 μm)

④ Neutron (n^0)

↳ produced by nuclear rxn

↳ charged particle $\xrightarrow{\text{high E}}$ material $\longrightarrow n^0$

⑤ Heavy charged particles

↳ nuclei of elements (C, N, Ar, Fe)

↳ accelerate to high E

↳ radiotherapy

Radioactive Decay

decay constant = $1/\tau$, τ = lifetime

$$N = N_0 e^{-kt} = N_0 e^{\frac{-\ln(2)\, t}{t_{1/2}}}$$

N: # of radioactive atoms

Activities

rate of decay $\Rightarrow$ $A = -\frac{dN}{dt} = kN = \frac{N}{\tau}$

Application of α, β, γ rays

- α
  - low range $\Rightarrow$ highly localized
  - tumour treatment
- β
  - cancer therapy
- γ
  - γ-knief surgery for large tumours
  - Diagnostic $\rightarrow$ PET / SEPT $\Rightarrow$ fludeoxyglucose $\rightarrow$ positrons $\rightarrow$ 2 γ photons
  - Sterilizes

Ionizing Radiation Units

- **Activity**: # of nuclear decays per time (Bq, Becquerel)
- **Exposure**: Charge ionized (C) per unit mass of air (kg) (Roentgen, R)
- **Absorbed Dose**: Energy absorbed (J) / mass of material (kg) (Gray, G)
- **Dose Equivalent**: Absorbed dose $\times$ RBE (Sievert, Sv)

Attenuation of Radiation

- radiation on matter ⇒ absorption or scattering ⇒ attenuated
- fluence: $\phi = \frac{dN}{dA}$

Interaction cross section: σ (prob. of interaction)

↳ the area to be hit by the particle for interaction

$$\Rightarrow -d\phi = \sigma \phi \frac{dN_c}{dA} = \sigma \phi \frac{dN_c}{dN} dx$$

$$dN_c = \frac{dm}{m_a} N_A = \frac{C\,dv}{m_a} N_a = C N_A\, dv$$

$$\therefore -d\phi = \sigma \phi C N_A\, dx$$

$$\phi = \phi_0 e^{-\sigma C N_A x} = \phi_0 e^{-\varepsilon C x} = \phi_0 e^{-\mu x} \Rightarrow \text{Lambert-Bear Law}$$

- ε is the molar extinction coefficient
- C : concentration
- x : in cm

} Absorbance = $\varepsilon C x$ (A)

- $\sigma = \frac{\varepsilon}{N_A}$ ⇒ strong absorber, $\varepsilon \approx 10^4\ M^{-1}\ cm^{-1}$

↳ σ is size of an H-atom

- $\mu = \varepsilon C$ ⇒ linear attenuation coefficient ⇒ how quickly it attenuates

$1/\mu$ ⇒ mean free path

- In radiation dosimetry, energy are transferred (μ_{tr}) or absorbed (μ_{ab})

$$\mu_{tr} = \mu \frac{E_{tr}}{hf} \qquad \mu_{ab} = \mu \frac{E_{ab}}{hf}$$

$$\text{Dose} = \frac{\Delta E_{ab}}{\Delta m} \Rightarrow \text{absorbed per mass of material}$$

$$\mu_{ab} = \mu_{tr}(1-g)$$ ← radiative fraction lost to heat

Photon Radiation

- Attenuation: $N = N_0 e^{-\mu x}$
- Type of photon interaction:
  - σ for each depends on energy & Z

① Coherent / elastic scattering
  - No energy interaction, no biological effect.

② Excitation ⇒ non-ionizing radiation

③ Ionizing Radiation:
  - Photo electric Effect
  - Compton Scattering Effect
  - Pair Production

④ photonuclear ⇒ with very high E (~100 MeV)

- Exciation By Photon Absorption
  - 3 types of transition:
    - Electronic transitions ⇒ By Vis / UV
    - Vibrational transitions ⇒ By IR
    - Rotational transitions ⇒ By MW

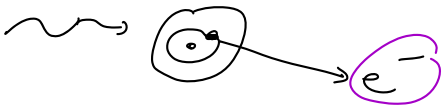
- **Photoelectric Effect (PE)**

$$E_e = hf - E_B$$ ← Binding energy

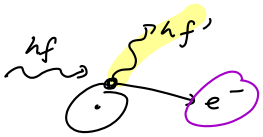
- Strongly dependent on Z

$$\sigma_{pe} \approx Z^n / (hf)^3$$

* photon vs. atom



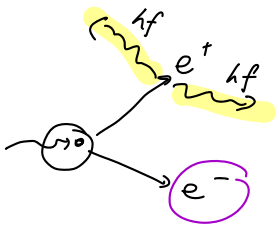
- **Compton Effect**



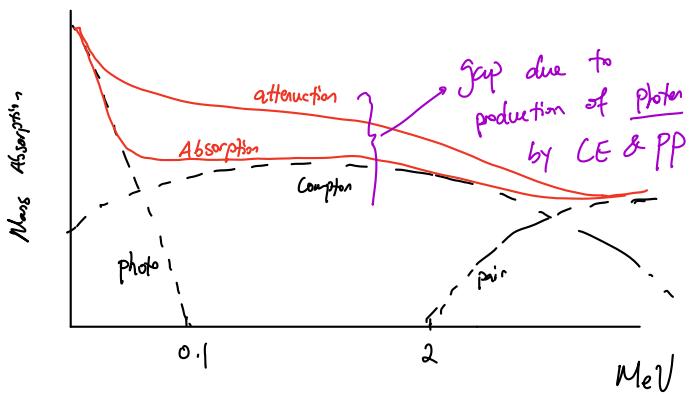
- Single electron event ⇒ does not depend on Z as much * photon vs. e^-

- **Pair Formation**

- $\sigma = Z^2 \cdot hf$ * photon vs. atom



Total interaction $(\sigma_{total}) = Z\,\sigma_{compton} + \sigma_{PE} + \sigma_{pair}$

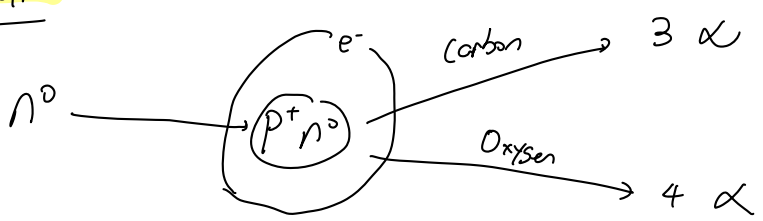


Diagnostic: Low E ⇒ PE ⇒ dependent on Z

Radiotherapy: uniformly absorbed ⇒ independent on Z

* photon radiation is indirect ionizing ⇒ production of 2° e^-

- **Neutron**



- **Ions**

E increase ↓

① e^- capture ⇒ neutralization

② collision w/ atomic e^-

③ Nuclear collisions & nuclear rxn.

Direct ionization is more significant

- **Electrons**

① collision w/ shell electrons

② **Bremsstrahlung** ⇒ deceleration of e^- ⟶ emission of radiation

③ **Cherenkov radiation** ⇒ high E (exceed 500 keV in H_2O) ⇒ UV light production
- ↳ e^- exceed phase velocity in medium.
- ↳ detection of labelled biomolecule
- ↳ image substances in the body

④ **Nuclear rxn.**

- **Charged Particles (e^-, p^+, ions) Interaction**

- Continuous slowing down approximation ⇒ assume interaction does not Δ E of e^-

$$R = \int_0^{E_0} \frac{dE}{dE/dx}$$

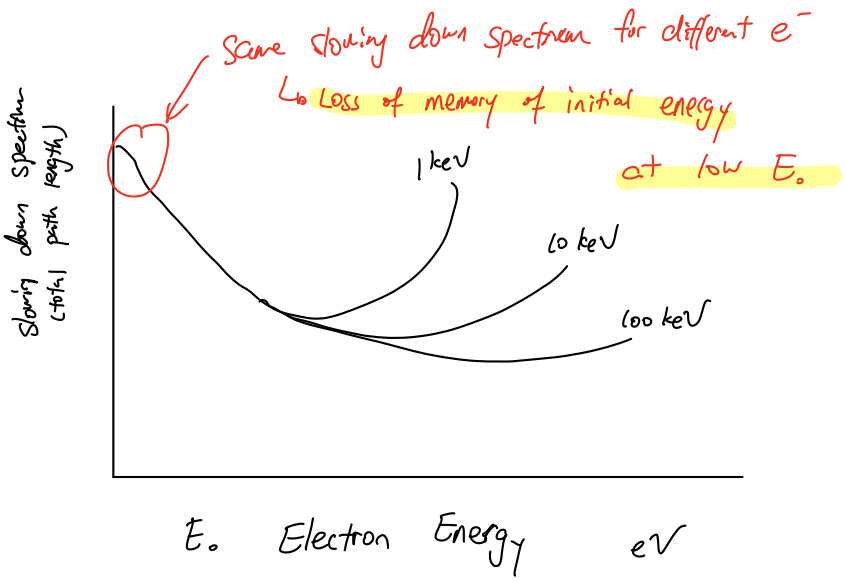
- ↑ E = ↑ Range (R) travelling in material.

2° Particle Equilibrium

$$y(E, E_0) = I(E)\, n'(E, E_0)$$

- $y(E, E_0)$: slowing down spectrum
- $I(E)$: mean free path length
- $n'(E, E_0)$: # of e^- with energy E

total path length of e^- with energy E produced by E_0



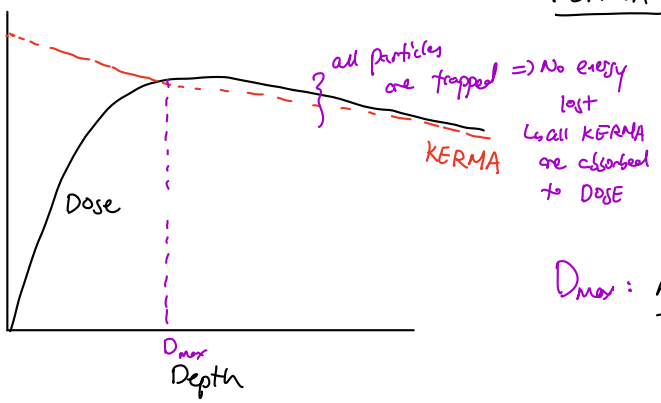
∴ For low E_0, total path lengths are the same

↳ e^- will travel a similar distance b/w each collision

Deposition of Radiation

- non ionizing photon radiation ⇒ selective absorption
- ionizing radiation ⇒ majority is absorbed by H_2O

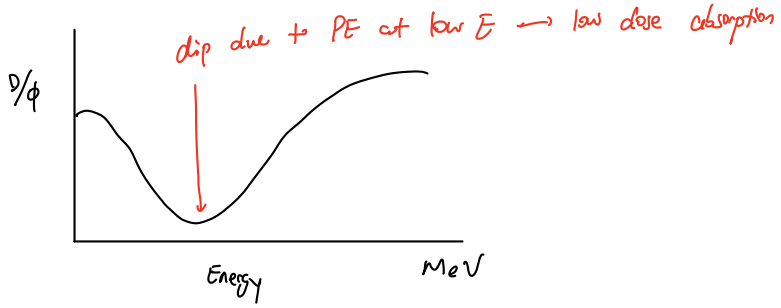
Ionizing Radiation



KERMA: $\sum$ initial kinetic energies of all 2° particles per unit mass element

- Large at surface: Some 2° particle will escape from surface.
  ↳ KERMA > DOSE

D_{max}: Average range of the most energetic 2° particle

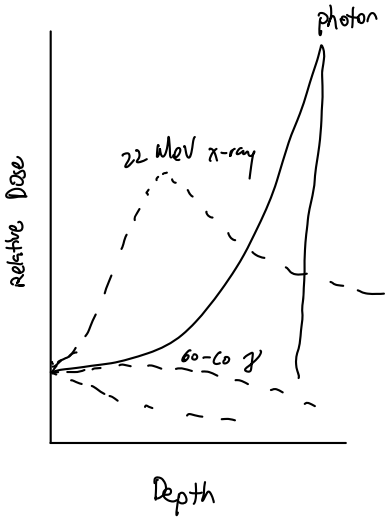


- **Stopping Power** (dE_{tr}/dE)
  - ↳ Energy loss of the particle per unit length ⇒ transferred E / unit length
    - ↳ include bremsstrahling & collision

- **Linear Energy Transfer (LET)**
  - amount of locally absorbed energy per unit length
  - low E = high LET
  - heavier = higher LET

  } high LET = less cellular repair

- **Depth Dose Curves**



  - Amount of absorbed E ∝ fluence & LET
  - Highest energy deposition at end of rage
    - ↳ higher energy = longer penetration rage

- **Relative Biological Efficiency (RBE)**
  - ↑ LET = more dead cell per Gy
  - RBE = $\frac{\text{Dose of ref. radiation}}{\text{Dose of test radiation}}$ ← low LET radiation (250 keV x-rays)
  - RBE ↑ w/ LET, then ↓ due to **overkill**

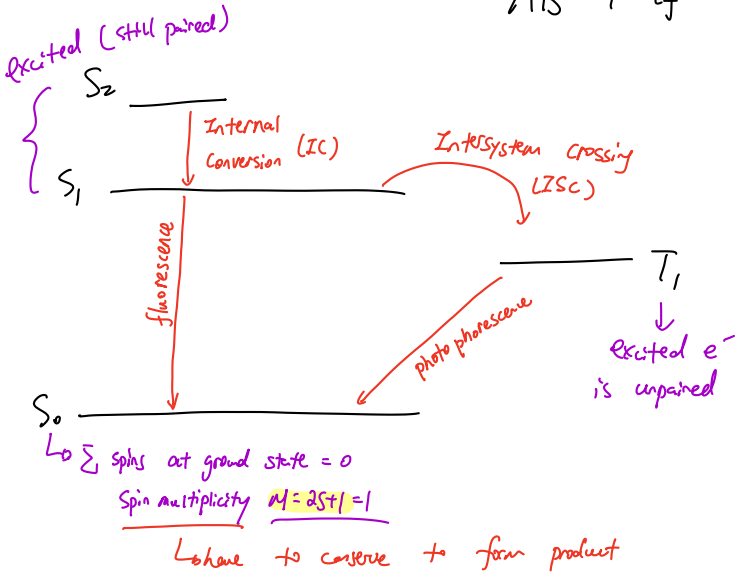
Microdosimetry

- high amount of E is locally deposited in a very small volume
  - ↳ alteration or destruction of individual biological molecules
  - ↳ scale of micrometers

Photochemistry

- rxn initiated by interactions of molecules w/ photon radiation
- **Quantum yield (Q)**: # of entities altered per quantum absorbed
- Absorption of light ⇒ excitation ⟹ first excited state in singlet or triplet

$$AB + hf \longrightarrow AB^*$$



↳ $\sum$ spins at ground state = 0

Spin multiplicity $M = 2S+1 = 1$

↳ shown to conserve to form product

- $S_1 \longrightarrow S_0$: fluorescence ($\tau_r \sim ns$)
- $T_1 \longrightarrow S_0$: phosphorescence ($\tau_r \sim ms$)

- **Fluorescence Quantum Yield**

$$\text{fluorescence intensity } (I) = I_0 e^{-t/\tau}$$

$$k = k_r + k_{nr} = \frac{1}{\tau_r} + \frac{1}{\tau_{nr}} = \frac{1}{\tau}$$

τ_r: radiative lifetime; τ_{nr}: non-radiative lifetime

$$\text{fluorescence Quantum yield } (Q) = \frac{k_r}{k_r + k_{nr}} = \frac{\tau_{nr}}{\tau_{nr} + \tau_r} = Q_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2}$$

OD: optic density; R: reference; n: reflect indexes

⇒ # of photons emitted / photon absorbed

- **Photosensitization**
  - a non-absorbing molecule is photochemically altered via the rxn. w/ an excited chromophore
  - Types:
    - $PS + A \rightarrow PSA$ ⇒ changes spectrum of A
    - $PS + hf \rightarrow PS^*$
      $PS^* + A \rightarrow PS + A^*$ — Excitation transfer
      * more likely from triplet state ↳ due to longer time
    - $PS^* + A \rightarrow PS^+ + A^-$ — electron transfer
    - $PS^* + A \rightarrow PS^*A \rightarrow PS + A^*$ — exciplex
    - $PS^* + B \rightarrow PS^* + B$
      $B + A \rightarrow B + A'$ — Indirect rxn. (if $B = O_2$ ⇒ PDT)

- **Singlet with O_2**

$M^* + S \xrightarrow{k_0} M^\circ$

$M^* + O_2 \xrightarrow{k_{O_2}} P$

if $[O_2] \gg [M^*]$ & $[S] \gg [M^*]$

$\Rightarrow \frac{d}{dt} M^* = k_{O_2}[M^*][O_2] + k_0[M^*][S]$ (with O_2) → Singlet $\left(\tau = \frac{1}{k_0[S] + k_{O_2}[O_2]}\right)$

$\frac{d}{dt} M^* = k_0[M^*][S]$ (no O_2) → triplet $\left(\tau_0 = \frac{1}{k_0[S]}\right)$

$$I = \int_0^\infty [M^*]\, dt$$

absence of O_2: I_{total}

presence of O_2: I'_{total}

to calculate k_{O_2}

For $k_{ox}^T = \frac{1}{9} k_{ox}^S$

① ISC to the singlet encounter pair from spin states of different M_s are slow relative to diffusion apart of the encounter partners

↳ ie: the lifetimes of the encounter pairs are shorter than time required for ISC

② All singlet encounter pairs proceed to forming the singlet Oxygen

Molecular Mechanism of PDT

$^1M \xrightarrow{h\nu} {}^1M^* \Rightarrow {}^1M^* \longrightarrow {}^3M^*$

Type I: $^3M^* + H_2O \longrightarrow {}^{\cdot}OH$ or H_2O_2

Type II: $^3M^* + {}^3O_2 \longrightarrow {}^1M + {}^1O_2$

ROS → Biological Damage

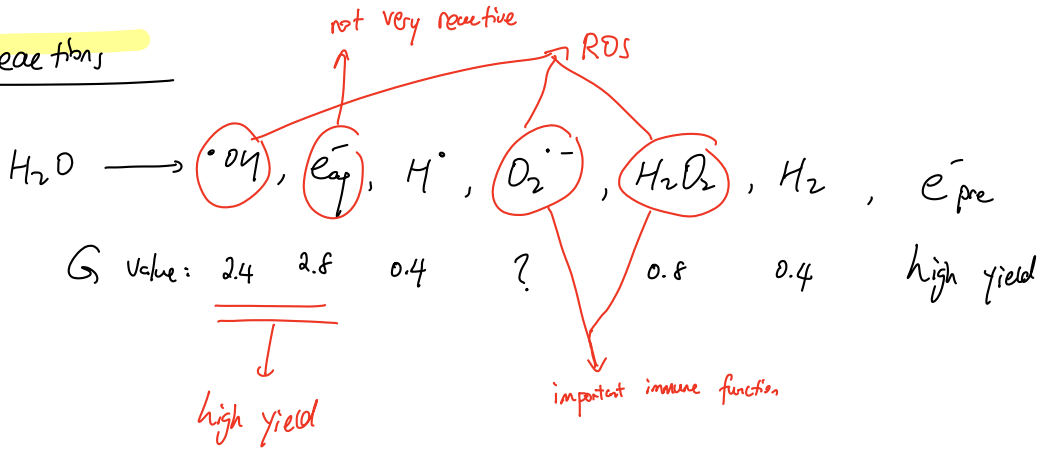
- **Radiation Chemistry**
  - Chemical reactions caused by ionizing radiations
  - Absorption is the whole rxn. mixture
  - 2° particles are formed
  - Quantum yield = "G value"
    - # of altered entities / 100 eV
    - also: molar concentration / unit dose ($\frac{c}{Dc}$)
    - No chain rxn for $G \leq 10$
  - absorbed in H_2O → radiolysis of H_2O → free radical process.
    - highly reactive
- **Radiolysis of H_2O**

$e^- \longrightarrow H_2O \xrightarrow{\text{Ionization}} H_2O^{\cdot+} + e^- \rightsquigarrow \begin{cases} H_2O^{\cdot+} + H_2O \longrightarrow H_3O^+ + {}^{\cdot}OH \\ e^- + nH_2O \longrightarrow e^-_{aq} \end{cases}$

$e^- \longrightarrow H_2O \xrightarrow{\text{Excitation}} H_2O^*$

- **Spur**
  - radiation is locally absorbed on the size of nm.

2° Reactions



- Harber-Weiss Reaction

$$O_2^{\cdot -} + H_2O_2 \xrightarrow{Fe^{2+}/Cu^{+}} H_2O + O_2 + {}^\cdot OH$$

$O_2^{\cdot -} + H_2O_2$: ROS

${}^\cdot OH$: can cause oxidative damage

- Radical Rxn

  - Hydrogen Abstraction

$$RH + H^\cdot \longrightarrow R^\cdot + H_2$$
$$RH + {}^\cdot OH \longrightarrow R^\cdot + H_2O$$

$R^\cdot$: can do damage to biological species

  - Dissociation

$$R-NH_3^+ + e^-_{aq} \longrightarrow R^\cdot + NH_3$$

  - Addition

Add $\cdot OH$ to $C=C$

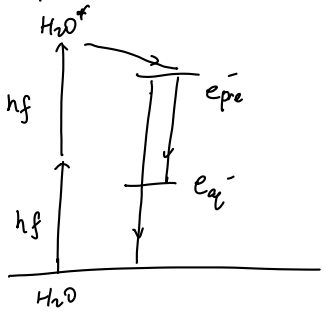
- Solute Radicals form stable products

  - Dimerization, addition of O_2, hydrogen transfer

O_2 fixation

- **Scavenging of e^-_{aq} & $^{\cdot}OH$**
  - Using DMSO & alcohol to capture $^{\cdot}OH$
  - Using N_2O to convert e^-_{aq} to $^{\cdot}OH$

- **e^-_{pre}**



  - e^-_{pre} has a very short lifetime (in fs)
    - ↳ very reactive
    - ↳ Absorption max at IR

- **Radiosensitizer**
  - enhance radiation therapy
    - ↳ XdU, X = Cl, Br, I
  - Radiosensitizing Effect: IdU > BrdU > CldU, no for FdU

$$e^-_{pre} + XdU \longrightarrow XdU^{*-} \longrightarrow X^- + dU^{\cdot}$$
$$\searrow\ XdU^{*-} \longrightarrow XdU^-$$

F^- is very unstable
↳ need +3eV for e^- energy

- **Reductive damage**
  - e^-_{pre} can do reductive damage ⇒ cause DSB & SSB

- KNO_3 is a strong scavenger of e^-_{pre}

- **DNA Packaging**
  - chromatin = DNA + protein (histone) + RNA
    ↓
    package & order the DNA to nucleosomes

- **Absorption Spectrum of DNA**
  - DNA absorption peak: 260 nm
    Protein absorption peak: 280 nm
    } $A_{260\,nm} : A_{280\,nm} = 1.6 \sim 1.8$

  - Absorption is due to bases
    ↳ Thymine has lowest triplet state => primary candidate for photo-induced changes
    ↳ longer life time

- **UV-induced Base Alteration**
  - UV b/w 200 & 300 only affect the bases
  - Absorption at 254nm main product:
    a) pyrimidine dimers => especially T-T dimers, stable
    ↳ most yield ↳ easy to repair
    b) hydrates
    ↳ unstable => easily revert back
    c) DNA-protein cross link => significant yet to be defined
    d) Spore photoproducts
    e) (6-4) pyrimidine adducts => smaller yield, hard to repair
    ↳ very lethal
    f) thymine glycole
    g) Strand break => rare

- **Photosensitized Reaction**

① Bromouracil (BU) / Bromodeoxyuridine (BrdU)

↳ extending spectrum to 313 nm

↳ cause SSB without cause DNA damage

② Ketones

↳ UV light beyond 300 nm ⇒ triple excitation ⇒ Thymine dimers

③ Furocoumarins

↳ PDT of skin disease

↳ absorption at 365 nm

↳ denature / inter cellular DNA replication

- **Direct & Indirect Action of Radiation**

  - Direct ionization of DNA

    ↳ cation radicals ⇒ change G base to FaPy

    ↳ lose of base ⇒ Apurinic / Apyrimidinic Sites

  - Rxn with e^-_{pre} or e^-_{aq}
  - Rxn with $^{\cdot}OH$ or H_2O^+
  - Rxn with $O_2^{\cdot -}$, H_2O_2, $H^{\cdot}$

  } Indirect

⇒ Most damage is via indirect effect

⇒ SSB: easily to repair DSB: hard to repair

- **SSB**
  - Break b/w C3-C4 or C4-C5

- **DSB**
  - Single energy deposition (linear term $\propto$ dose)
  - two SSB (quadratic dependence)

$\Rightarrow$ result in cell killing

- **MDS**
  - mixture of lesions if assume $^{\cdot}OH$ have lifetime ~0.5 ns & diffuse by ~3nm

- **Effect of Oxygen**

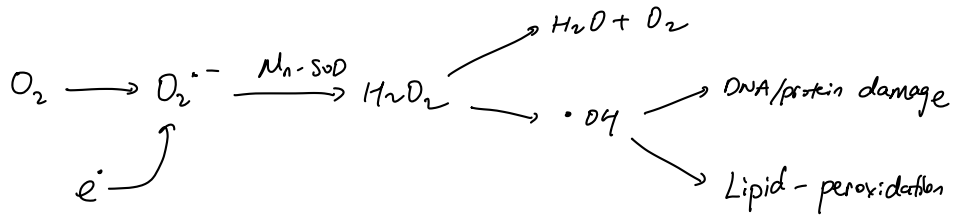
Absence: $R^{\cdot} + XSH \longrightarrow RH + XS^{\cdot}$

Presence: $R^{\cdot} + O_2 \longrightarrow RO^{\cdot}$

↳ stable, cannot be repaired

↳ damage fixation

- **Metabolic Oxidative Stress**



- **Reaction of e^-_{pre}**

$$e^-_{pre} + M \xrightarrow{k_o} e^-_{aq} \Rightarrow Y' \text{ (yield of no scavenger)}$$

$$e^-_{pre} + S \xrightarrow{k_s} S^- \Rightarrow Y \text{ (yield of w/ scavenger)}$$

$$\Rightarrow P_{sc} = \frac{Y - Y'}{Y}, \quad P_{Survival} = 1 - P_{sc}$$

- **Scavenger**
  - DMSO / isopropanol : all $^{\cdot}OH$, 50% & 75% e^-_{pre}
  - KNO_3 : all e^-_{pre}, produce $^{\cdot}OH$

DNA Repair Mechanisms

① Nucleotide excision Repair (NER)
- fix pyrimidine dimers (T-T) & (6-4) photoproducts by UU (more biological damage)
- first discovered in bacteria when irradiated w/ blue light ⇒ photolyase
  - human equivalent to photolyase helps us to set the circadian clock.
- removes bulky adducts via
  - global genome repair (GG-NER) ⇒ whole genome
  - transcription coupled repair (TC-NER) ⇒ actively transcribed genes

UU ⇒ non-ionizing
Mutation does not lead to ionizing radiation sensitivity

② Base excision repair (BER) ⇒ repairs radiation damage
- fix damaged bases & SSB
- defects lead to ↑ mutation rate, not cellular radio sensitivity ⇒ exception: XRCC I

③ Mismatch repair (MMR)
- correct mismatched nucleotides during replication
- defects increase the risk of hereditary colon cancer.
- recruit MMR factors

④ Homologous Recombination (HR) ⇒ repairs radiation damage

↳ Repairs DSB by using an undamaged DNA strand as a template

↳ error free

↳ occur during late S & G_2 phase.

⑤ Non-homologous end-joining (NHEJ) ⇒ repairs radiation damage.

↳ Repairs DSB by using end to end joining

↳ prone to errors

↳ occurs during G_1 ⇒ no template exists $G_2 \begin{pmatrix} M \\ S \end{pmatrix} G_1$

NOT mutually exclusive

⇒ Both are found & active during late S/G_2

⑥ Cross-link repair ⇒ repair ionizing radiation damage (low yield)

↳ repair DNA-DNA & DNA-protein crosslinks (CLs)

↳ NER + recombinational repair pathways are needed for CLs repair

Oxidative Stress

- imbalance between the production of ROS & repair of ROS damage
- Some ROS can act as messengers through redox signalling
- Oxidative stress can cause a lot of diseases
- ROS can always be beneficial in the immune system to kill pathogen
- Biological effects of oxidative Stress:
  - associated w/ ↑ production of oxidizing species such as ROS
  - can cause necrosis, ATP depletion, preventing cell apoptosis

- SOD: important antioxidant

$$O_2^{\bullet -} \xrightarrow{SOD} O_2 + H_2O_2$$

$H_2O_2 \rightarrow O_2 + H_2O$

$H_2O_2 \xrightarrow{\text{Haber-Weiss Rxn}} {}^{\bullet}OH$

Superoxide Dismutase (SOD)

① SOD 1 (with Cu / Zn) ⇒ cytoplasm ⇒ dimer

② SOD 2 (with Mn) ⇒ mitochondria ⇒ tetramer

③ SOD 3 (with Cu/Zn) ⇒ extracellular ⇒ tetramer

⇒ Lack of Mn-SOD (SOD 2) can lead to severe neurodegeneration

↳ require for protecting ROS-induced injury in O_2 metabolism

⇒ Lack of Cu/Zn-SOD (SOD 1) can lead to liver cancer, muscle atrophy, cataracts, reduced life span.

⇒ SOD is also important in IR ⇒ reduce oxygen radiosensitizing effect

⇒ Conclusion: $O_2^{\cdot -}$ participates in radiation induced injury (not necessarily in oxidative stress)

H_2O_2 (SOD) is required for normal physiological functions

⇒ Since $O_2^{\cdot -}$ is a weak oxidant & $^{\cdot}OH$ (a strong oxidant) can form from H_2O_2, their role in physiological functions are not limited to oxidative stress

↳ H_2O_2 participates in cell signalling

↳ Redox eustress

↳ H_2O_2 is also degraded by catalase to prevent the formation of $^{\cdot}OH$

Common ROS

$^1O_2$, $O_2^{\cdot -}$, H_2O_2, $^{\cdot}OH$, $^{\cdot}NO$, HOCl / HOBr / HOI

$^{\cdot}NO$ ↓ RNS

HOCl / HOBr / HOI ↓ RHS

Ionization in Cytoplasm

The cytoplasm is just as likely for radiation damage as in nucleus

⇒ { Nucleus: $\cdot OH$ & e^-_{pre} ⟶ SSB & DSB
  Cytoplasm: more complicated

⇒ Transient generation of ROS / RNS / RHS has been detected when cell exposed to IR

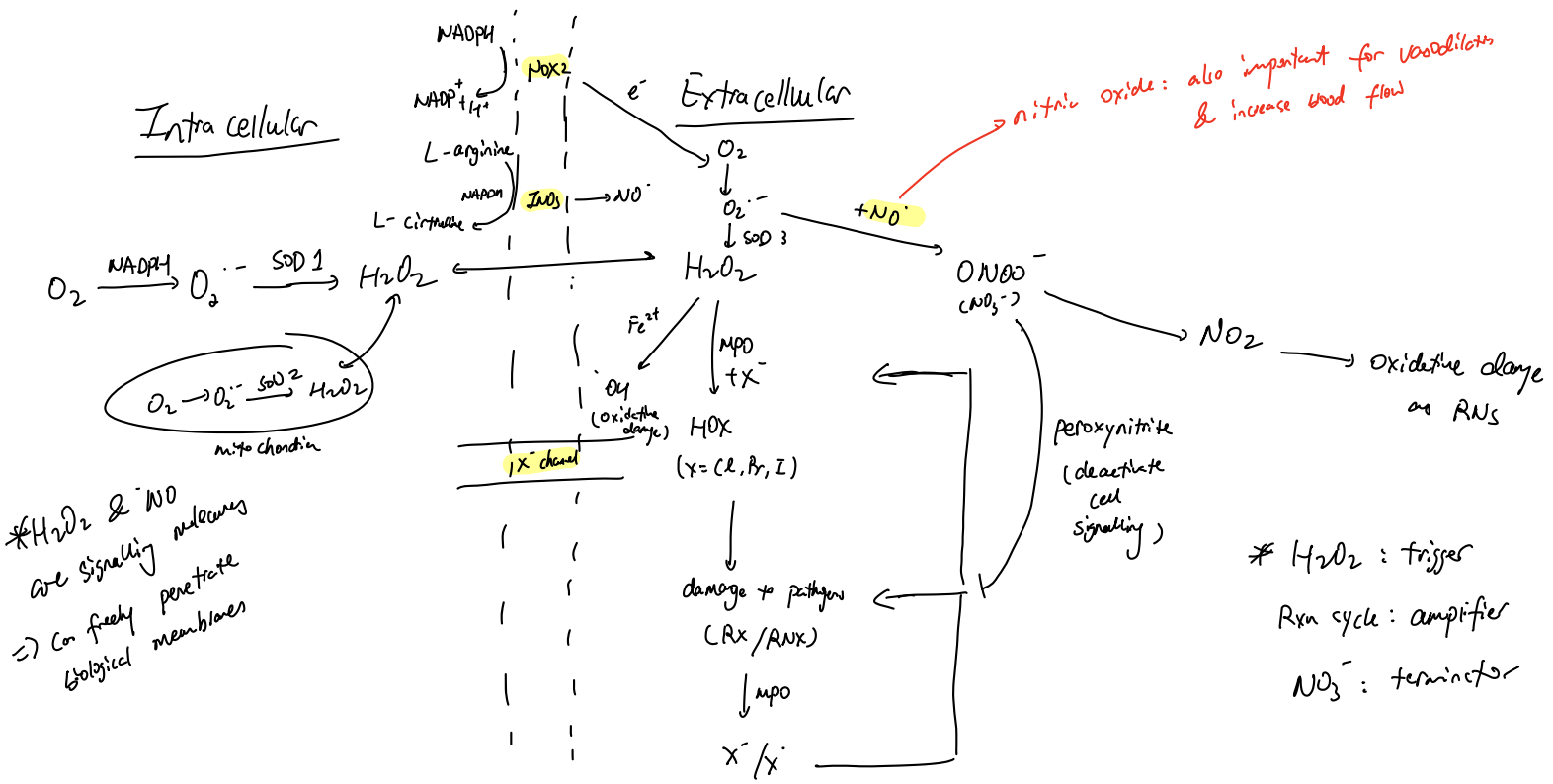
⇒ Mitochondrion & cytoplasm are sources for ROS / RNS / RHS

Big Q:

- ROS by IR at Clinically relevant dose (2 Gy) is ~1000 fold lower than the background ROS level by oxidative metabolism (lethal at 4 Gy)
- ANS: Cytoplasmic amplification mechanisms including ROS / RNS

Reaction Cycle of RHS

O_2-dependent degradation in phagocytosis



Respiratory Burst

- rapid release of reactive species → immune defense & cell signalling
- During IR → produce ROS
- Similar reactions are found by cosmic rays in atmosphere

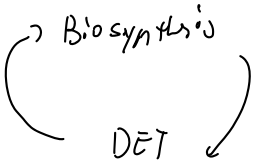
Biosynthesis & DET Rxns

Biosynthesis: produce halogenated organic compounds. (RX/RNX)

$$X^- + H_2O_2 \longrightarrow \underbrace{HOX/OX^-/OX^{\cdot}/X_2}_{\text{RHS}} \longrightarrow RX/RNX + e^-$$

Dissociative electron-transfer (DET) Rxn: produce reactive radicals

$$RX/RNX + e^- \longrightarrow RX^{\cdot-}/RNX^{\cdot-} \xrightarrow{+e^-} (X^{\cdot} + R^-/RN^-) \,/\, (X^- + R^{\cdot}/RN^{\cdot})$$



- The reduction or removal of nitrogen species (HNO_3 as final product) is a critical requirement for the formation of ozone hole in the atmosphere.

⇒ $O_2^{\cdot-}$ is lethal because if only have $O_2^{\cdot-}$ but no SOD

⇒ Halogenated aromatic drug could be used to treat COVID-19

Reproductive integrity

- Cell death means:
  - For non-proliferation cells: loss of specific function
  - For proliferation cells: loss of reproductive integrity ⇒ reproductive death
  - In radiobiology:
    - ↳ lose the ability to divide indefinitely & produce a large # of progeny ⇒ dead.
    - ↳ Survivors are clonogenic
    - ↳ For tumours, cells are killed if unable to divide
      - ↳ via mitotic cell death (dominant mechanism)
      - ↳ via apoptosis
    - ↳ 100 Gy to destroy cell completely, 2Gy for loss of proliferative capacity

In Vitro Cell Survival Curve

- Use of established cell lines
- plating efficiency (PE) ⟶ % cell seeded that grow into colonies

$$PE = \frac{\#\text{ of colonies counted}}{\#\text{ of cells seeded}} \times 100\%$$

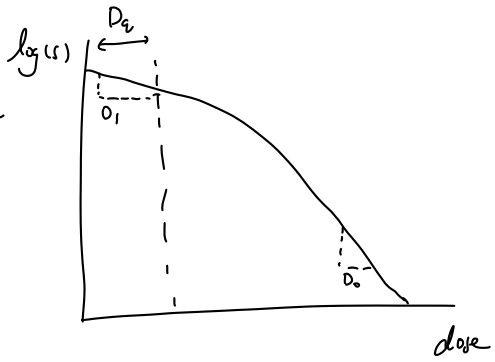
- After irradiation & incubate for 1-2 weeks:
  - ① seeded single cells are still single ⇒ nuclear deterioration
  - ② 1 to 2 division ⇒ tiny colony ⇒ not survived
  - ③ large colony ⇒ <u>survived</u> & retain reproductive integrity.

$$\text{Survival fraction} = \frac{\text{Colonies counted}}{\text{Cell seeded} \times PE}$$

⇒ For more dose, seed more cells ⇒ counting is easier

Shape of Cell Survival Curve

- low LET radiation (ex: x-ray)
  - ↳ starts out straight on log(S) vs. d at low dose
  - ↳ at higher dose, it bends
  - ↳ very high dose: straight again
- high LET (ex: α-particles, neutrons)
  - ↳ straight line for all doses



Analysis of Cell-Survival Curve

① Target theory

A number of critical targets have to be inactivated for cells to be killed

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

- If this is a single-hit, single target model ⟶ straight line for semi-log.
- On radiosensitive mammalian cells as responses to low-dose rates
- D_0: the dose that averges of 1 hit/target based on Poisson distribution ($f(n) = e^{-a}\frac{a^n}{n!}$) (or D_{37})
  - ↳ reduce survival rate to 37% ($n=0, a=1$) when $D=D_0$

average hit number per target

# of target receiving n hits

② Multitarget model

- For cells with more than 1 identical target:

$$S = N/N_0 = \left(1-(1-e^{-D/D_0})^n\right) e^{-D/D_1}$$

⇒ bent shoulder + straight line at higher doses

Size of shoulder (D_q) $= D_0 \ln(n)$ ⇒ $\ln(n) = \frac{D_q}{D_0}$

⇒ D_1: initial slope from single-event killing

D_0: final slope from multiple-event killing

n/D_q: size of shoulder

- To reduce survival fraction to 10% (ie: one decay of cell kill)

$$D_{10} = \ln(10) \times D_0 = 2.3\, D_0$$

③ Linear Quadratic Model

- Continuously bending ⇒ no straight portion

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

The contribution from linear & quadratic are equal at $D = \alpha/\beta$.

- Commonly used in clinic ⇒ accurate representation of radiotherapy dose data

Mechanism of Cell death

- Apoptosis:
  - programmed cell death, change in chromatin
  - DSB with DNA fragments (~ 185 b.p.) ⇒ ladders in gel
- Necrosis:

  traumatic cell distruction ⇒ acute cell death ⇒ no change in chromatin

- Senescene: ⇒ relatively unimportant for IR

  Shorten of telomeres ⇒ permanent cell-cycle arrest

- Autophagy:
  - long lived proteins & organelles are directed to lysosomes for degradation ⇒ relate to amplification mechanism
  - Disruptance could lead to disorders that appear in the elderly
- Mitotic cell death

  aberrant mitosis ⇒ cell death or non-viable cells

  ↳ chromosome aberrations

Radiation induced Cell-death

- In normal tissue:
  - High proliferative capacity ⇒ apoptosis
  - Fibroblast ⇒ growth arrest
- In tumour:
  - less likely apoptosis ; more likely mitotic catastrophe or senescence-like irreversible growth arrest
  - Apoptosis might be important in early stage
  - Mitotic cell death is the cause post radiotherapy or chemotherapy
    - frequently followed by apoptosis in apoptosis-competent cells

Mitotic cell death

- Exchange-type aberrations need two chromosome breaks.
  - low dose: single electron → probability ∝ dose
  - high dose: 2 electrons → probability ∝ dose^2 ⇒ Survival curve bends
- We use linear-quadratic relationship to describe this
- Curved in a log-liner plot, with a broad initial shoulder.
  - Also: dose-rate effect for low & high doses

Apoptotic death

- Straight line on a log-linear plot

⇒ Most cells are in between

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

mitotic, apoptosis

Effective Survival Curve for Multifraction Regimen

- For multifraction, effective dose-survival curve becomes an exponential function of dose.
- D_0/D_{37}: the dose required to reduce fraction of cell survival to 37% (1/e)
- 90% probability of tumour control: depopulation of 10^{-x-1}

  i.e.: for a colony of 10^8 → depopulation of $10^{-8-1} = 10^{-9}$
- $D_{10} = 2.3\ D_0$ → kill 90% of population

Autophagic Cell Death by IR

- Signalling from PERK
- Induction of ER stress → inducing autophagic cell death → radiosensitization

Senescence Cell death by IR

↳ results in permanent cell cycle arrest

↳ will not eliminate the mitogenic or cytokine contribution to tumour growth

Dogma of Radiation Biology

① Nucleus was much more radiosensitive than the cytoplasm → DSB by IR

② damage by α-particles is very localized

③ Certain radiosensitizer via substitution (ex: BrdU) modifies cellular radiosensitivity.

④ Damage to DNA/chromosome is correlated to cell lethality

⑤ positive correlation b/w nucleic acid volume & radiosensitivity

By Stander Effect

- Induction of biological effects in cells that are not directly traversed by a charged particle, but in proximity to cells that are
- Idea: H_2O_2 as a signaling molecule ⇒ cross membrane

Tumour Environment

- All causes resistance to chemotherapy

① High interstitial fluid pressure (IFP)

- caused by formation of high contractile ECM
- reduce transcapillary fluid flow & transportation of therapeutic molecules

② low oxygen tension (or hypoxic)

- caused by poor O_2 delivery in defective vasculature ⇒ disorganized vasculature
- Oxygenation is heterogeneous both spacially & temporally
- affect effectiveness & is more present in apoptosis-resistant cells

③ Low extracellular pH (pHe) ⇒ acidic

- induction of carbonic anhydrase IX & XII & impaired clearance of metabolic product
- shift in glucose metabolism to anaerobic glycolysis ⇒ 200× higher
- increased glucose intake ⇒ generate lactic acid
- HIF-1 regulates glycolysis under hypoxia environment. → recruit VEGF
- reduced gradient ⇒ barrier for drug delivery

HIF

Normoxia:

$$HIF\text{-}1\alpha \xrightarrow{O_2} O_2\text{-}HIF\text{-}1\alpha \xrightarrow{VHL} VHL\text{-}O_2\text{-}HIF\text{-}1\alpha \longrightarrow \text{degredation}$$

Hypoxia:

HIF-1α, ARNT → HRE gene → EPO → RBC production → Brings out more O_2

*Drugs can interfere w/ disease state by activating or blocking the O_2-sensing machinery

Warburg Cancer Hypothesis

Cancer is a metabolic disease

↳ mitochondrial dysfunction ⟶ malignant transformation

↳ instead of mutations in tumour suppressor genes

Radiotherapy & hypoxia

- Tumour hypoxia ⇒ O_2 is radiosensitizer; hypoxia reduces effect (2.5-3 for x-ray)

↳ Oxygen enhancement ratio (OER)

$$OER = \frac{\text{Radiation dose in hypoxia}}{\text{Radiation dose in air}}$$ for same biological effect

Mechanism for O_2 Effect

- Oxygen fixation hypothesis (OFH)

$R \longrightarrow R^{\cdot}$ — In biological system → $DNA + {}^{\cdot}OH \longrightarrow {}^{\cdot}R$

${}^{\cdot}R + O_2 \longrightarrow {}^{\cdot}RO_2$ → irreparable

${}^{\cdot}R + O_2 \longrightarrow$ inactive product

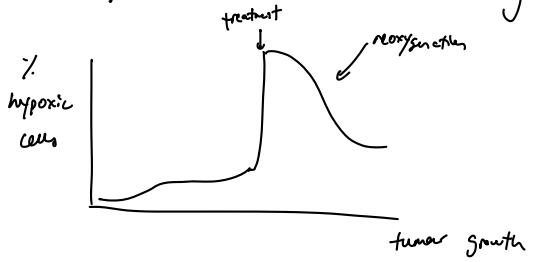
- Yet, similar chemical species do not perform as well ⇒ OFH might not be the full picture

Acute vs. Chronic hypoxia in tumour

- Chronic hypoxia: due to cells locating very far from vasculature ⇒ short life span & radio resistant
- Acute hypoxia: intermitten flow in blood vessels; shorter period of hypoxia

↳ blood vessels are temporarily occluded.

- Reoxygenation: give fractions to enhance radiation killing of initialy hypoxic cells



Radiosensitization

① Increasing oxygen-carrying capacity in blood to reduce hypoxia

② Anti-vascular endothelial growth factor (VEGF) to normalize blood vessel

③ Decreasing tumour oxygen consumptions.

④ Mild hyperthermia (41°C - 42°C) improves oxygenation

⑤ Block Ras signalling to increase oxygenation

Drugs for Hypoxia

① Hypoxic Sensitizers

- mimic radiosensitizing properties of O_2
- Electron affinity from O_2 to form radicals when IR hits ($O_2 + e^- \longrightarrow O_2^{\cdot -}$) (e⁻ from IR)
- ex: nitromidazoles ⇒ yet, trial results are not satisfactory
- Not this is not OFH ⇒ We are not using O_2 to fix damage. ⇒ purpose other than OFH

② Hypoxic Cytotoxins

- Use bioreductive drugs that are only toxic under hypoxic conditions
- ex: TPZ ⟶ TPZ radical ⟶ reduce O_2 if O_2 is present ($O_2 \rightarrow O_2^{\cdot -}$)
  ↘ no O_2, reductive damage on molecules

↳ However, $O_2^{\cdot -}$ is not a strong oxidizing agent, but could produce H_2O_2 & to be amplified in the RHS cycle.

Fractionation

- produces better tumour control for a given level of normal tissue toxicity than a single large dose.
- Why? ⇒ 4 Rs in Radiobiology

Four Rs in Radiobiology

① Repair of sublethal damage
  - ↳ DSB is repaired by enzyme ~ hours

② Repopulation
  - ↳ cell growth ~ weeks

} for normal tissue ⇒ divide dose in fractions to spare normal tissue

③ Redistribution (reassortment of cells)
  - ↳ cell cycle effect ~ days

④ Reoxygenation
  - ↳ oxygen effect ~ days

} for tumour ⇒ divide dose in fractions + increase dose to tumour

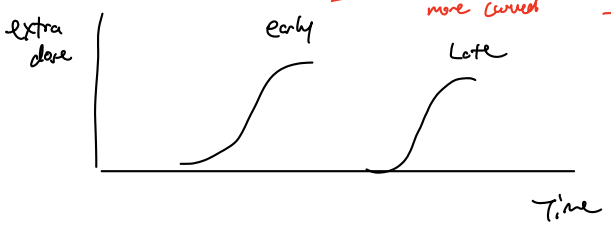
Proliferation

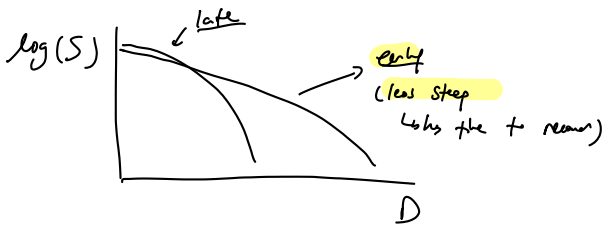
- In human skin, proliferation kicks in at around week 4 ⇒ need additional dose
- Different for different organ: Late-responding or early-responding

$(S = e^{-\alpha D - \beta D^2})$ → larger β = more curved

Small α/β → more curved

large α/β





Early & Late-Responding Tissue

- Hyperfraction → small dose at high freq.
  - ↳ reduce late effects
- Hypo fraction → large dose few fractions
  - ↳ more severe late effects
- Late tissues are more sensitive to changes in fractionation pattern than early tissues.
  - ↳ In fast growing (early) tissues, S occupies a majority of cell cycle → acutely responding → self-sensitizing
  - ↳ In slow growing (late) tissues, cells are in G_0 resting → radio resistant
- Iso effect curve:
  - ↳ dose vs. # fractions for same biological effect
  - ↳ steeper for late effect. → fractionation determines late effect
    less steep for acute (instant) effect.
- Early response tissues are easier to treat than late response tissues w/ radiation
  (large $\alpha/\beta \sim 10$ Gy) (small $\alpha/\beta \sim 2$-3 Gy)

Fractionation in Linear Quadratic Models

- We repeat shoulder ⇒ linear on semi-log
- Biological effect (E) $= -\ln(S) = nd(\alpha + \beta d)$

  Biological effective dose (BED) $= \frac{E}{\alpha} = (nd)\left(1 + \frac{d}{\alpha/\beta}\right)$
- For early responding tumour: prol: fraction

$$\frac{E}{\alpha} = nd\left(1 + \frac{d}{\alpha/\beta}\right) - \frac{\ln(2)(T - T_k)}{\alpha T_D}$$

(single fx dose; start time; # of doses; doubling time)

- For multiple treatment, $BED = \sum_i BED_i - \frac{\ln(2)(T - T_k)}{\alpha T_D}$

Cancer Survival Rates

- High rates are for Cisplatin treatable cancers & reproductive cancers
- Low rate: pancreas, stomach, CNS/PNS, liver, lung
- Oncology drug success rate: 10%, cost 1.8 billion, over 13 yrs.
- Radiation therapy only treats 12.5% of patients ⇒ rest relys on chemo, target, hormone, or immunotherapy

Problems with Chemotherapy

- Toxicity to normal tissues
- Intrinsic & developed drug resistance

Chemotherapy Basis

- Affecting DNA Synthesis
  - ↳ however, limited by fraction of cells in active cycle (S)

Chemotherapy Targets

- Cell cycle specific agent ⇒ target S or M.
  - ↳ ex: cisplatin → inhibit DNA Synthesis in S phase
  - ↳ ex: Paclitaxel → mitotic inhibitor in G_2/M phase
  - ↳ Agents that target S-phase → ineffective in slow growing cells (ie: in G_0 mainly)
- Cell cycle non-specific agent ⇒ independent of cell cycle
  - ↳ ex: Alkylating agent → agent for proliferative tumors

Classes of Agents

① Alkylating

↳ highly reactive, substitute alkyl groups in DNA

② Antibiotic

↳ direct binding to DNA

③ Antimetabolites

↳ interact with metabolic enzymes

Target Therapy

- Target epidermal growth factor receptor (EGFR)
- Monoclonal antibody binds to EGFR & inhibit cell growth
  - ↳ induction of apoptosis & decrease VEGF
- Ex: Cetuximab
  - ↳ increased survival when combined w/ radiotherapy
- Ex: Bevacizumab
  - ↳ neutralizes effect of VEGF to improve dry deliver
  - ↳ increased survival when applied with chemo.

Oxygen Effect of Chemotherapy

- Some like aerobic, some like hypoxic, others have no selectivity

Drug Resistance

- Intrinsic or develop overtime.
- Strategy: combine different drugs w/ different mechanisms
  - ↳ yet, multidrug resistance & cross-resistance is also possible
- Yet, cells are usually not resistant to radiation

Combination Therapy

① Chemo + Radio

- Radiation on large tumor; chemo on metastases; or
- Chemo on large tumor; radiation on sanctuary sites unreachable by chemo.

② Interactive b/w chemo & radio

- Use chem drug to change cell cycle, then treat w/ radio.

③ Combine w/ non-toxic agents

- ↳ ie: hypoxic radiosensitizers => increase radiosensitivity selectively
- ↳ ie: selective protection via radioprotectors
- ↳ w/ chemotherapeutic drug

Cisplatin Problems

- Side effect & drug resistance
- Utilize dissociative electron transfer (DET) from Cisplatin to G-base (with help of Pt)
  - ↳ in radical, e^- from radiolysis of H_2O
- Develop novel combination therapies via DET to make Cisplatin safer & more effective.

Photodynamic Therapy (PDT)

- Use photosensitizer & light to produce singlet O_2 & kills cells
- need specific wavelength of activation
- Adv.
  - no long term effect
  - less invasive than surgery
  - short treatment time
  - precise target
  - can be repeated many times (unlike radiation) at the same site
  - No scarring
  - cost less

Mechanism

① Produce $^{\cdot}OH$ & H_2O_2 from H_2O

② Produce $^1O_2$ from $^3O_2$

⟹ Cytotoxicity directly or activating immune responses

Actions:

① Cellular
- in plasma membrane → necrosis
- in mitochondria → apoptosis

② Vascular
- damage damage on blood vessle ⇒ tumor hypoxia & prevent nutrient transport.

③ immunological
- activate immune system to attack tumor cells ⇒ lysosome

- Can treat esphagus & non-small cell lung cancer, & actinic keratosis (AK)

- Limitation
  - Low penetration depth
    - Light is absorbed by hemoglobin for visible light
  - Need O_2 → less effective for solid tumour
  - Local therapy & not on metastasized cancer
  - patient become very sensitive to light (temporary)
  - Side effects (burns, pain, scaring → temporary)
  - Cannot be used in patients w/ certain diseases.

⇒ Future: more selective, deeper, and collect more quickly in cancer.

Femtomedicine

- Use of FMD compound → no pt → non-toxic to normal cells/tissue
  - (to treat cancer directly)
  - → non-toxic in blood
  - ⇒ replacement of cisplatin
  - → specifically kill cancer cells
  - → induce apoptosis in cancer cells but not normal cells
  - → inhibit tumour growth
  - → Can be coupled w/ radiotherapy for targeted
    - FMD as radiosensitizer
  - → High LD_{50} w/ high max tolerance dose
- FMD is halogen → induce rxn. cycle