4 Rs of Radiobiology

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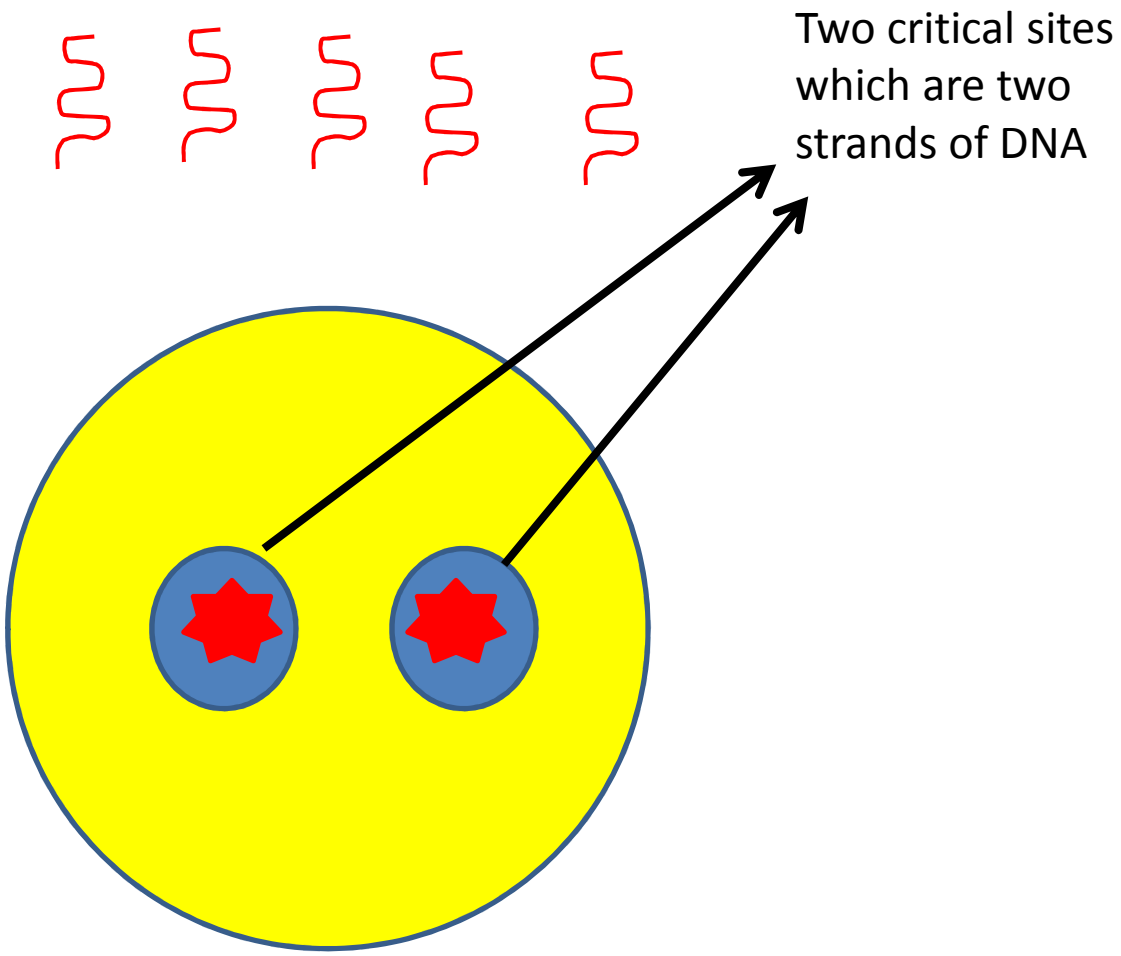
Forms the basis of fractionated radiotherapy

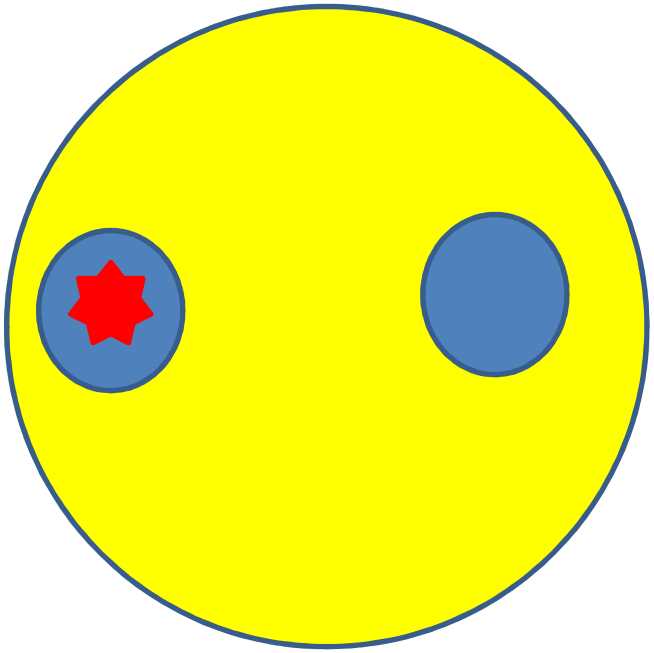
- **R**epair
- **R**e-oxygenation
- **R**e-distribution
- **R**e-population

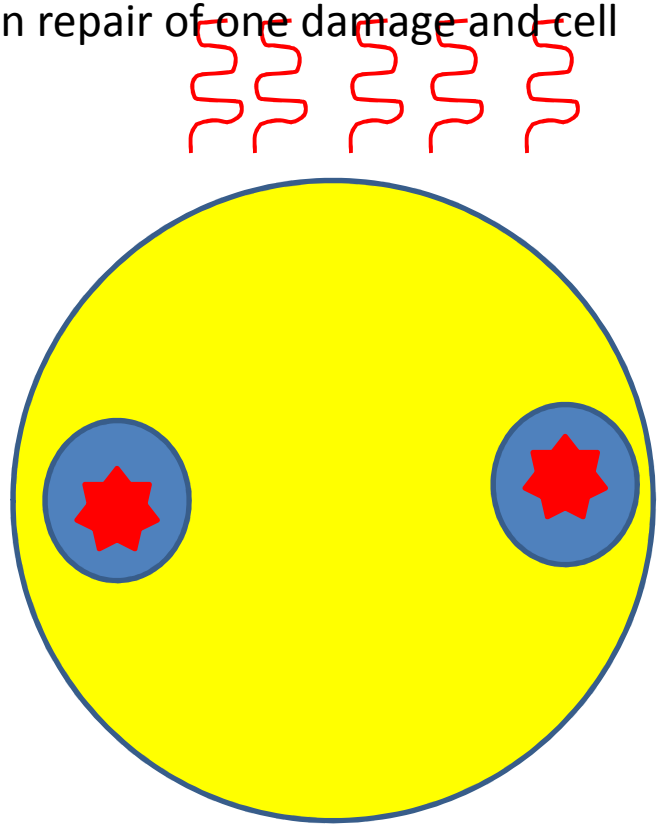
Lethal Damage=Irreversible=irrepairable Damage



Sub Lethal Damage = Repairable Damage

- Interaction between two hit sites results into cell death
- If sufficient gap between two radiation events then repair of one damage and cell does not die, k/as SLDR

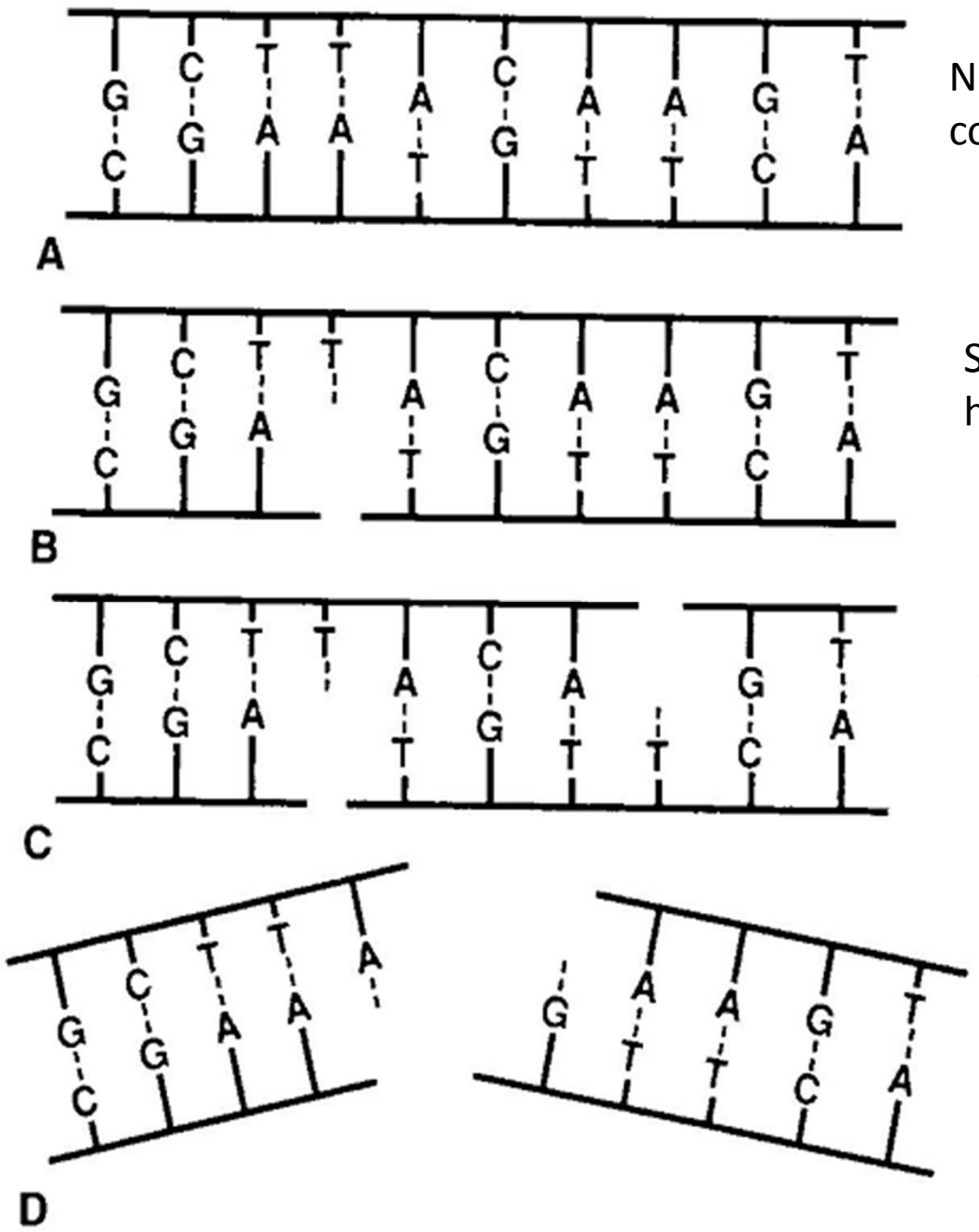




Sub Lethal Damage Repair (SLDR)

- *How does this SLD repair occur?*

- **TEMPLATE THEORY.**
- **CHEMICAL POOL THEORY.**

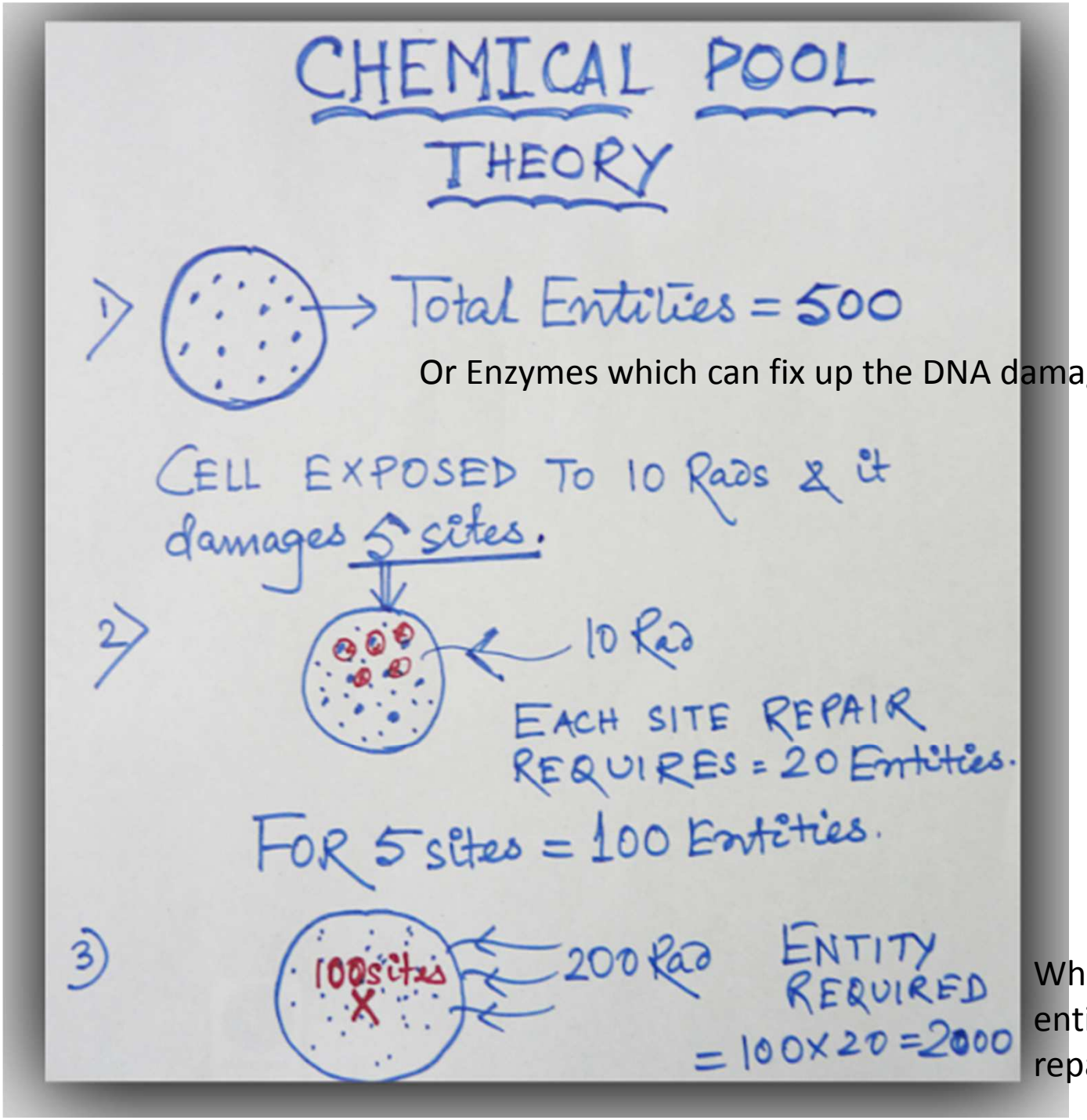


Normal DNA helix. The base pair are complimentary

Single strand break. Repair using other helix as template.

Double strand break at different level. Again will repair as two different single strand break.

Double strand break facing each other will not be repaired and lead to cell death.



Or Enzymes which can fix up the DNA damages

While cell have only 500 entities so damages are not repaired and cell will die

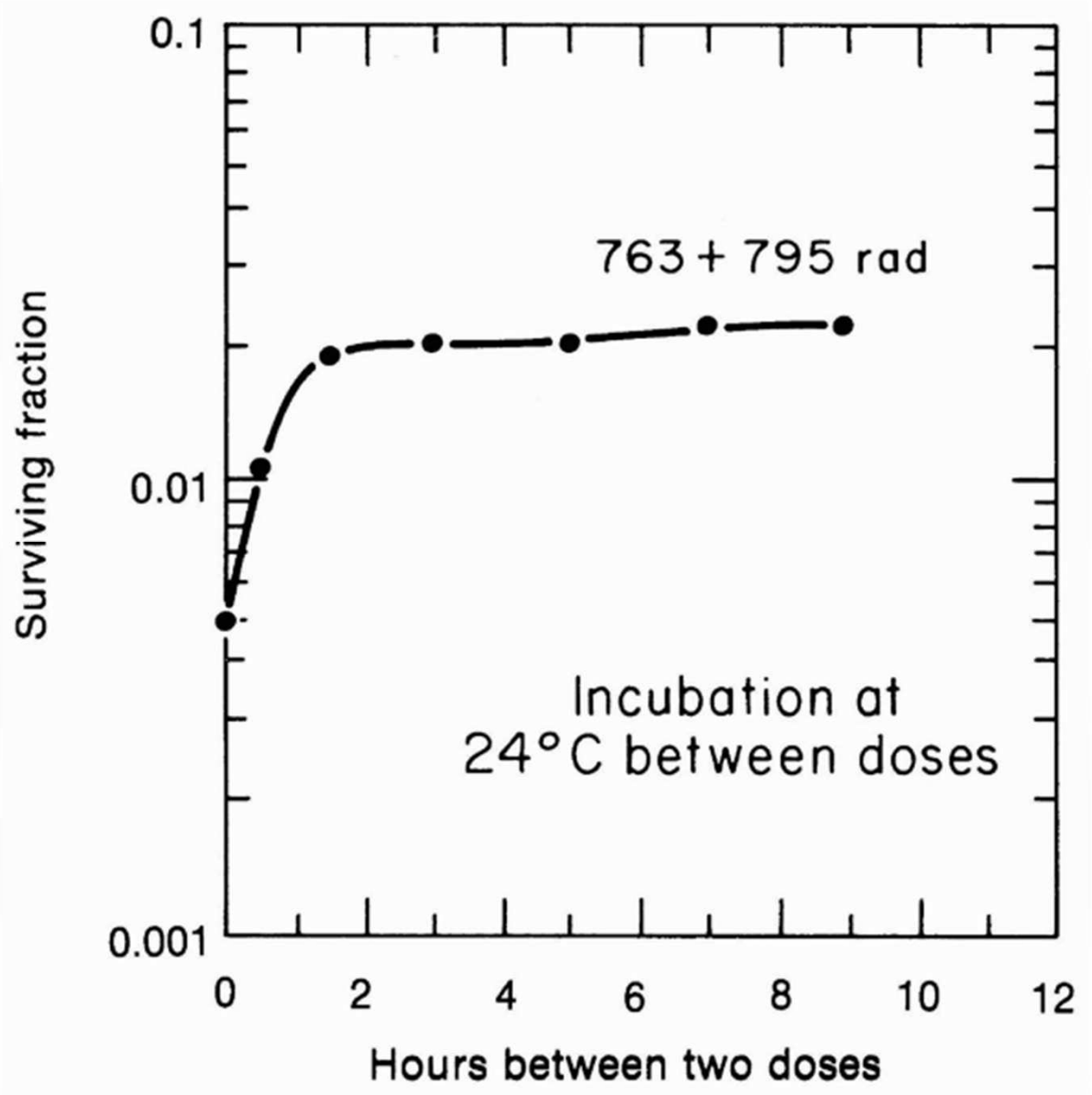
Multiple Targets

- Chemical pool theory defy two target theory.
- There are multiple targets in the cell (N)

- N targets hit simultaneously
- Accumulate SLD & their interaction.

Cell death.

- If (n-1) targets are hit repair of SLD occurs :
- **ELKINDS RECOVERY.**



First experiment on Chinese hamster cells to show the repair of SLD.

Single 1558 cGy results into a survival fraction of .05

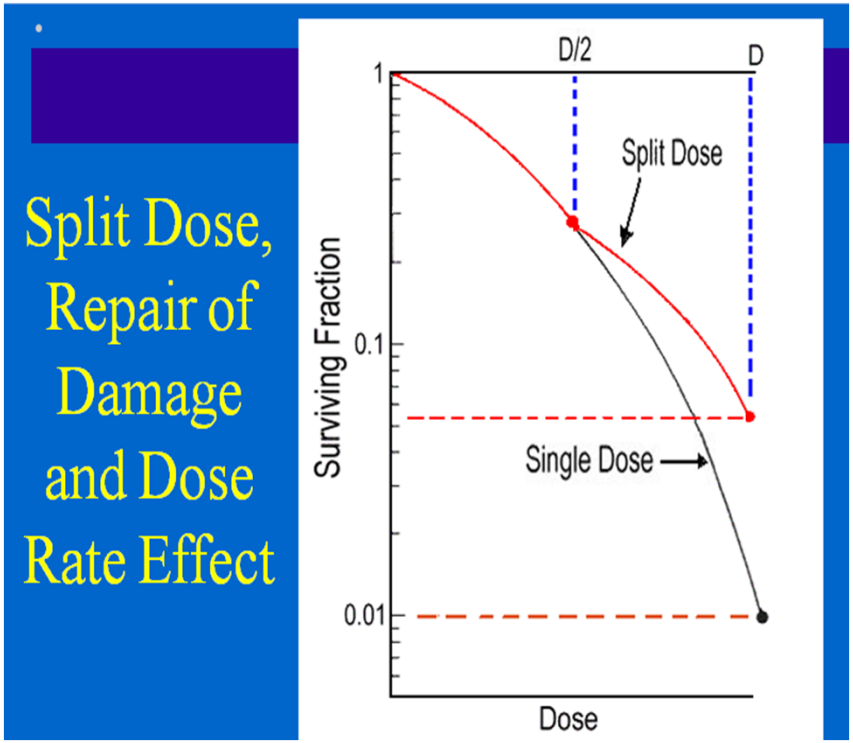
But when given in two divided doses few minutes apart the survival fraction increases

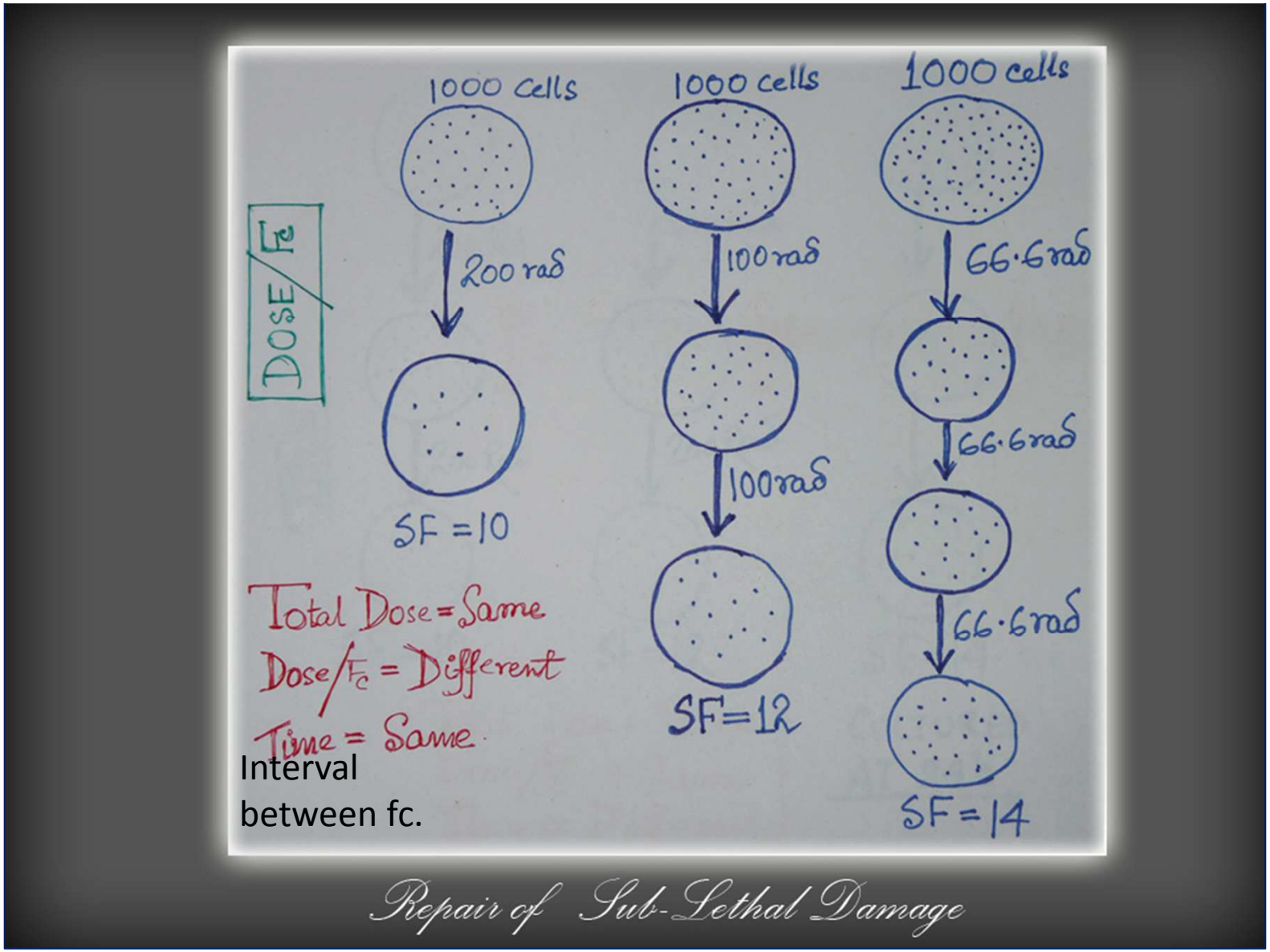
As the time interval between two doses increase the survival fraction also increases till it reaches a plataeu at time interval of 2 hours.

ELKINDS RECOVERY

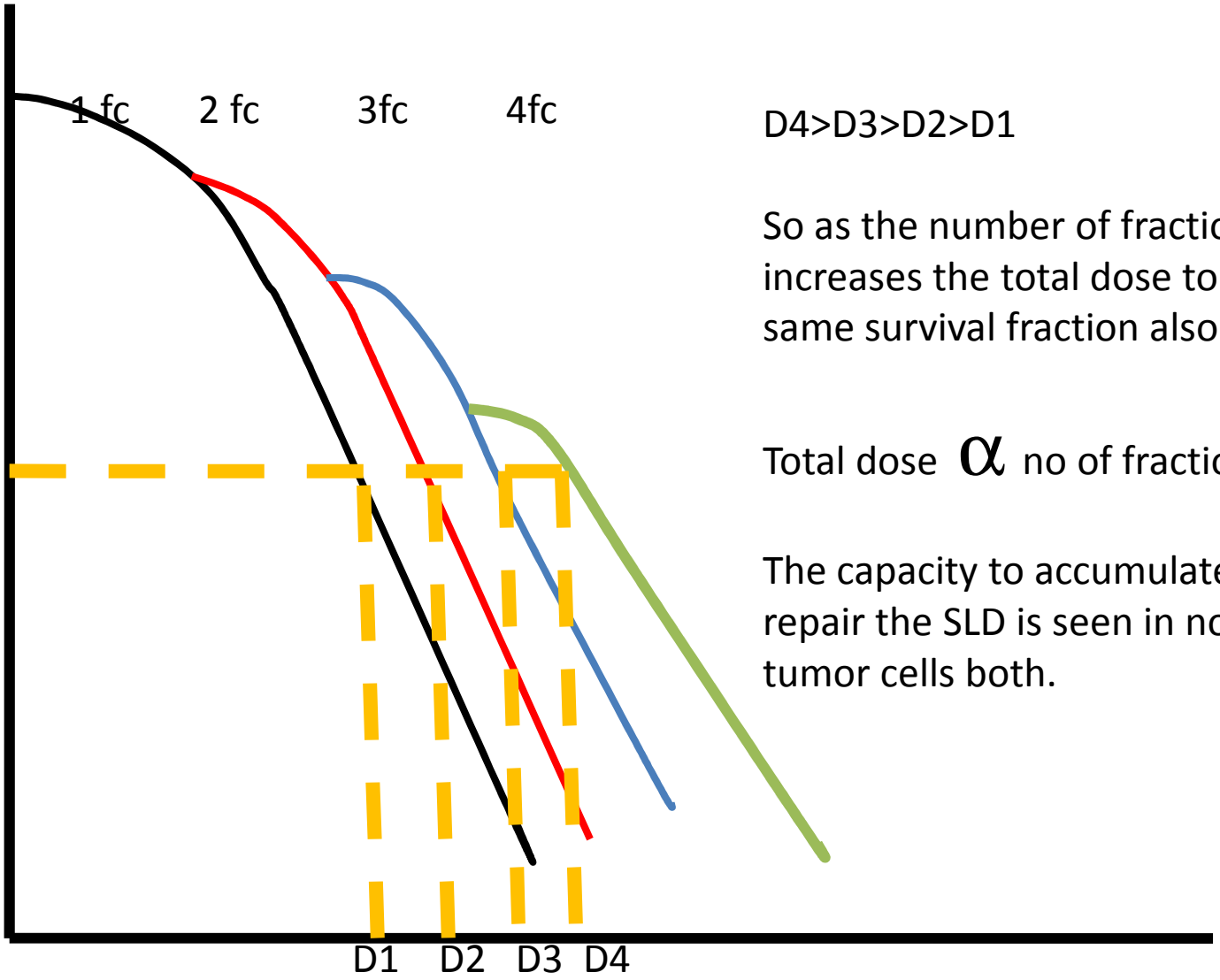
- Elkind & Sutton showed that when two exposures were given two hours apart the shoulder reappeared on cell survival curve.
- (n-1) targets repaired completely.

- Fresh cells without any radiation injury.





As the same total dose is delivered in fractions, the no of colonies counted increased with increasing no of fractions. This is because of repair of sub lethal damages.



D4>D3>D2>D1

So as the number of fraction increases the total dose to achieve same survival fraction also increases

Total dose α no of fractions

The capacity to accumulate and repair the SLD is seen in normal and tumor cells both.

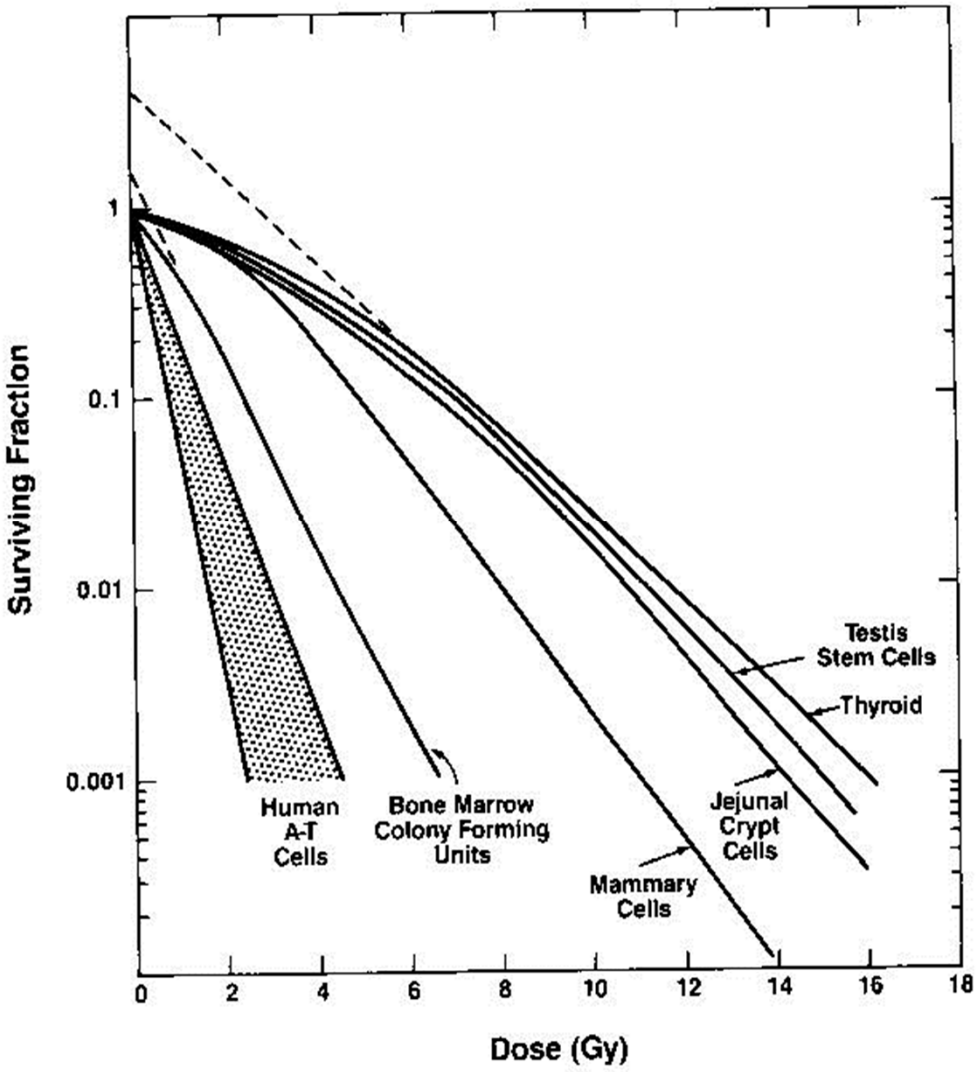
Effect of SLDR on Cell Survival Curve

Intracellular repair

- Studies have shown that although repair can be an ongoing process, the vast majority of the repair is finished by 6 hours post irradiation.

- Once repair is complete the remaining cell population will respond to subsequent dose of radiation as though the original irradiation had not occurred

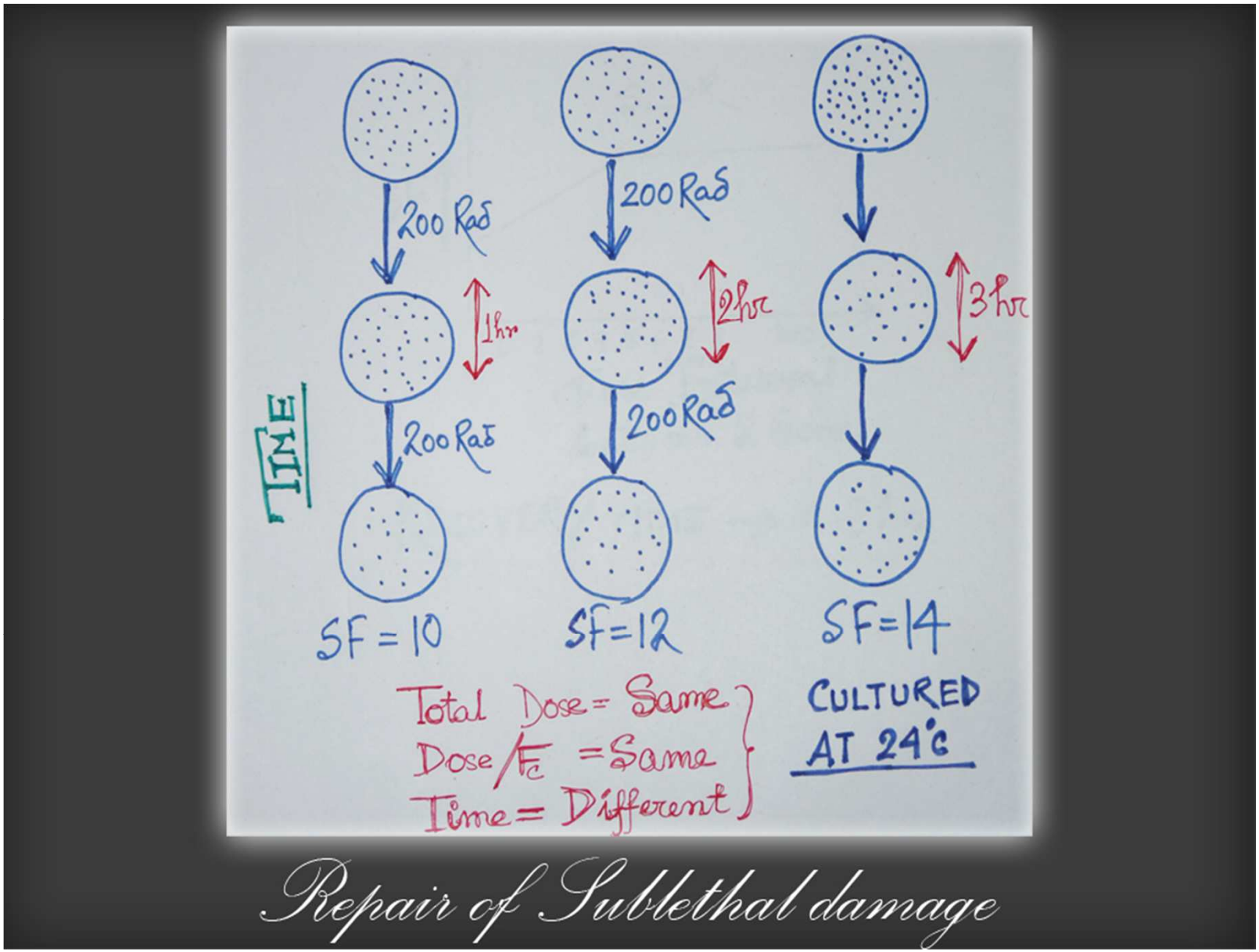
Repair capacity also influences radiosensitivity



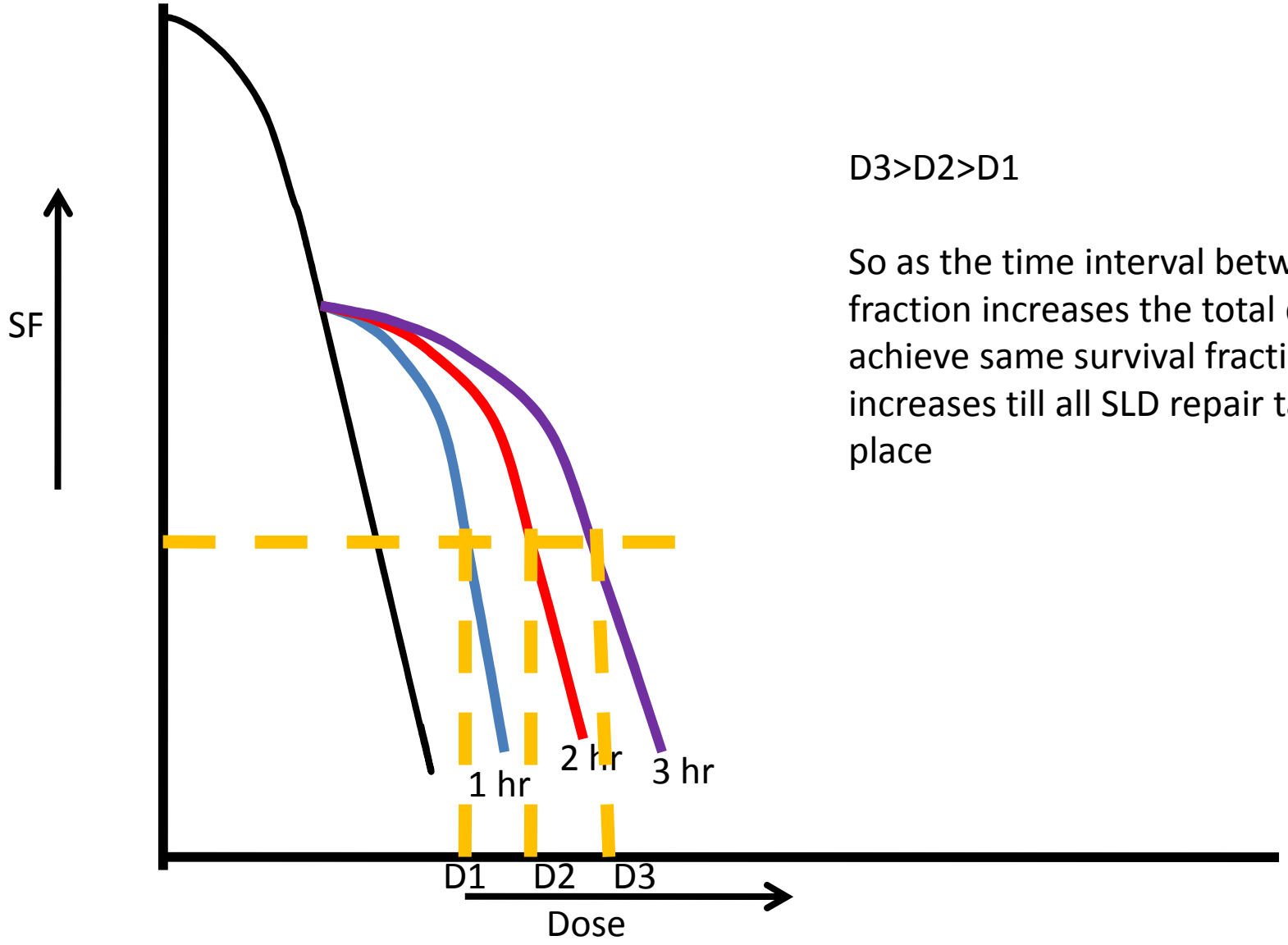
- Human A-T cells and other cells with deficits in repair pathways that repair DNA damage induced by radiation are hypersensitive to radiation
- Hematopoietic cells, including leukemias and lymphomas, have poor repair capacity. That is why they are also very sensitive to radiation.

Timing of sub lethal damage Repair

as the timing between the fractions increased the colony counted also increased indication that complete repair require some fixed time.

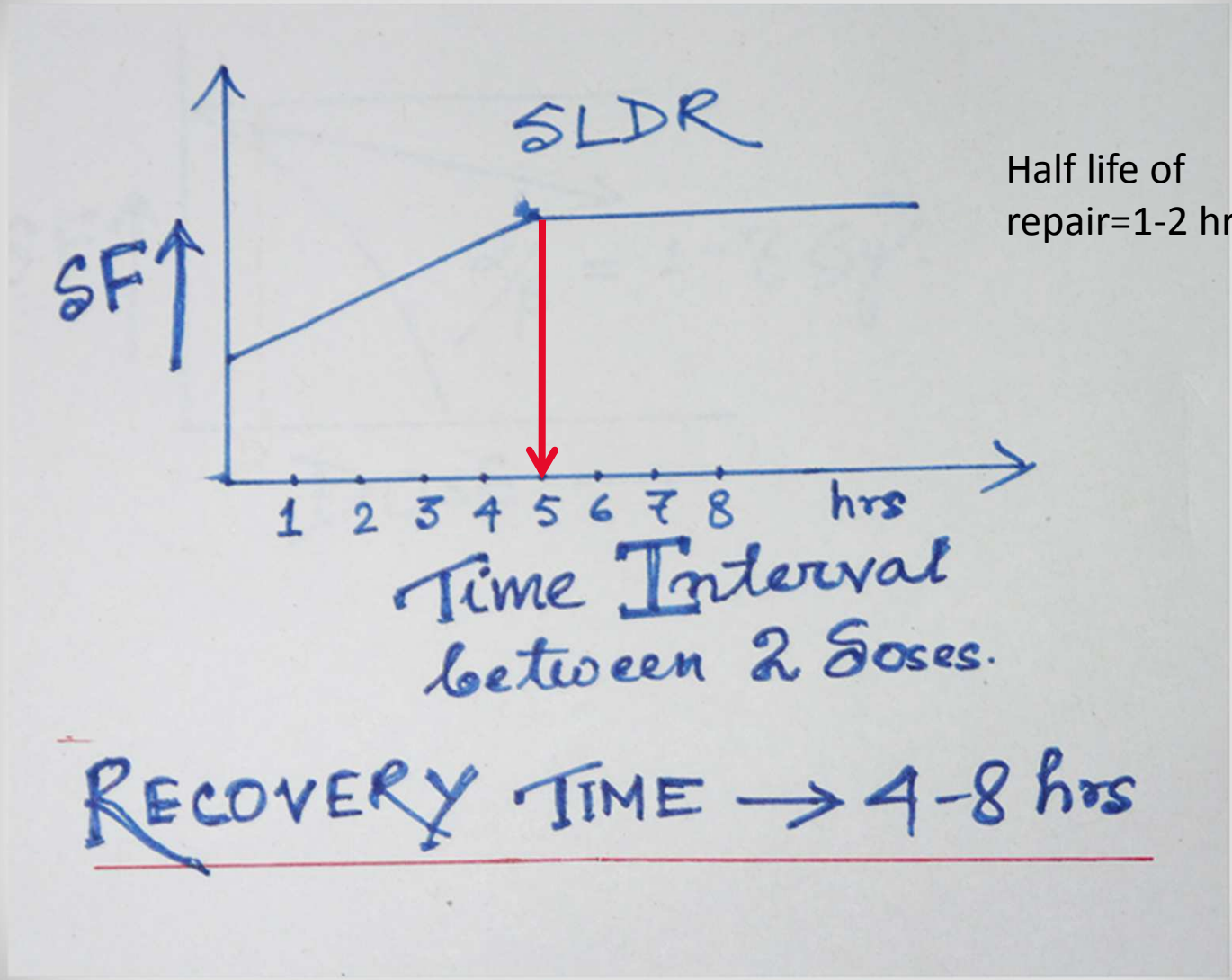


Effect of time interval between two fraction on Cell Survival Curve



D3>D2>D1

So as the time interval between two fraction increases the total dose to achieve same survival fraction also increases till all SLD repair takes place



EFFECT OF TIME INTERVAL ON SUBLETHAL DAMAGE REPAIR

- **R**epair
- **R**e-oxygenation
- **R**e-distribution
- **R**e-population

Oxygen and cell survival curve

1. Less damage to tightly pressed skin, Swartz 1912,

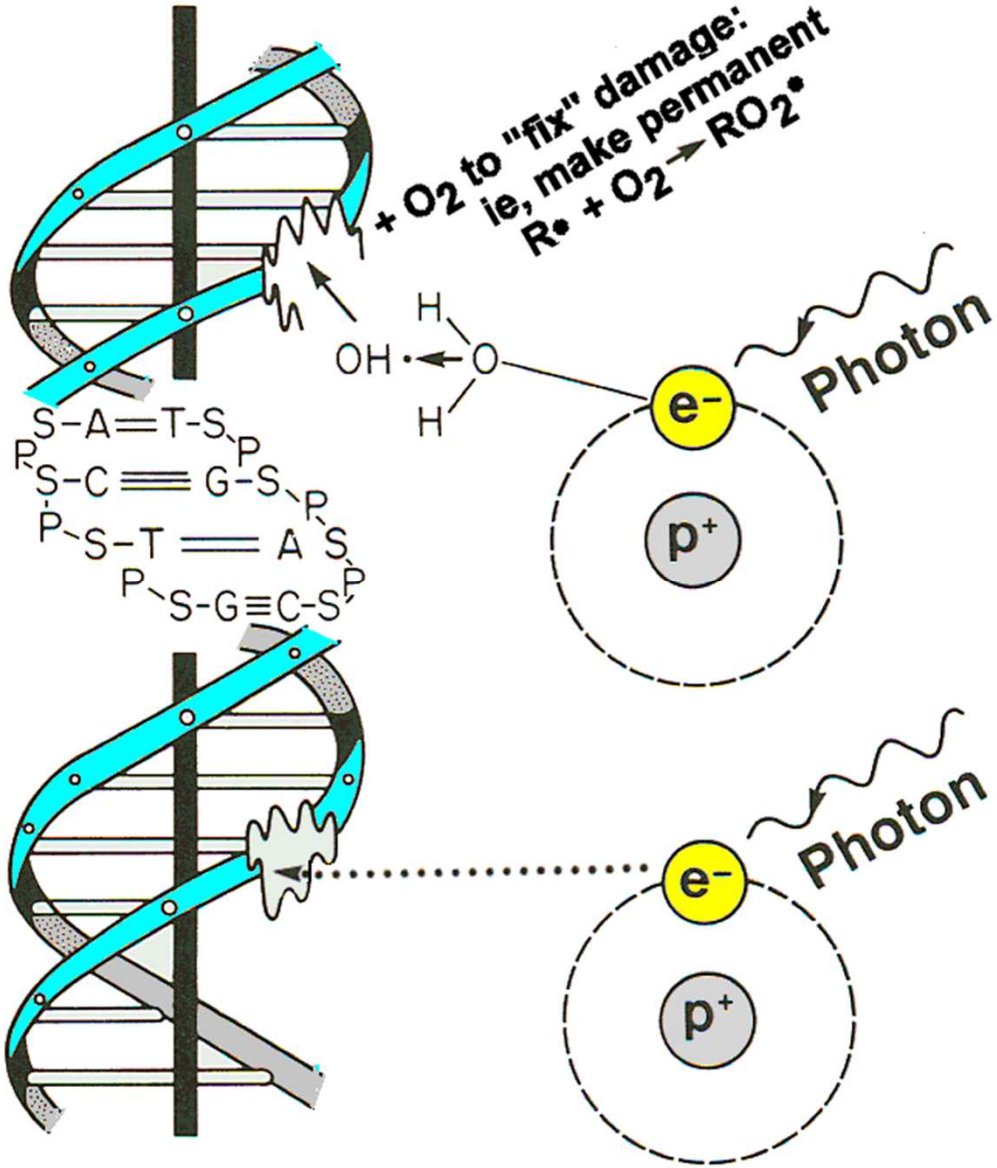
2. Radiation inhibited germination of vegetable seeds
   only in the presence of O_2, Petry 1923

3. Oxygen effect on tumor radiosensitivity
   championed by Mottram 1930s

4. 1st quantitative study on O_2 effects on radiation-induced
   growth inhibition of broad bean, Gray 1953

5. O_2 levels decrease in respiring tumor cells located
   away from blood vessels, Tomlinson and Gray 1955

Mechanism of Action



Oxygen fixes the damage. But HOW?

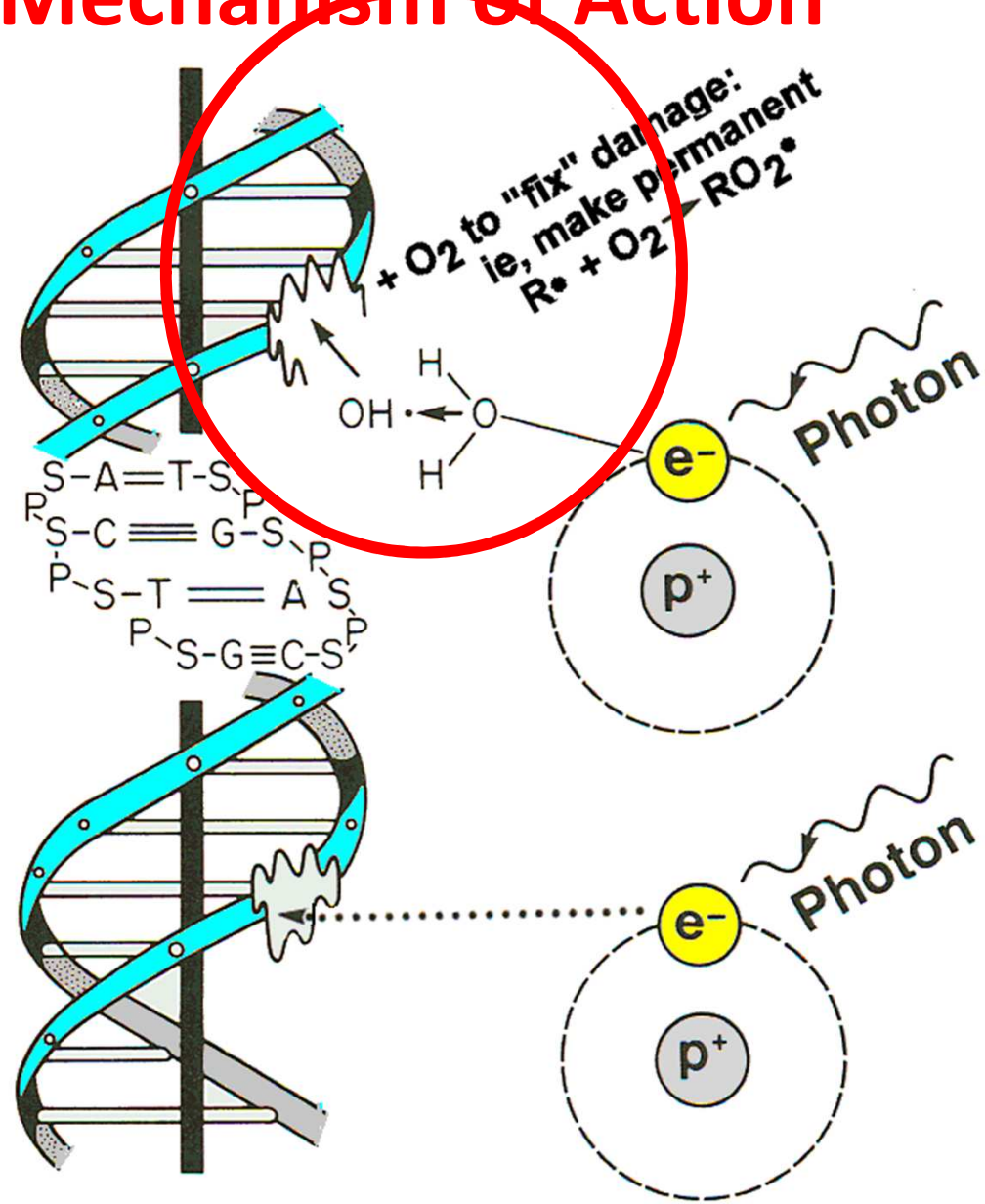
The absorption of radiation leads to the production of fast charged particles .

The charged particles , in passing through the biologic material, produce a number of ion pairs.

$Rad + H_2O = H_2O^+ + e^-$

These ion pairs have a very short life spans(about 10^{-10} sec)

Mechanism of Action

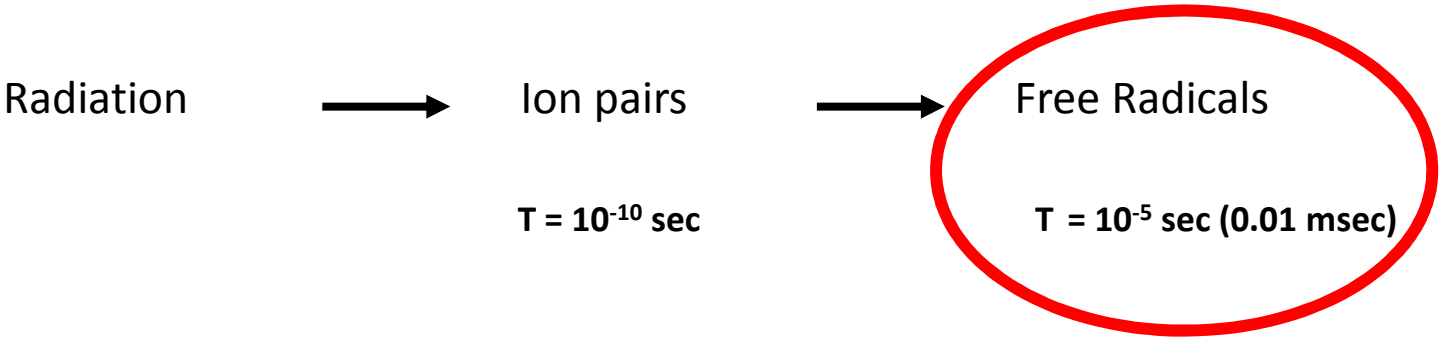


- These ion produce free radicals which are highly reactive molecules because they have an unpaired valence electron.
- H_2O^+ H_2O= H_3O^++ OH(Free RADICAL)
- The free radicals have life span of about 10^{-5} seconds and they break chemical bonds , produce chemical changes and initiate the chain of events that results in final expression of biologic damage.
- Oxygen fixes the damage by free radical to DNA.

4. Oxygen effect is time-dependent

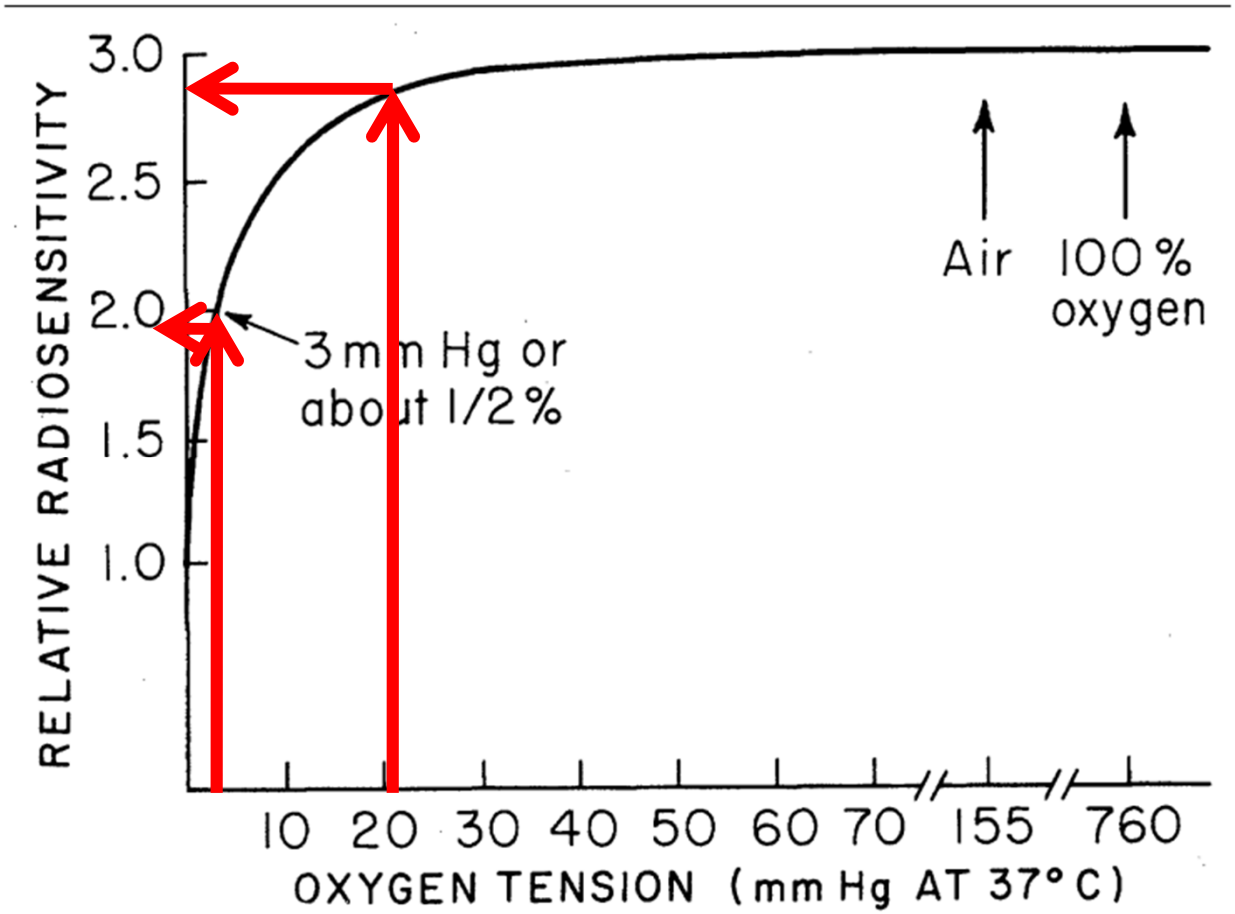
1. O_2 needs to be present DURING radiation

2. O_2 has to be present WITHIN milliseconds after radiation



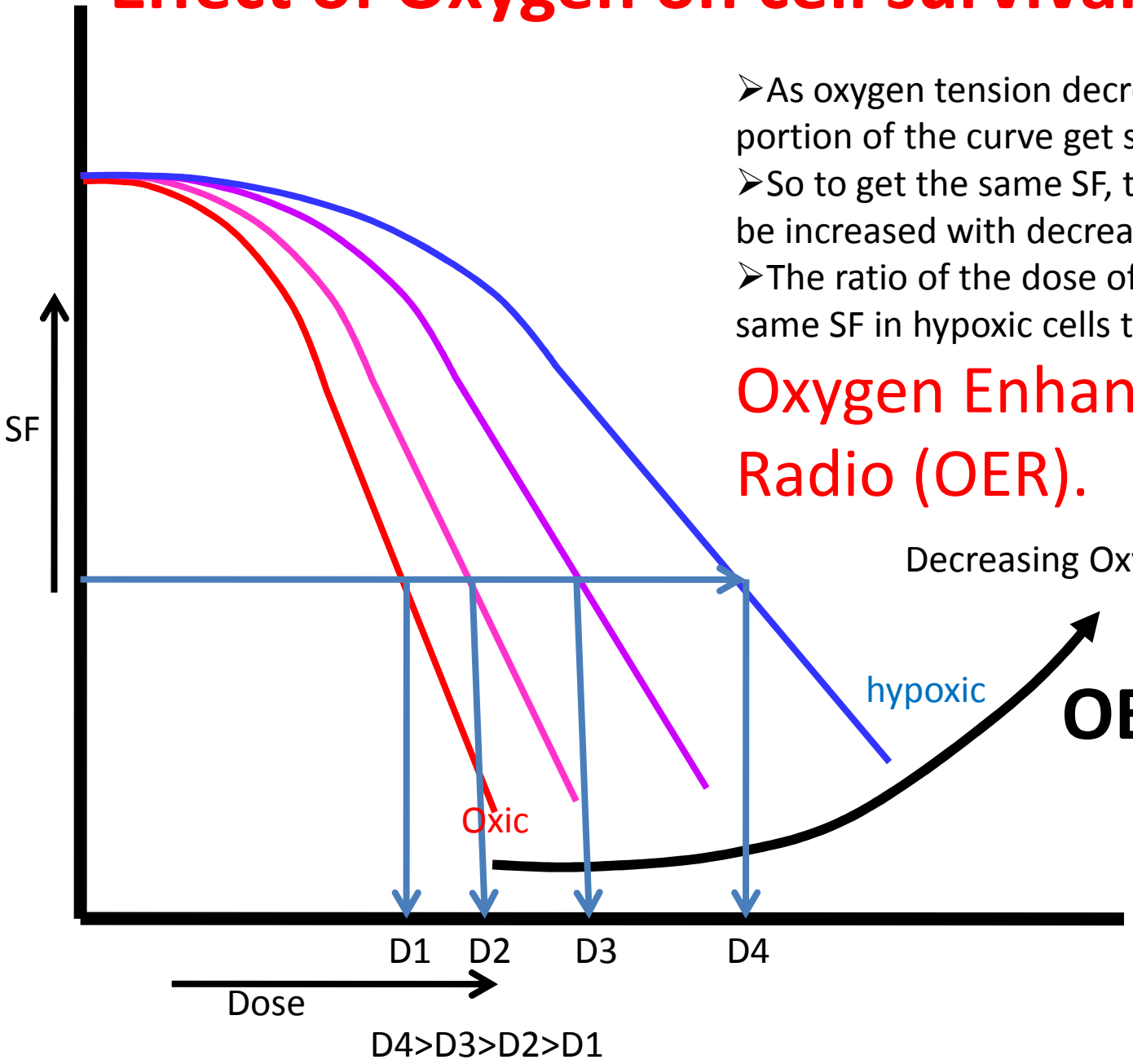
Oxygen is natural radio sensitizer

RADIOSENSITIVITY & O_2 CONCENTRATION



- Radiation sensitivity decreases very little upto 20mm of Hg of O_2 tension but reduced to half at 4 mmof Hg pressure.
- Maximum radioresistance is observed at partial pressure of oxygen at 1/100 mm of Hg.
- Oxygen tension in most of tissue is similar at VENOUS BLOOD O_2 Tension ie. 20 - 40 mm of Hg.
- So most of the normal tissues have good sensitivity to radiation.

Effect of Oxygen on cell survival curve



- As oxygen tension decreases the terminal portion of the curve get shallower.
- So to get the same SF, the dose of RT is to be increased with decreasing ox tension.
- The ratio of the dose of RT to get the same SF in hypoxic cells to oxic cells is k/as

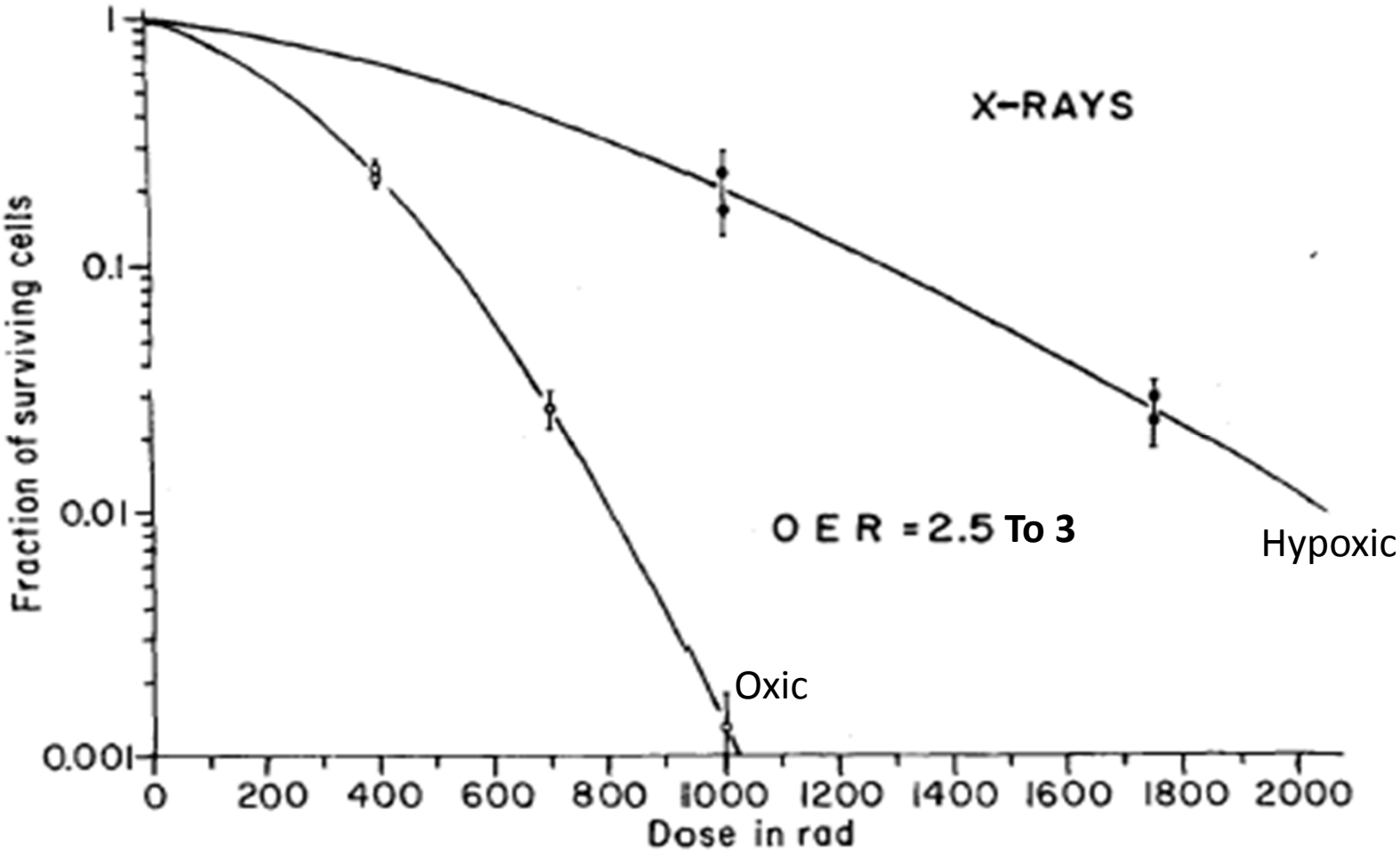
Oxygen Enhancement Radio (OER).

Decreasing Oxygen concentration

OER = D4/D1

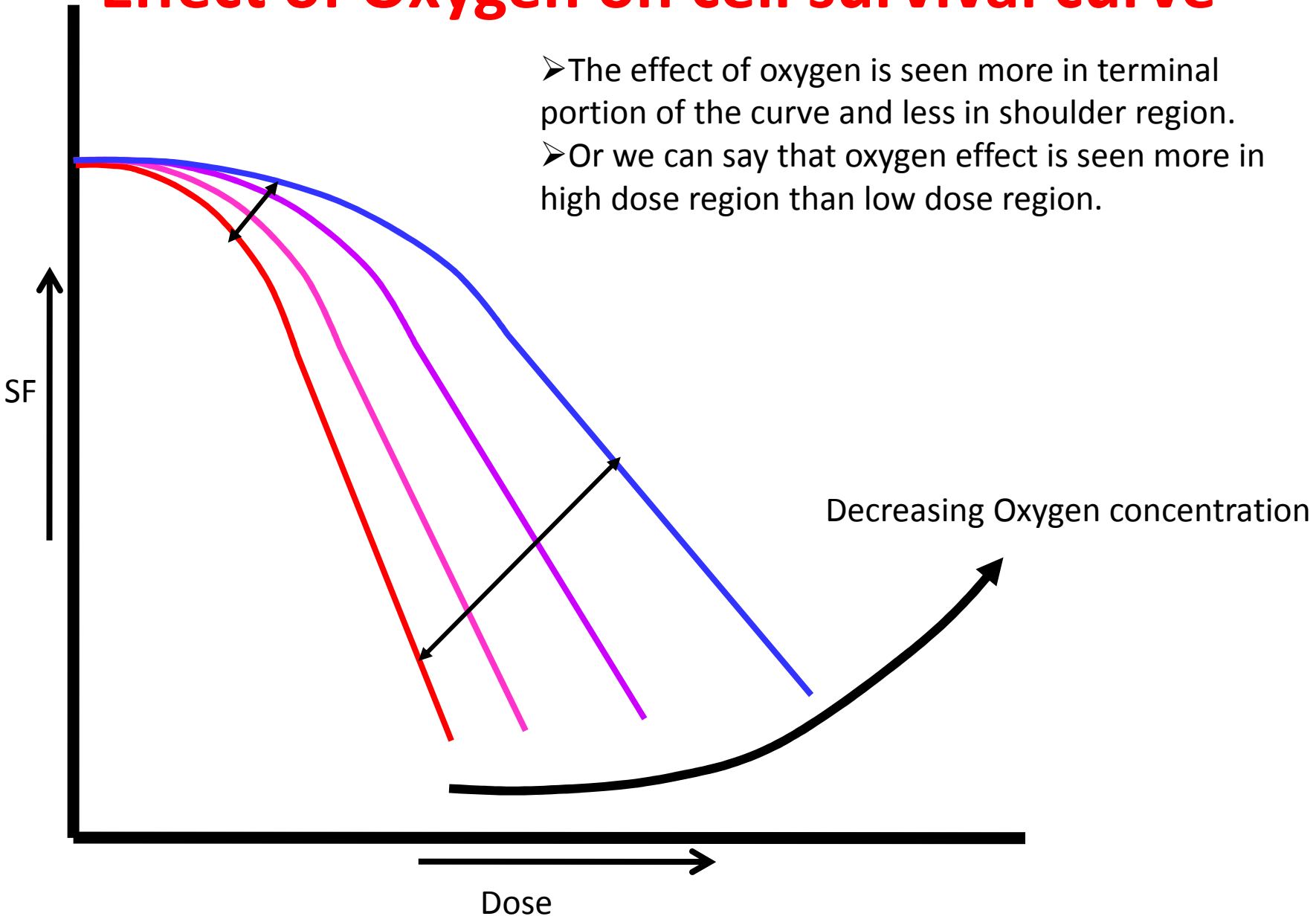
The ratio of HYPOXIC to AEROBIC IR doses needed to achieve the SAME biological effects is called Oxygen Enhancement Ratio.

$$OER = \frac{D_0 \text{ (hypoxic)}}{D_0 \text{ (aerobic)}}$$ = 2.5 to 3 for x-rays and γ-rays

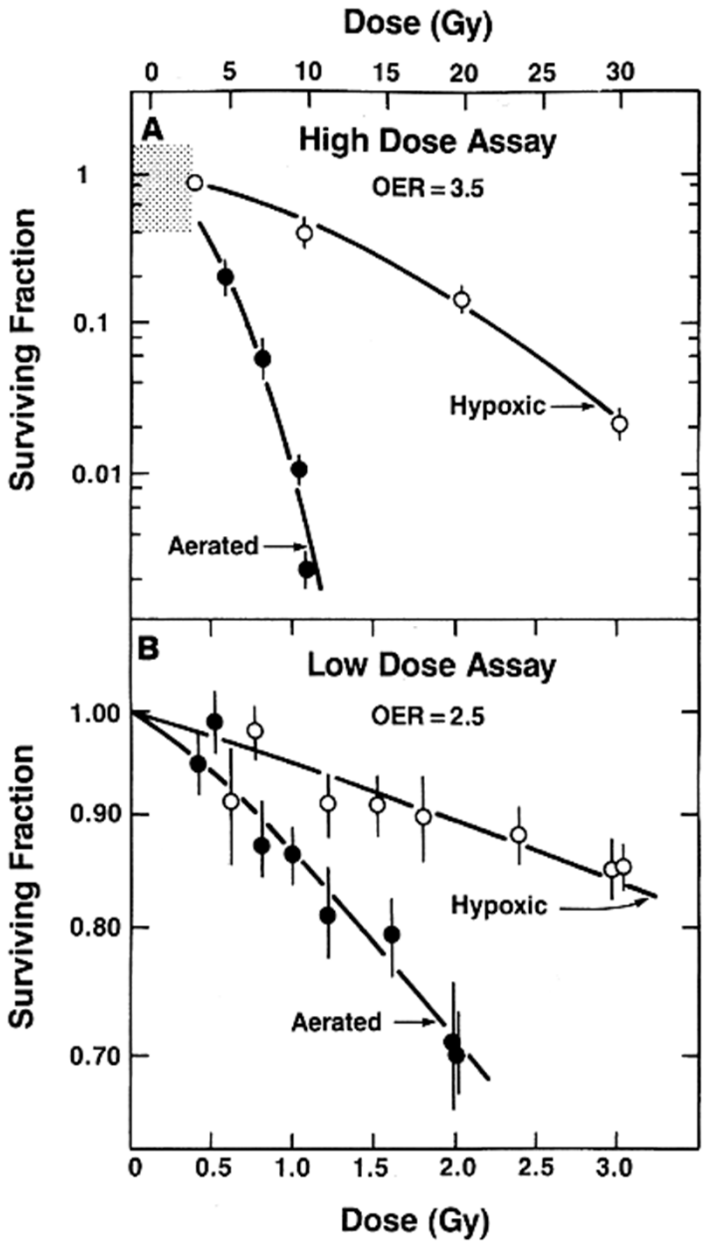


Effect of Oxygen on cell survival curve

- The effect of oxygen is seen more in terminal portion of the curve and less in shoulder region.
- Or we can say that oxygen effect is seen more in high dose region than low dose region.



Why OER Variation with Doses



Ans: **Because in the terminal portion of the cell survival curve the cell kill is predominantly by MHE.**

So in this region the cells are capable of accumulating SLD which can be repaired . So Oxygen will fix up the damage and repair get slowed down.

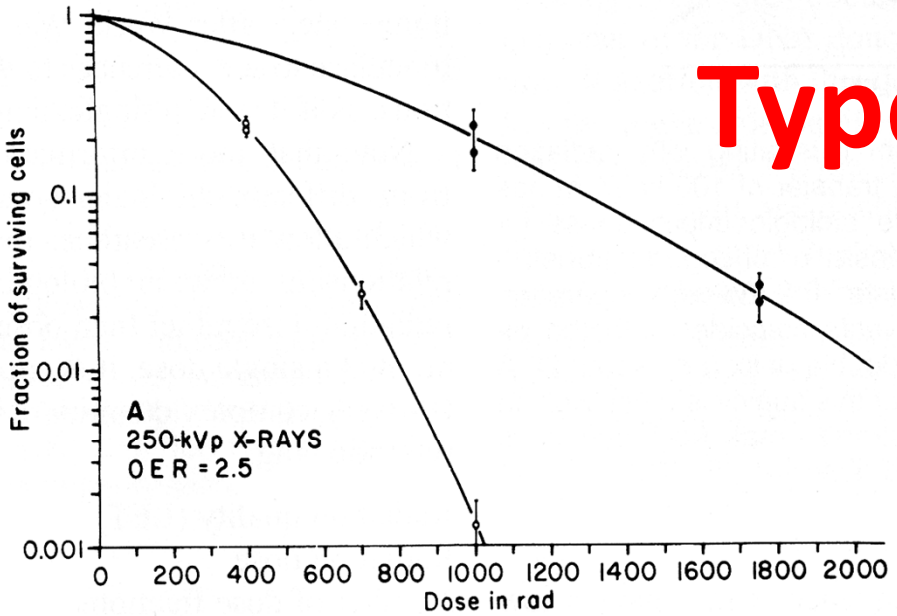
But in low dose region it is found that OER is less?

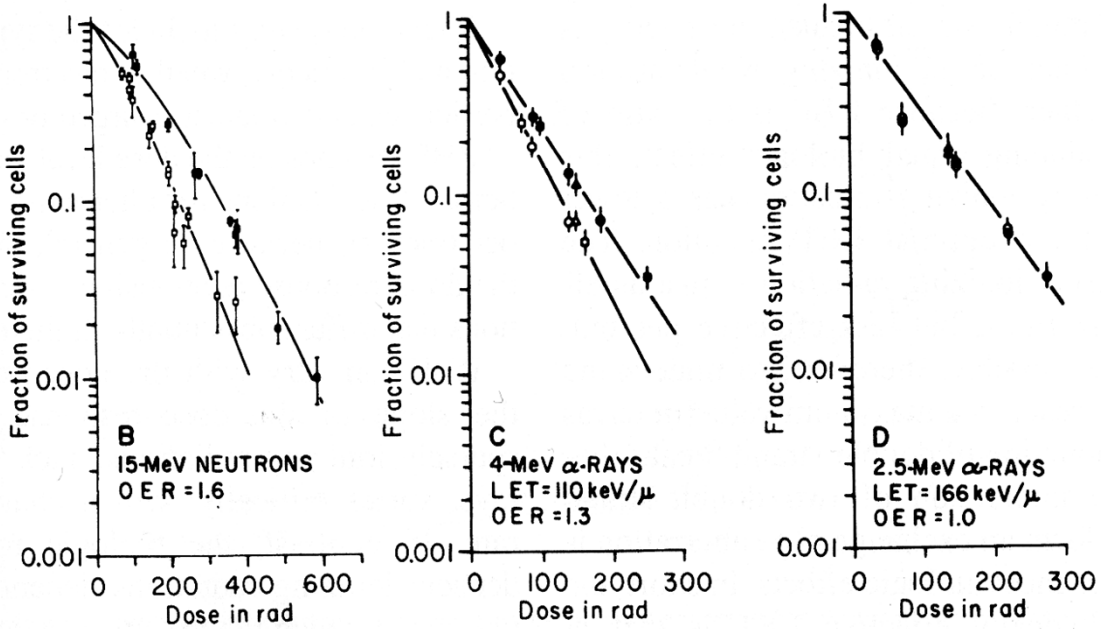
Ans: **Because , in low dose region the cell killing is predominantly by SHE.** So in low dose region OER= 2(X or γ ray).

Parameters Affecting OER

- Dose per fraction. Already discussed, low dose region less and high dose more.
- Type of Radiation (x-ray, neutron, alph particle,)
- pO_2
- Cell Cycle (cells in S-phase are more resistant.)

Type of Radiation

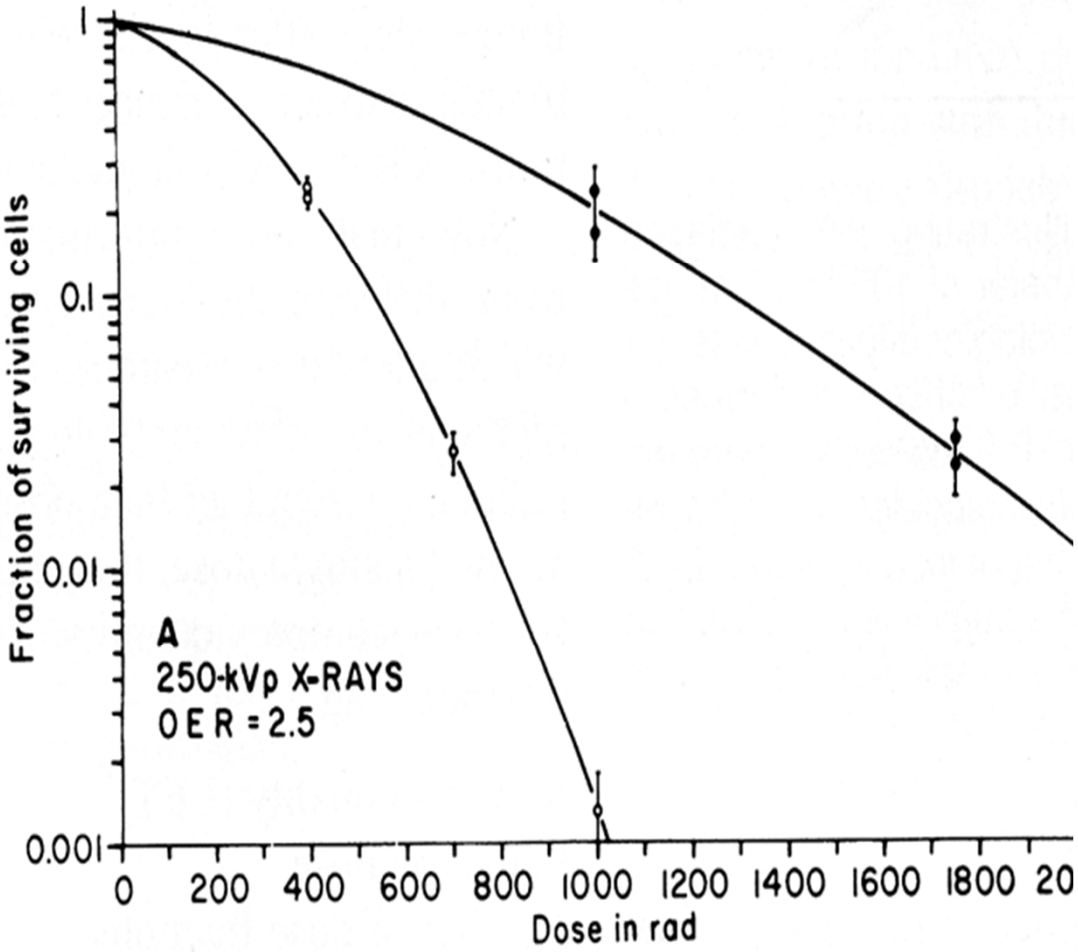


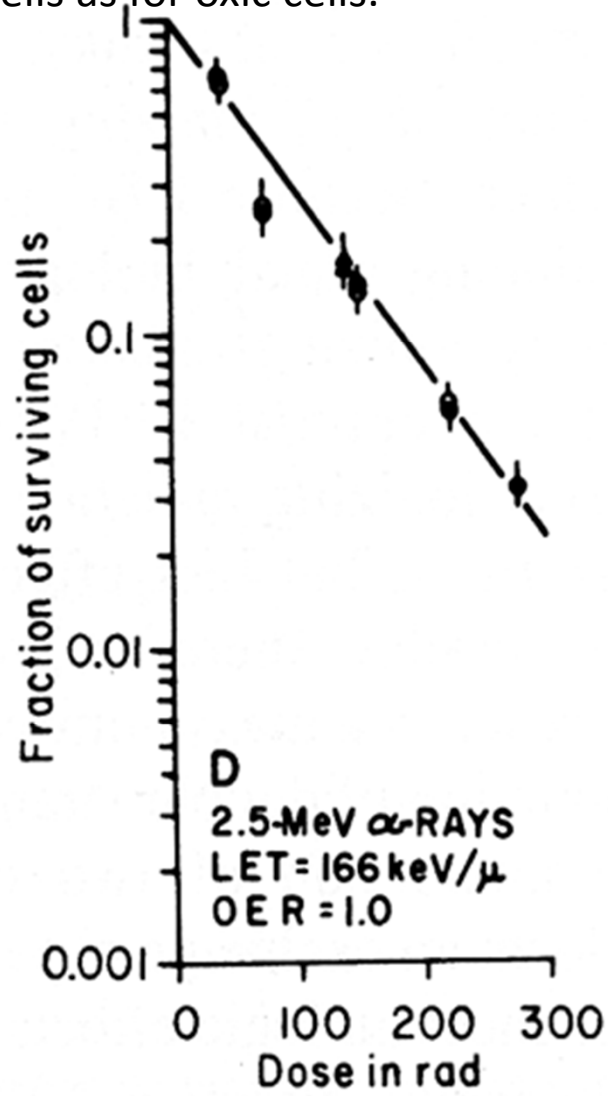


➢For a densely ionizing radiation , such as low energy α particles, the survival curve does not have initial shoulder, which means that all hits result into cell kill and no sub lethal damage to be fixed by oxygen.

➢So OER is one

➢We can say α particle radiation is as effective for hypoxic cells as for oxic cells.

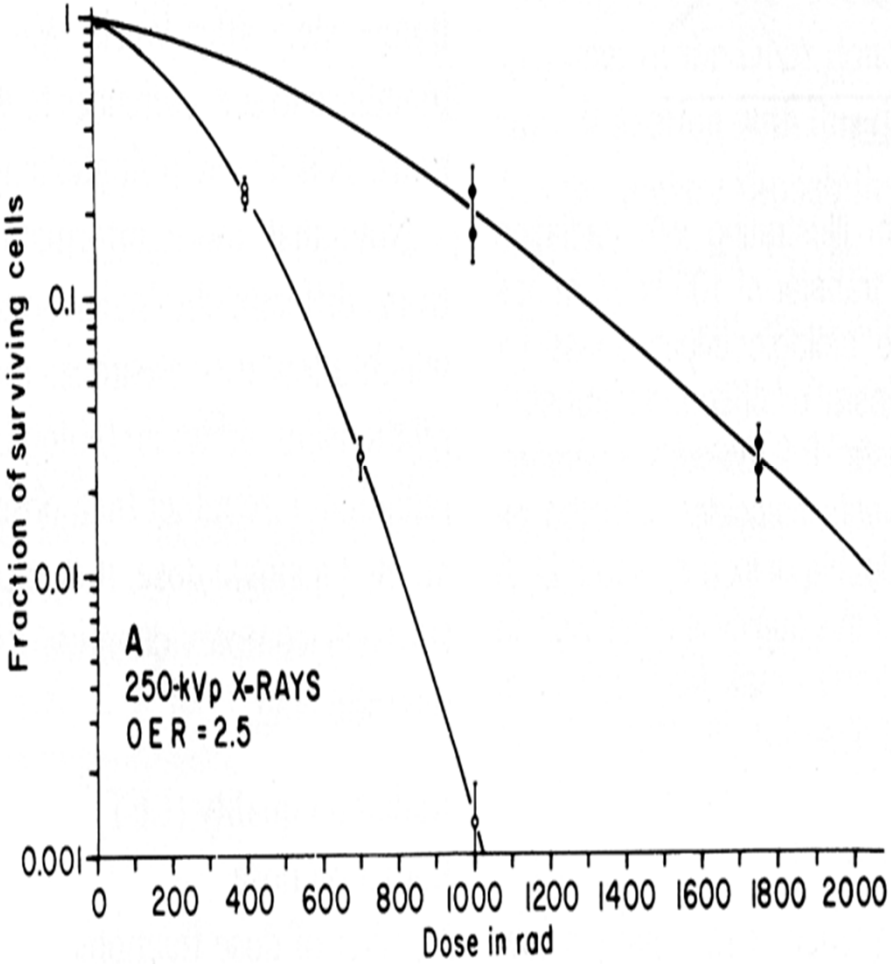


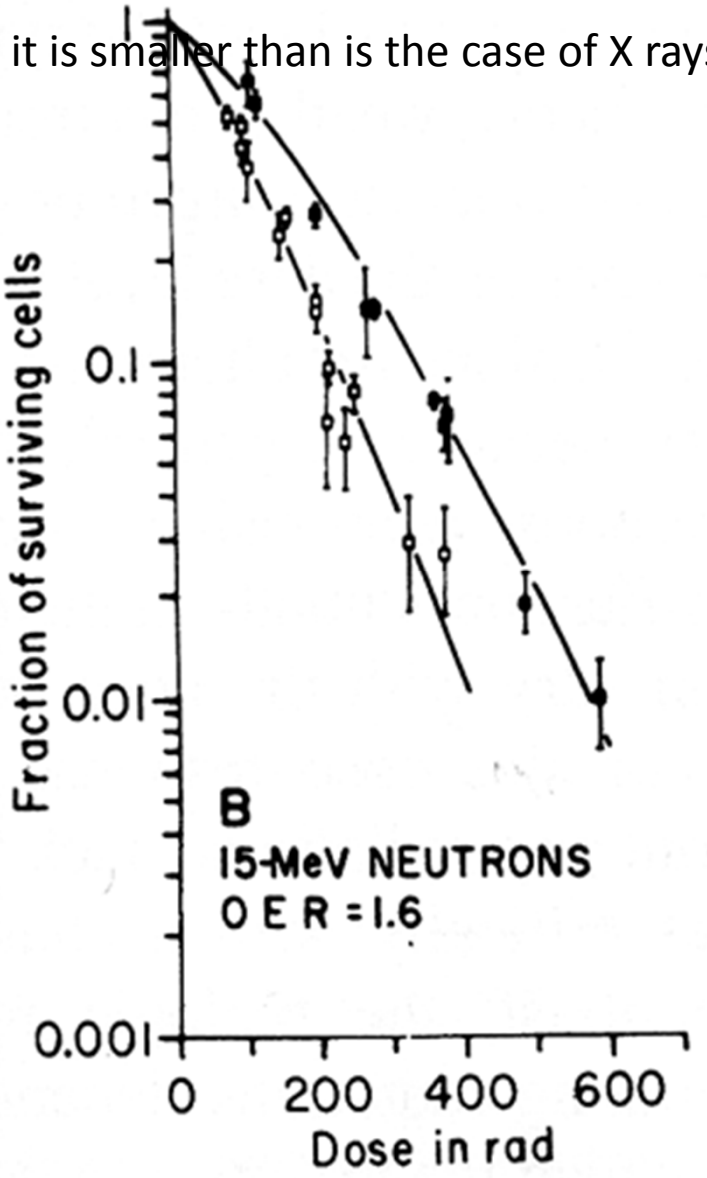


➢For radiations of intermediate ionizing density, such as Neutrons, the survival curves have a much reduced shoulder so very few SLD to be fixed while in case of x-rays, shoulder is large which means more SLD to be fixexd.

➢ In this case , the oxygen effect is apparent, but it is smaller than is the case of X rays.

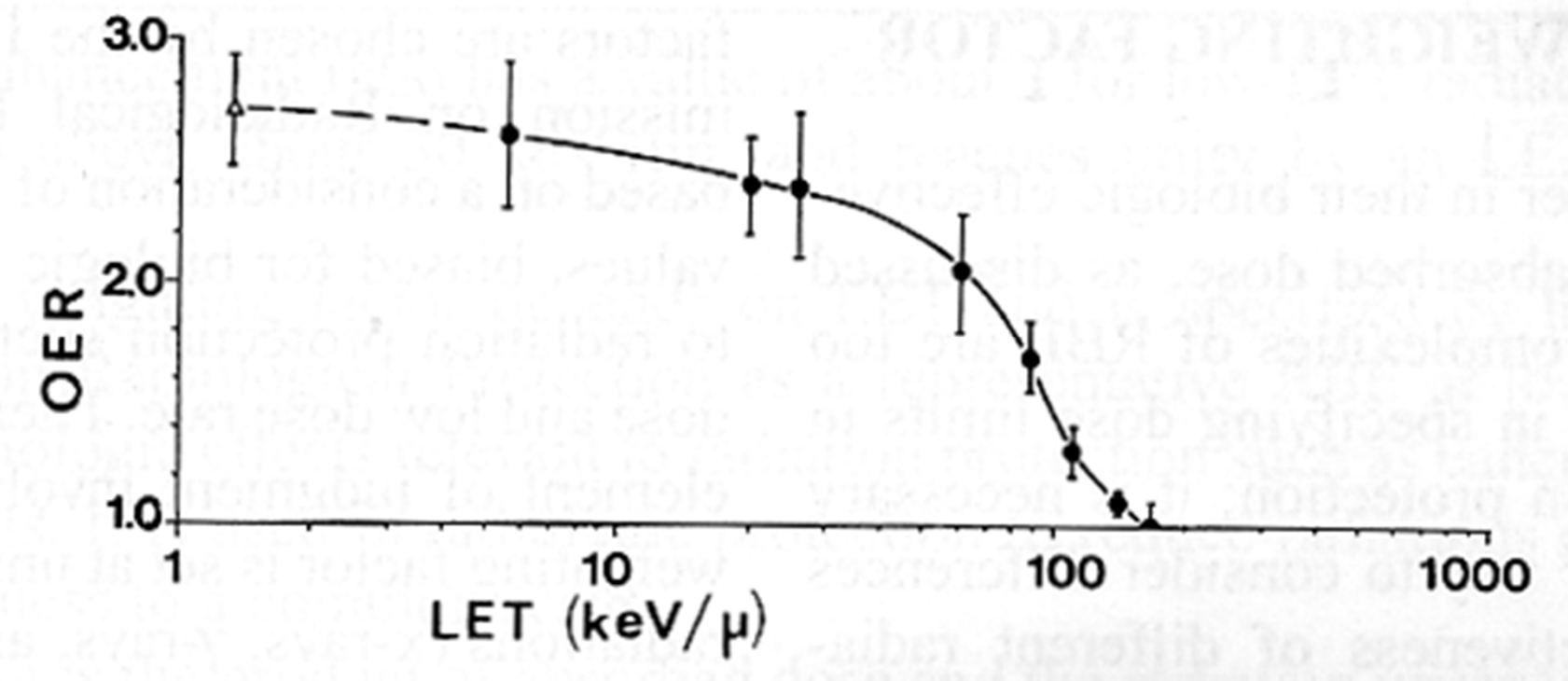
➢*The OER for neutrons is about 1.6*





LET and OER

As LET increases OER decreases

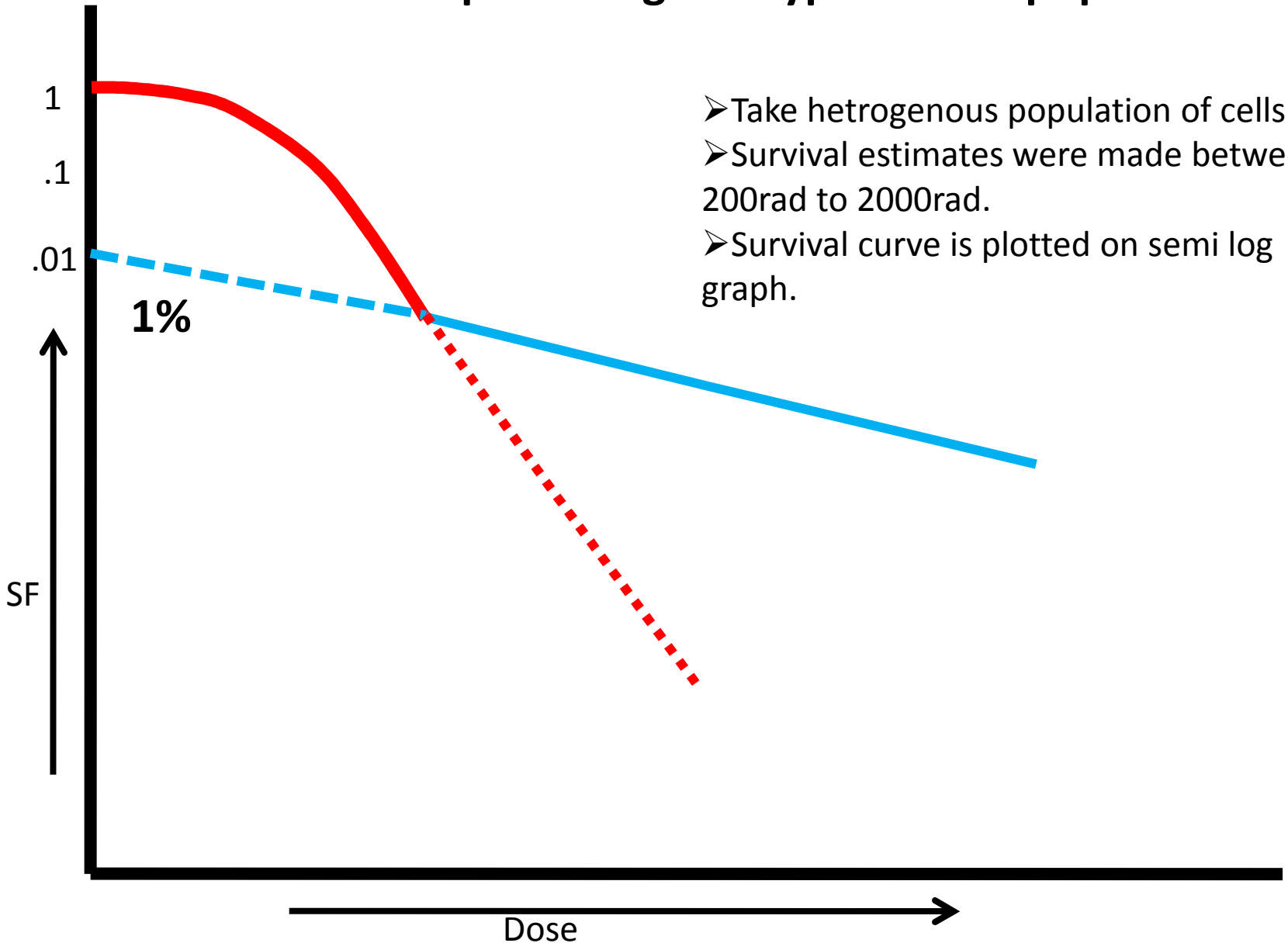


Cell Cycle and OER

OER varies slightly during cell cycle

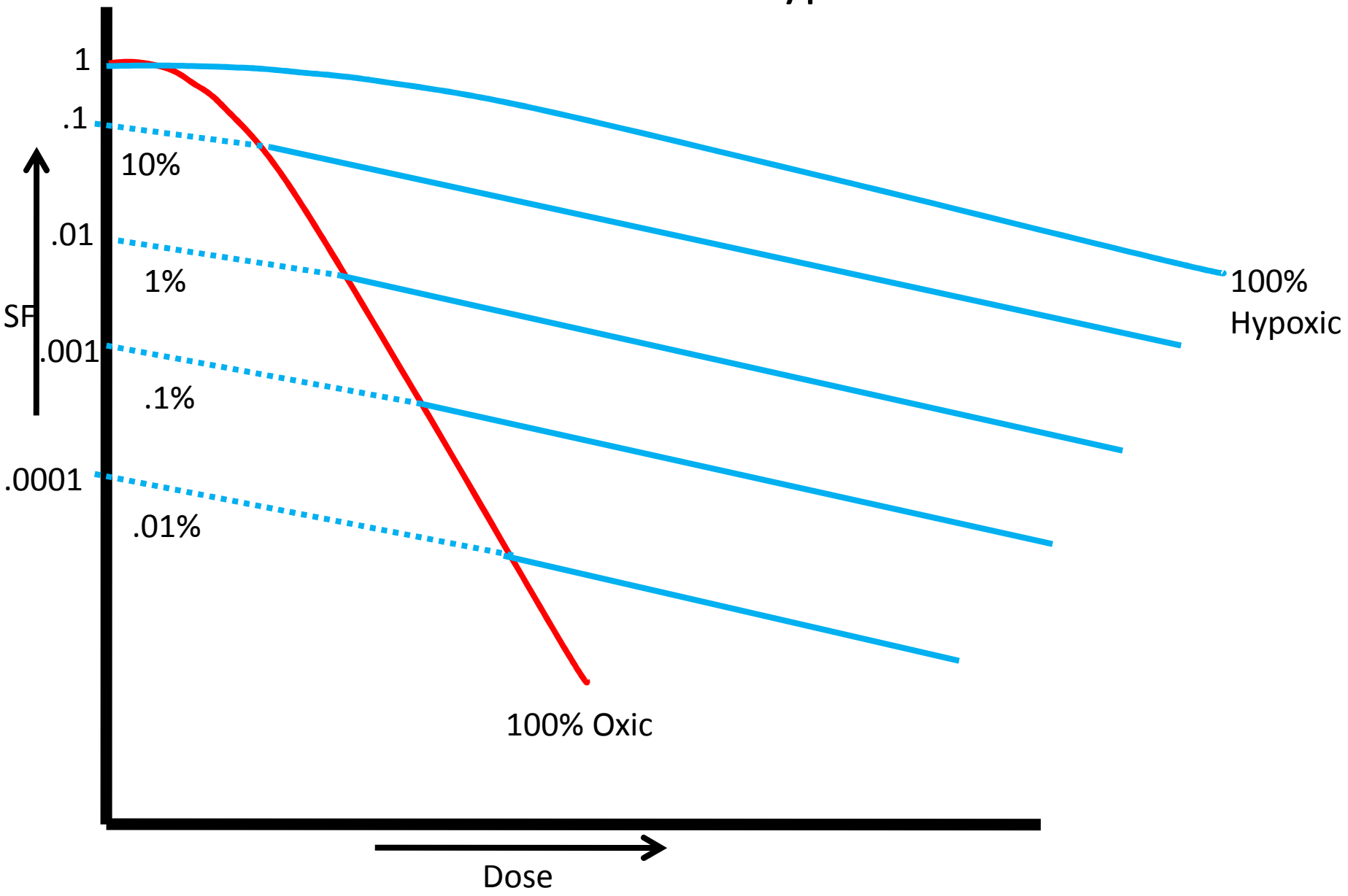
S phase (OER = 2.8-2.9) **>** G1 phase **>** G2/M phase (OER = 2.3-2.4)

Measurement of percentage of hypoxic cells population



- Take hetrogenous population of cells.
- Survival estimates were made between 200rad to 2000rad.
- Survival curve is plotted on semi log graph.

Cell Survival Curve for various hypoxic fraction in a tumor



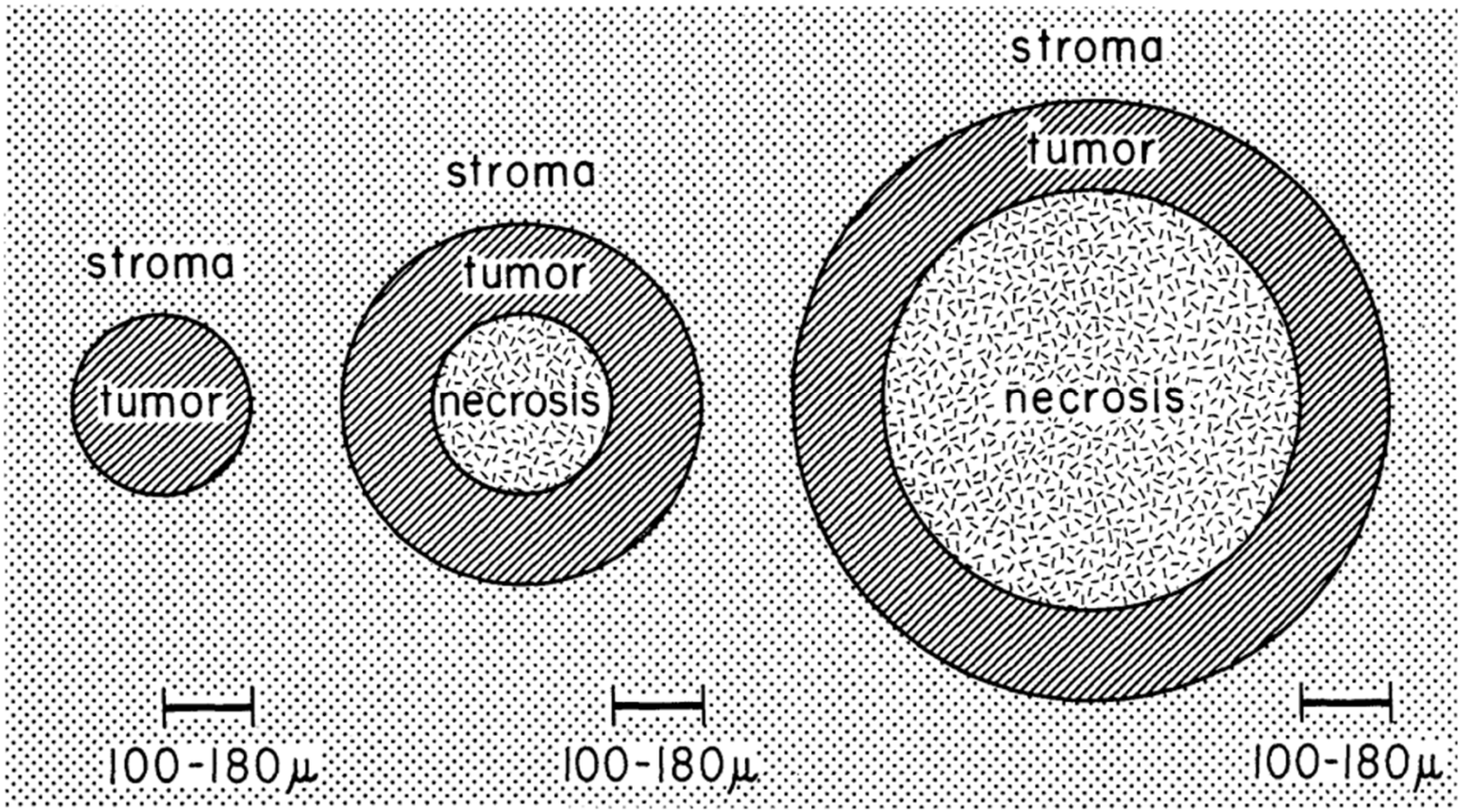
As % of hypoxic cell increases the curve becomes shallower

Clinical Implications

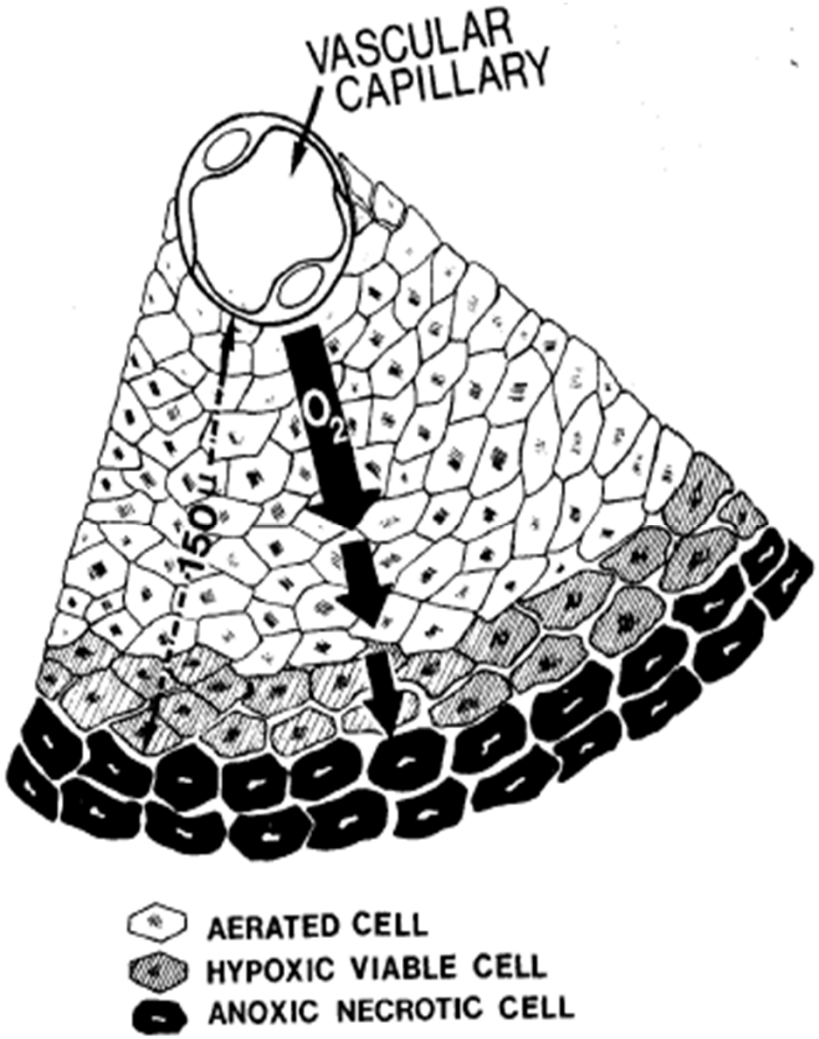
- Various experimental solid tumors in animals have shown to have hypoxic contents between 10 to 40%, which limit the radio curability.
- However, it should be remembered that even a minute proportion of hypoxic cells will limit the radio curability if treated with large single fraction.
- There are abundant evidences to support the existence of hypoxic cells in human tumor, but it is uncertain how frequently they limit the radio curability when conventional dose fraction is used as re-oxygenation comes into picture.

THOMLINSON & GRAY STUDY

- Study of histological section of bronchial carcinoma.
- No tumor cord with an average radius of < 160 micron showed necrosis.
- No tumor cord with a radius >200 micron was without necrotic centre.
- As tumor size increases the thickness of viable tumor sheath remains same.(about 100-180 micron)



THOMLINSON & GRAY STUDY



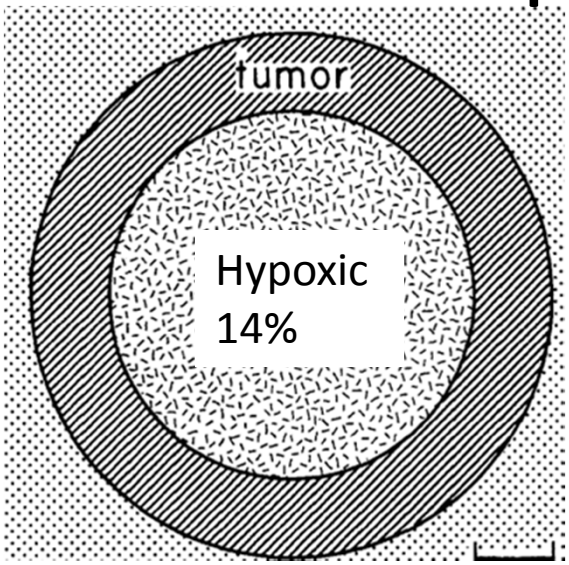
- They also calculated the distance to which oxygen can be diffused from the capillary and found that distance at which oxygen tension becomes zero is 150 to 200 micro meter from a capillary.
- So any sheath of tumor contain three zones.
  - Aerated cells
  - Hypoxic Cells
  - Anoxic Necrotic cells

In Hypoxic cells, the oxygen concentration is high enough to keep them alive but low enough to keep them protected from the effect of radiation and they may limit the radio curability of the tumor.

Experiment

If we have total 100cells

14 are hypoxic

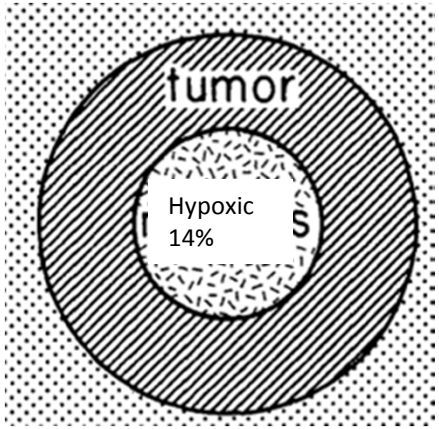


Treated with RT from Monday to Friday.

After 48 hours

Monday

After RT



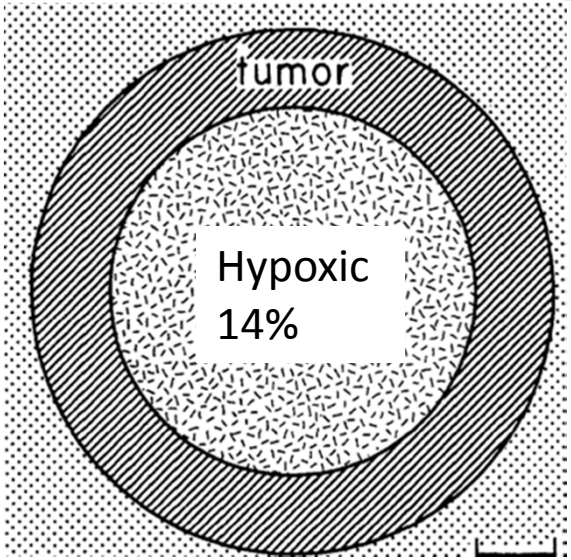
Proportion of hypoxic cell remain the same i.e. 14%

If total cells are 50
Hypoxic 07

Experiment

If we have total 100cells

14 are hypoxic



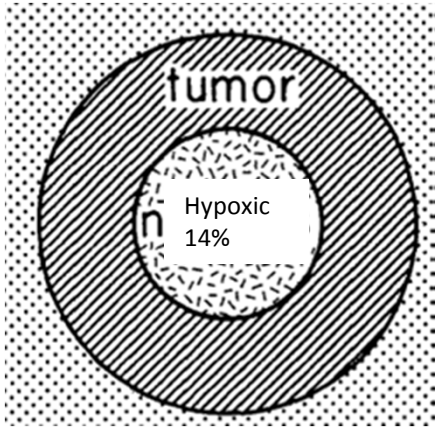
Treated with RT from Monday to Thursday.

After 24 hours

Friday

After RT

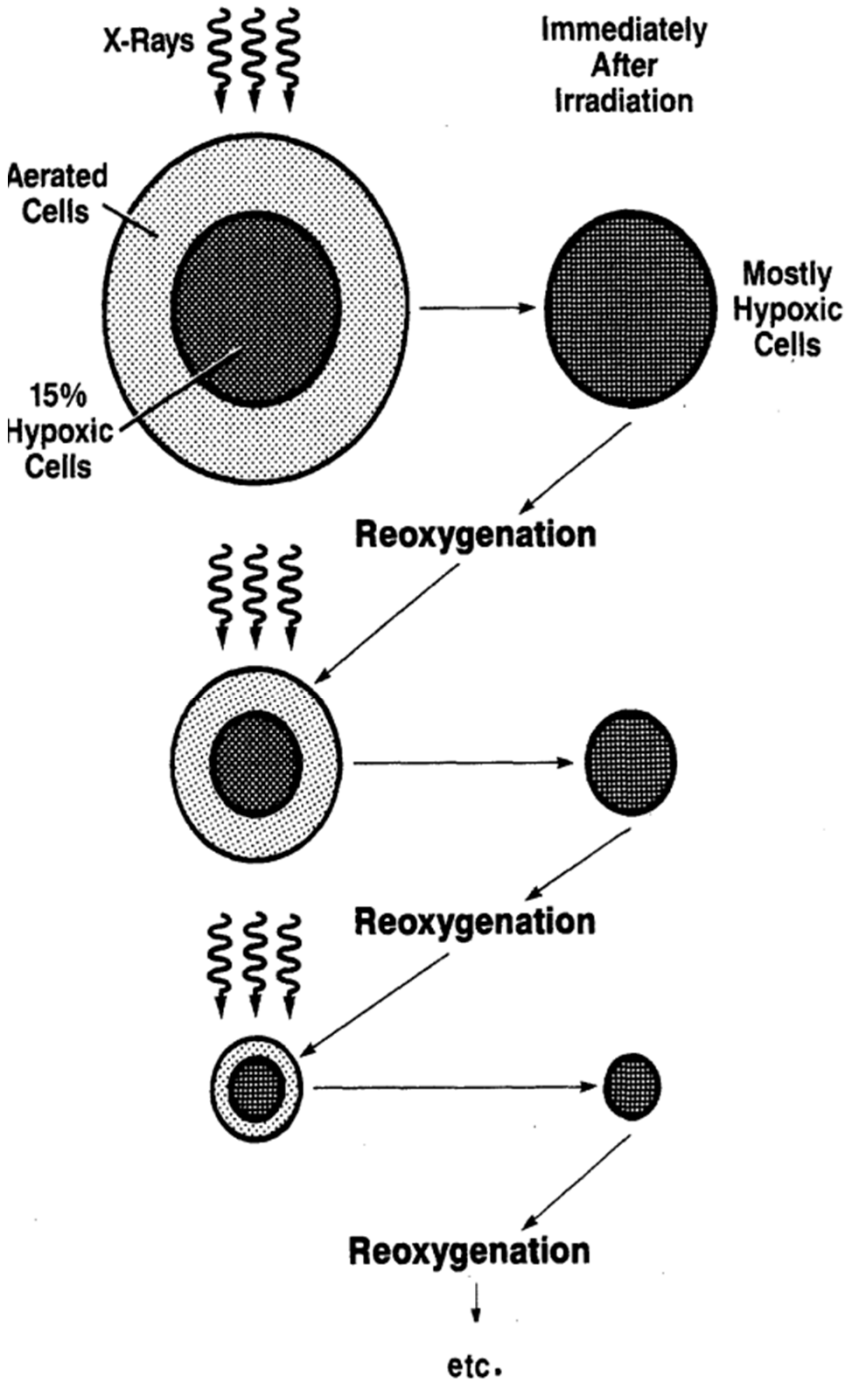
If total cells are 50
Hypoxic 07



Proportion of hypoxic cell remain the same i.e. 14%

Inference from Experiment

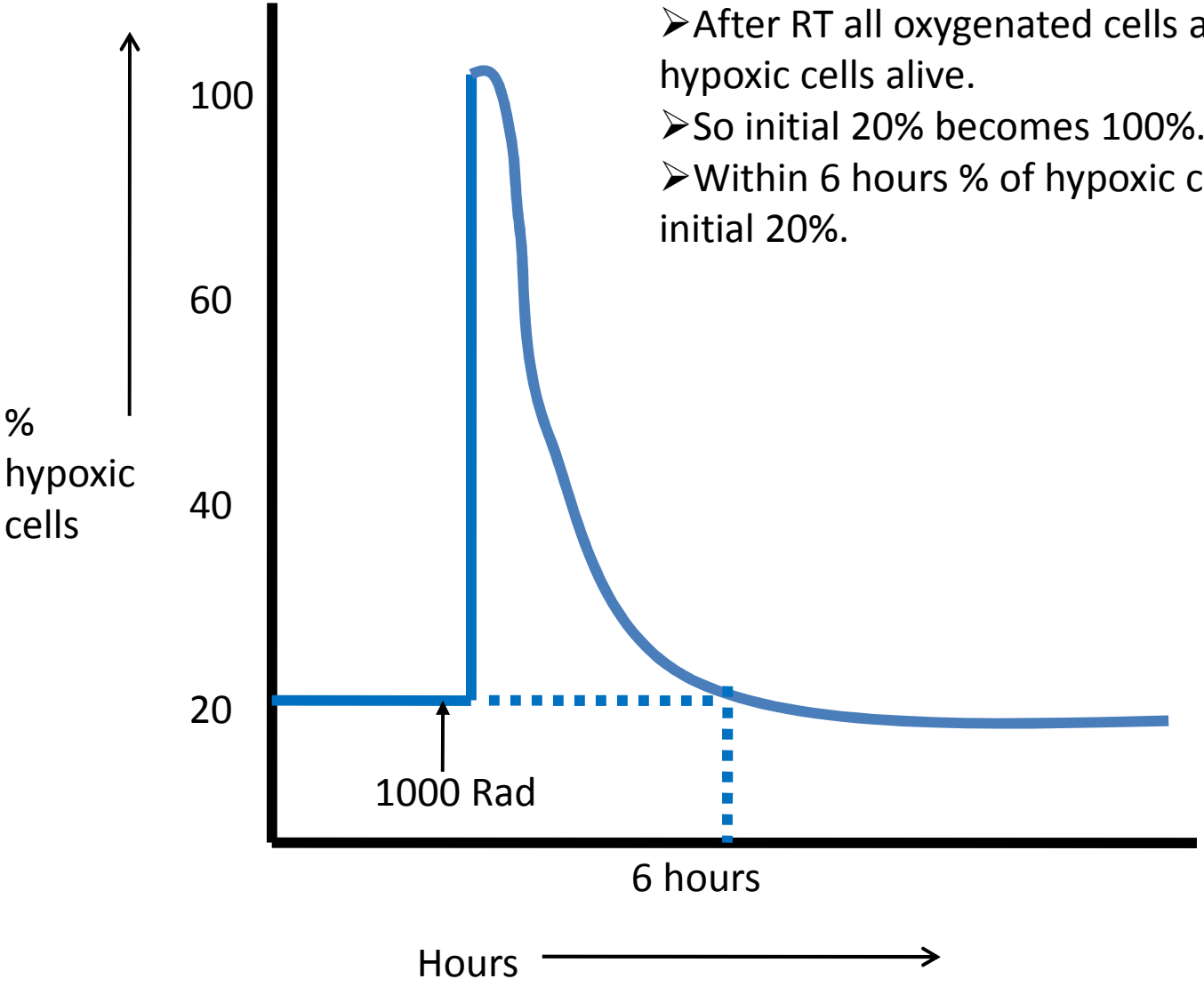
- Proportion of hypoxic cells returned to its pre treatment level in 24 hours after fractionated radiotherapy.
- This implies that cells moves from hypoxic compartment to oxic compartment during fractionated RT.
- If this were not the case then hypoxic cells proportion should have increased after each fraction of radiation.
- This process is known as Reoxigenation.



Reoxigenation

In between two exposure of radiation some of the hypoxic cells move into oxic compartment and become sensitive to radiation. This process goes on during fractionated radiotherapy.

Time Sequence of Reoxygenation



- After RT all oxygenated cells are killed and only hypoxic cells alive.
- So initial 20% becomes 100%.
- Within 6 hours % of hypoxic cells comes down to initial 20%.

Time Sequence of Reoxygenation

- Time sequence vary from one type of tumor to other.
- Some are rapidly oxygenated after RT while other takes few days.
- The only experimental tumor that does not show significant reoxygenation is osteosarcoma. That could be one of the reason for its radio resistance.

Mechanism of Reoxygenation

1. Reduction in ratio of total tumor cells to the surface area of blood vessels.

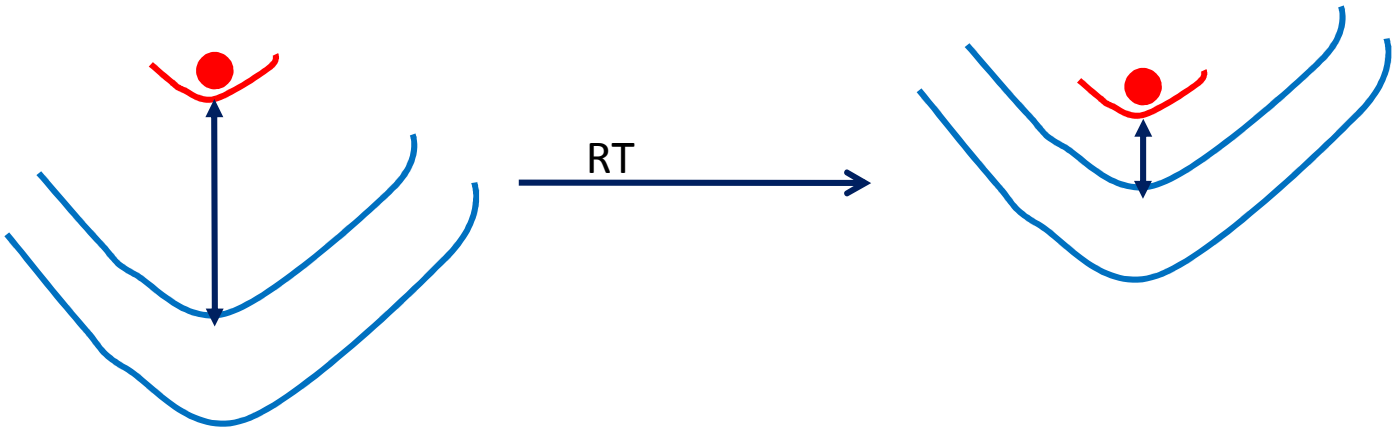
   for example if there are 10 capillaries supplying to 100 tumor cells the ratio of tumor cells to capillary is 10 which mean one capillary supplying 10 cells.

   After RT, 80 cells survived then ratio becomes 8 so now one capillary supplying to 8 cells

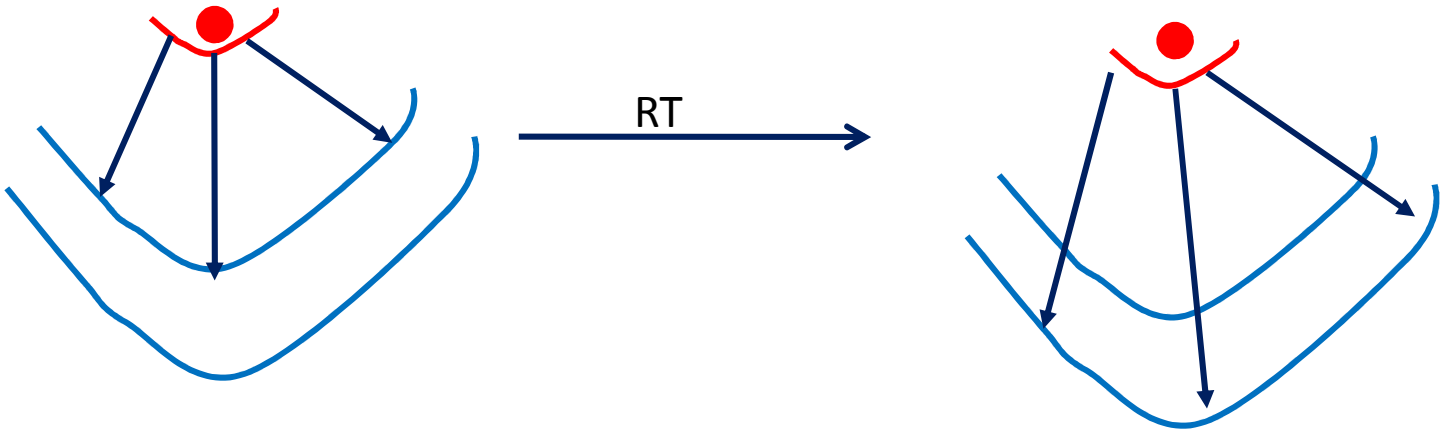
Mechanism of Reoxygenation

2. Distance of hypoxic cells from the blood vessels decreases because of preferential killing and lyses of oxygenated cells.



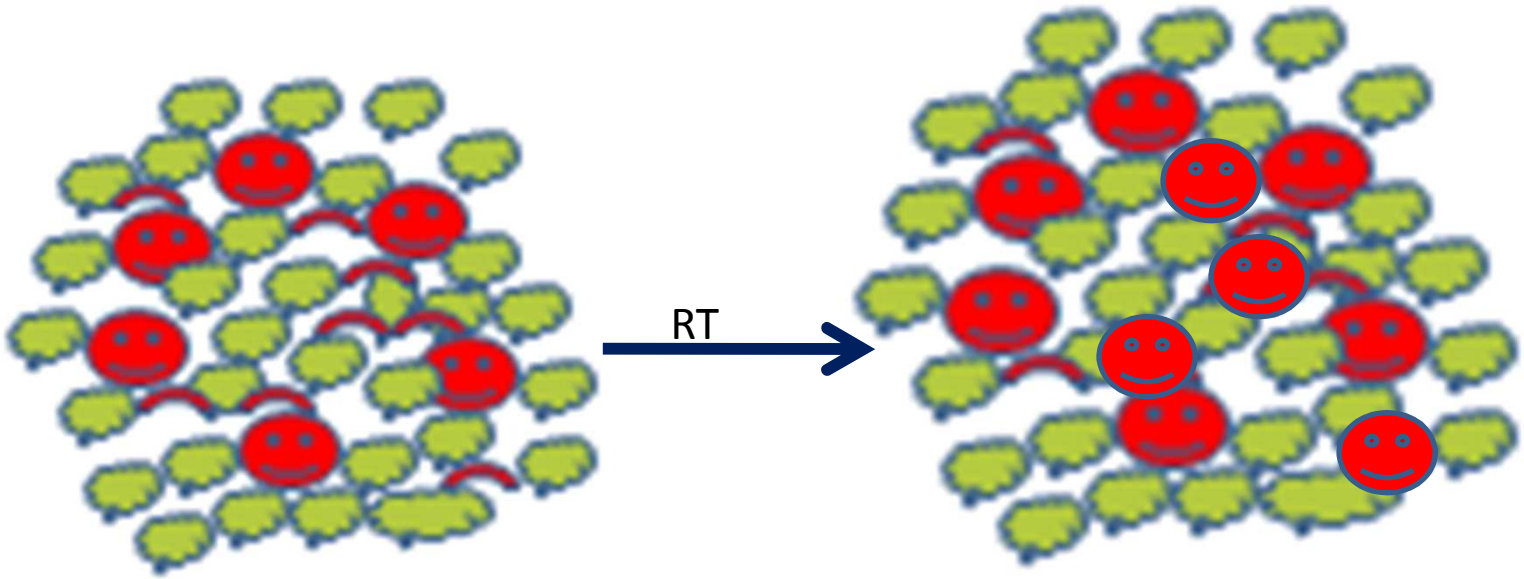
Mechanism of Reoxygenation

3. Increased radius of oxygen diffusion as total consumption of oxygen is decreased.



Mechanism of Reoxygenation

4. As oxygenated cells are depopulated, there is reduction in intra tumoral pressure permitting re opening of the some compressed blood vessels.



Clinical Significance of Reoxygenation

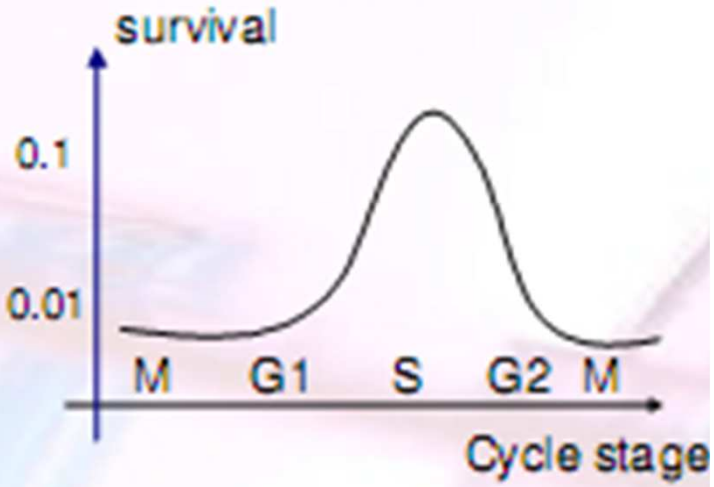
- Human tumor re-oxygenate between fractions and it forms one of the basis of fractionated radiotherapy.
- Timing of re-oxygenation vary from one tumor to other.
- Exact timing of re-oxygenation in human tumor is not known.
- If we know the exact timing of re-oxygenation of a particular tumor then we can schedule the radiation fractionation accordingly.

- **R**epair
- **R**e-oxygenation
- **R**e-distribution
- **R**e-population

Effect of cell cycle on cell survival curve

Age response

The changing radio sensitivity during the cell cycle is often called 'age response'.



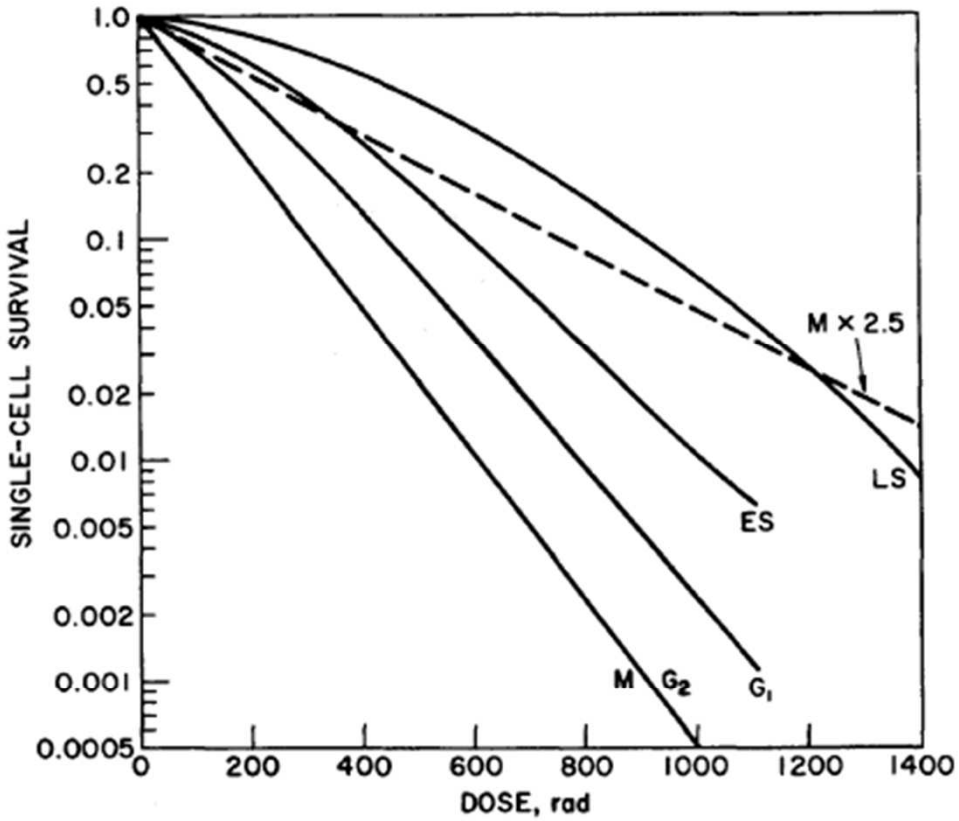
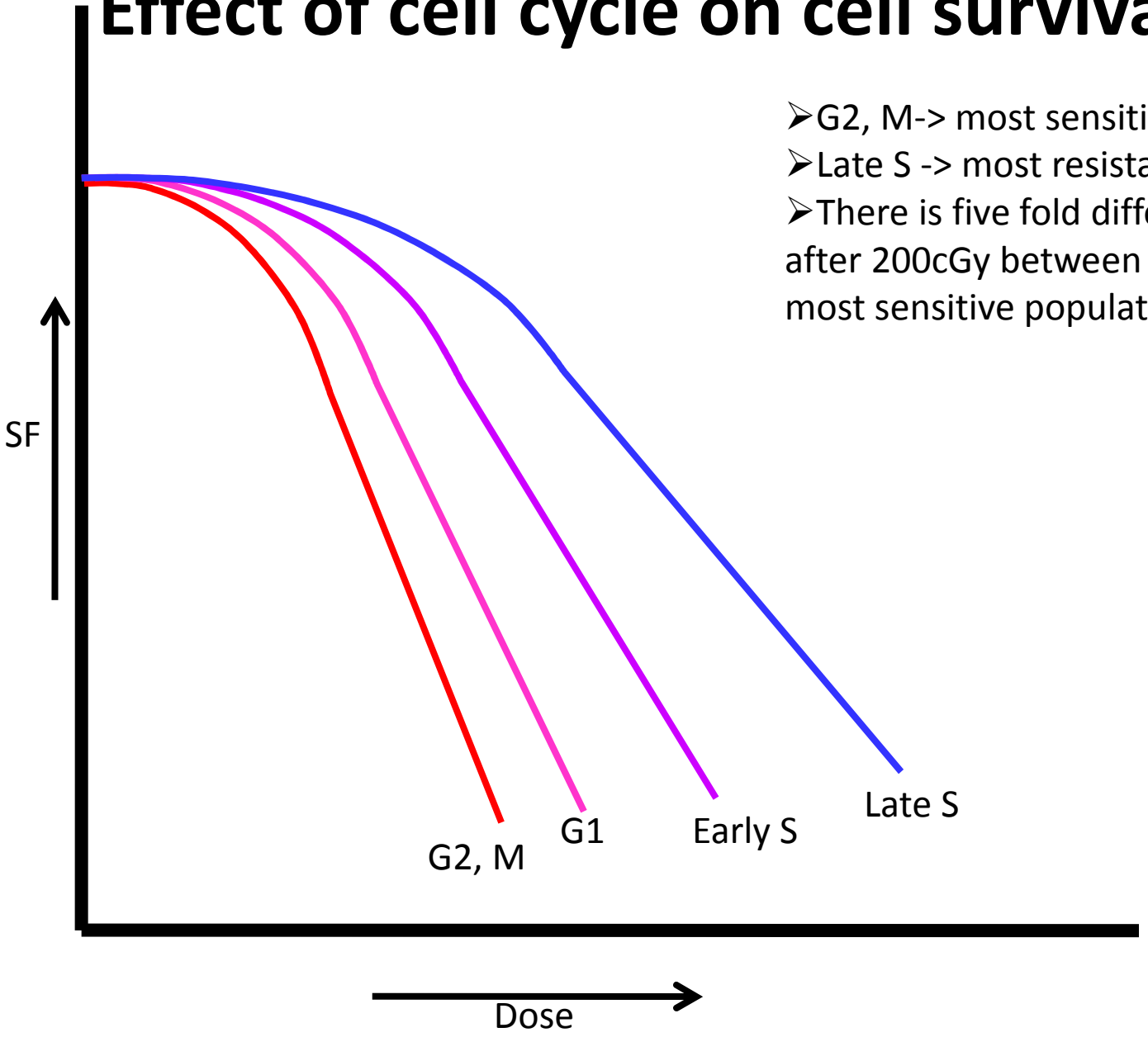


Figure 5–7. Cell-survival curves for Chinese hamster cells at various stages of the cell cycle. The survival curve for cells in mitosis is steep and has no shoulder. The curve for cells late in *S* is shallower and has a large initial shoulder. G_1 and early *S* are intermediate in sensitivity. The broken line is a calculated curve expected to apply to mitotic cells under hypoxia. (From Sinclair WK: Radiat Res 33:620–643, 1968)

Effect of cell cycle on cell survival curve



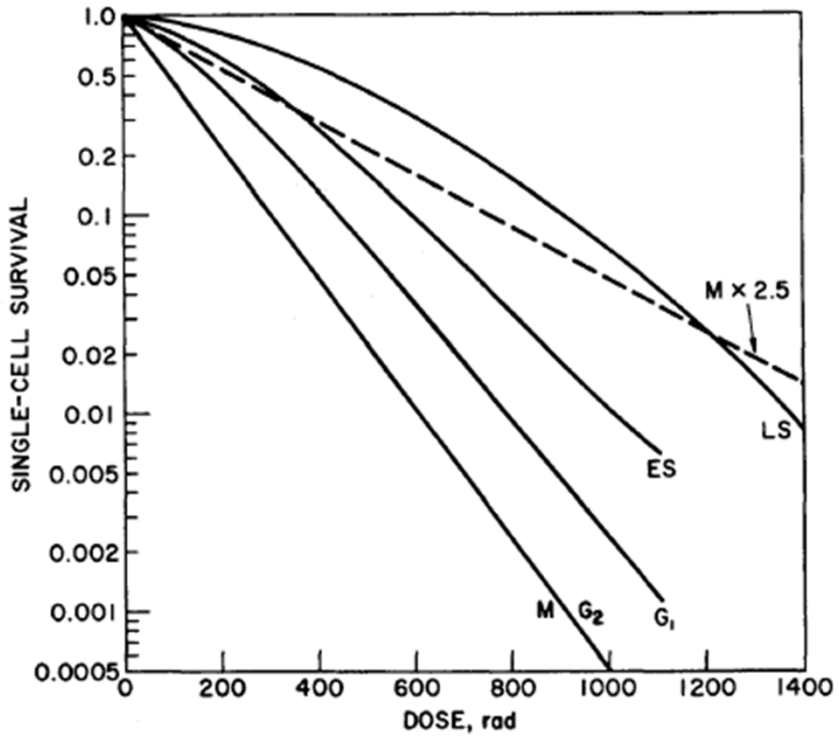
- G2, M-> most sensitive
- Late S -> most resistant
- There is five fold difference in survival after 200cGy between most resistant and most sensitive population of the cells.

Effect of cell cycle on cell survival curve

- Reasons for relative sensitivity during the cell cycle are not completely understood. Changes in the amount or form of DNA might be expected to influence sensitivity.

- The presence of naturally occurring radio-protectors also varies during the mitotic cycle. In particular, certain "sulfhydryl compounds" are effective at scavenging the free radicals responsible for indirect damage to DNA.

Redistribution or Reassortment



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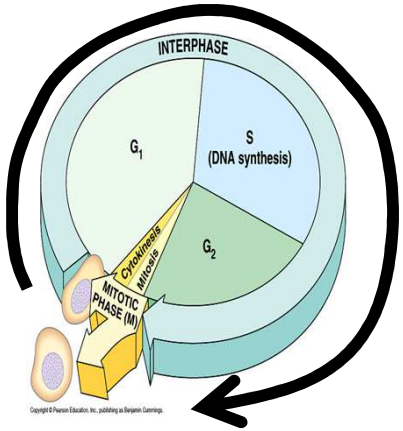
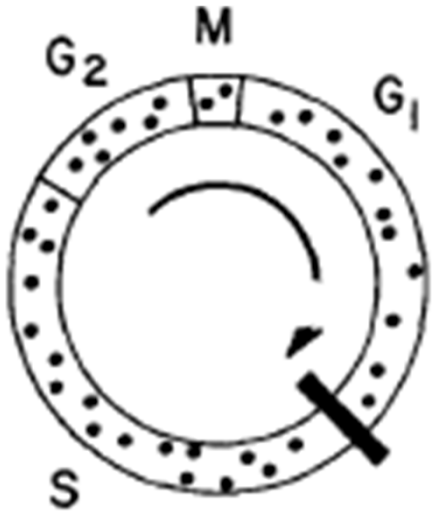
- During fractionation, after each fraction of RT, cells in sensitive phase are killed and before next fraction, cells progress through cell cycle and again come to sensitive phase.
- This process is known as

Redistribution.



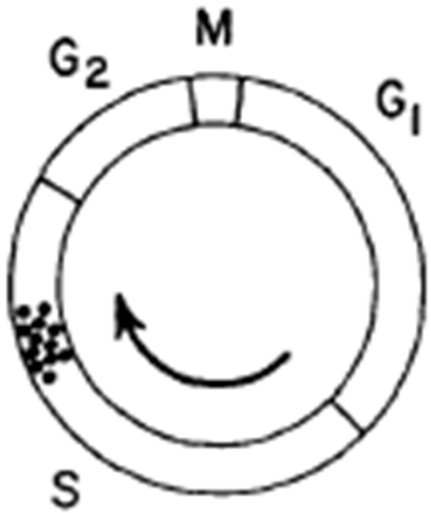
Redistribution or Reassortment

- Asynchronization:-
  - The dividing cells are distributed throughout all phases of cell cycle k/as asynchronization.



Redistribution or Reassortment

- Synchronization:-
  - If all the dividing cells occupy the same phase of the cell cycle and then all cells progress through various phases of cell cycle simultaneously.



This can be achieved by treating the cells with hydroxyurea.

When hydroxyurea is added, it kills all the cells in S-phase and impose a block at the end of G1-phase.

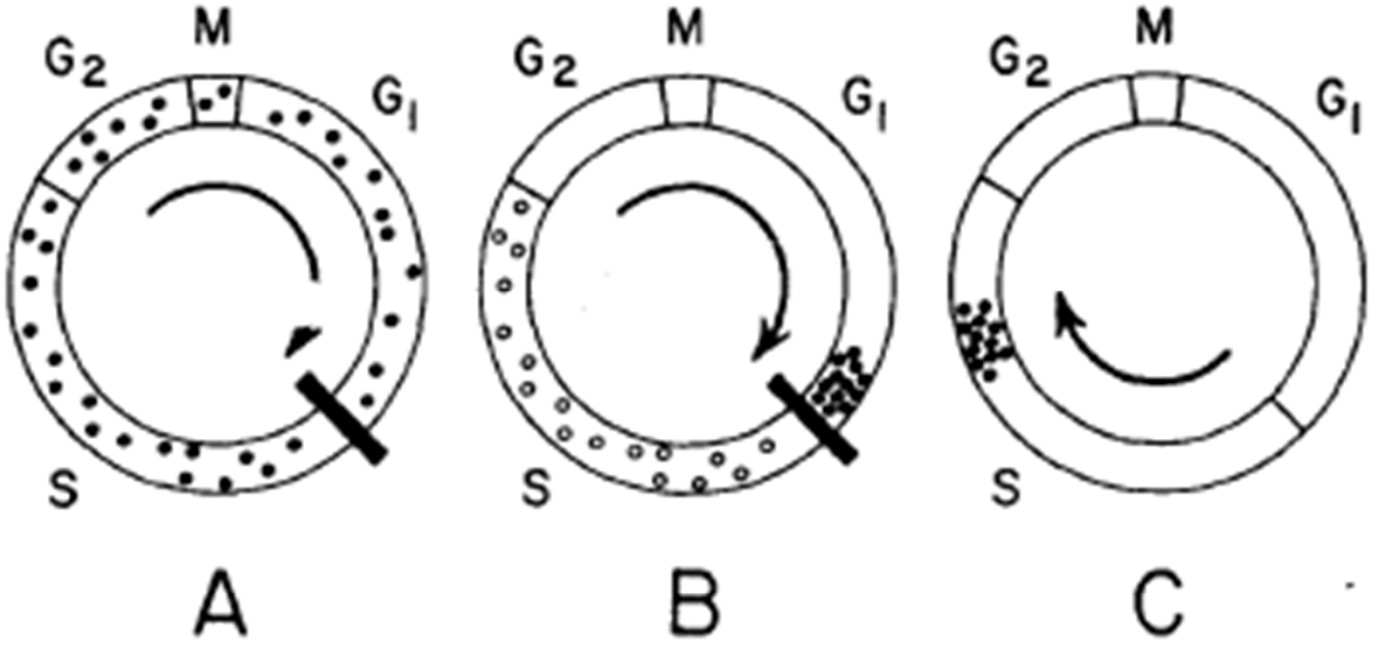


Figure 5–4. Mode of action of hydroxyurea as an agent to induce synchrony. This drug kills cells in *S* and imposes a "block" at the end of G_1. Cells in G_2, *M*, and G_1 when the drug is added accumulate at this block. When the block is removed, the synchronized cohort of cells moves on through the cycle.

Redistribution or Reassortment

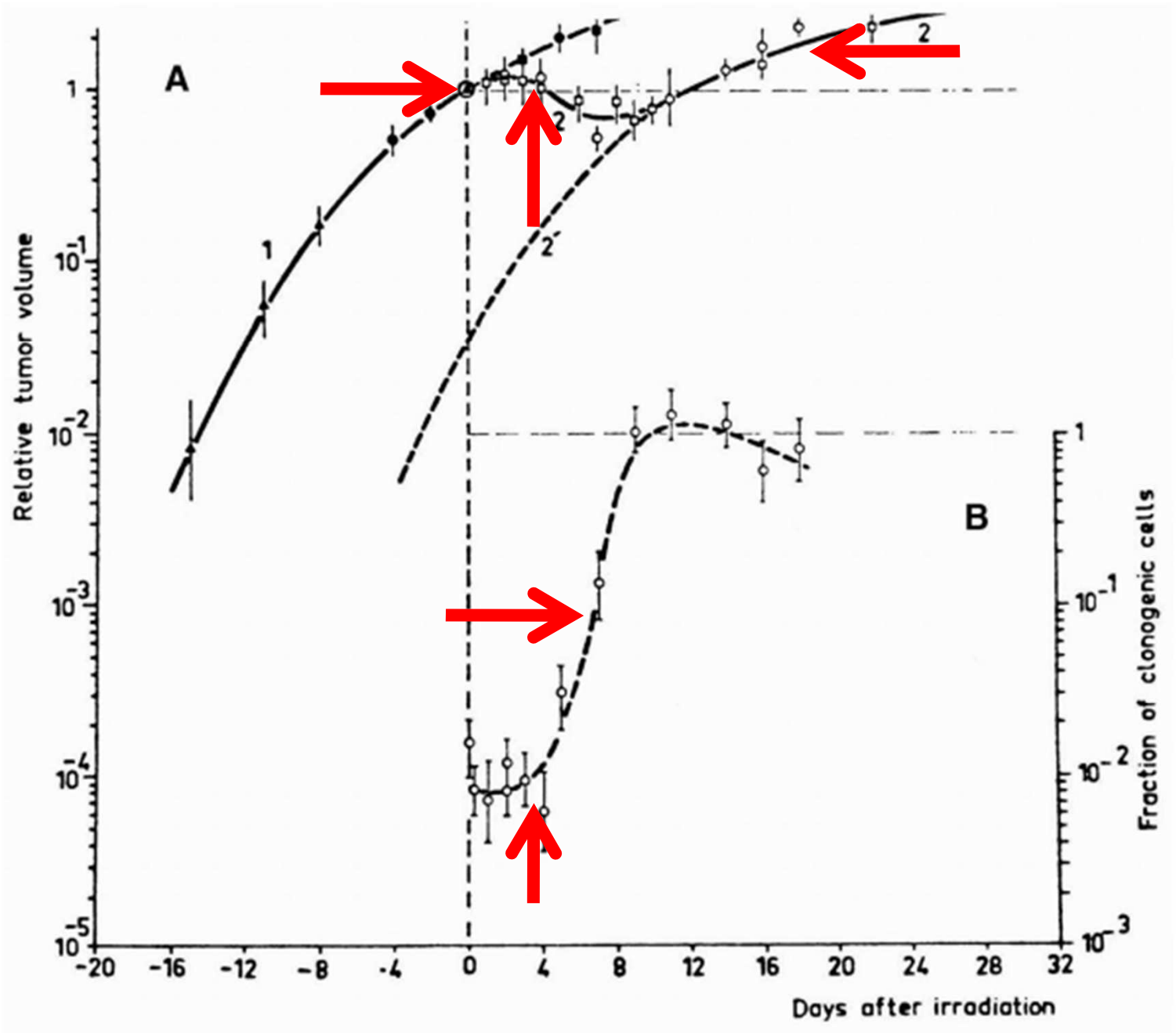
- So by making the cells in synchronization and knowing the time when they pass through G2, M phase, which is the most sensitive phase to radiation, we may achieve max kill by scheduling fractionation accordingly.

- **R**epair
- **R**e-oxygenation
- **R**e-distribution
- **R**e-population

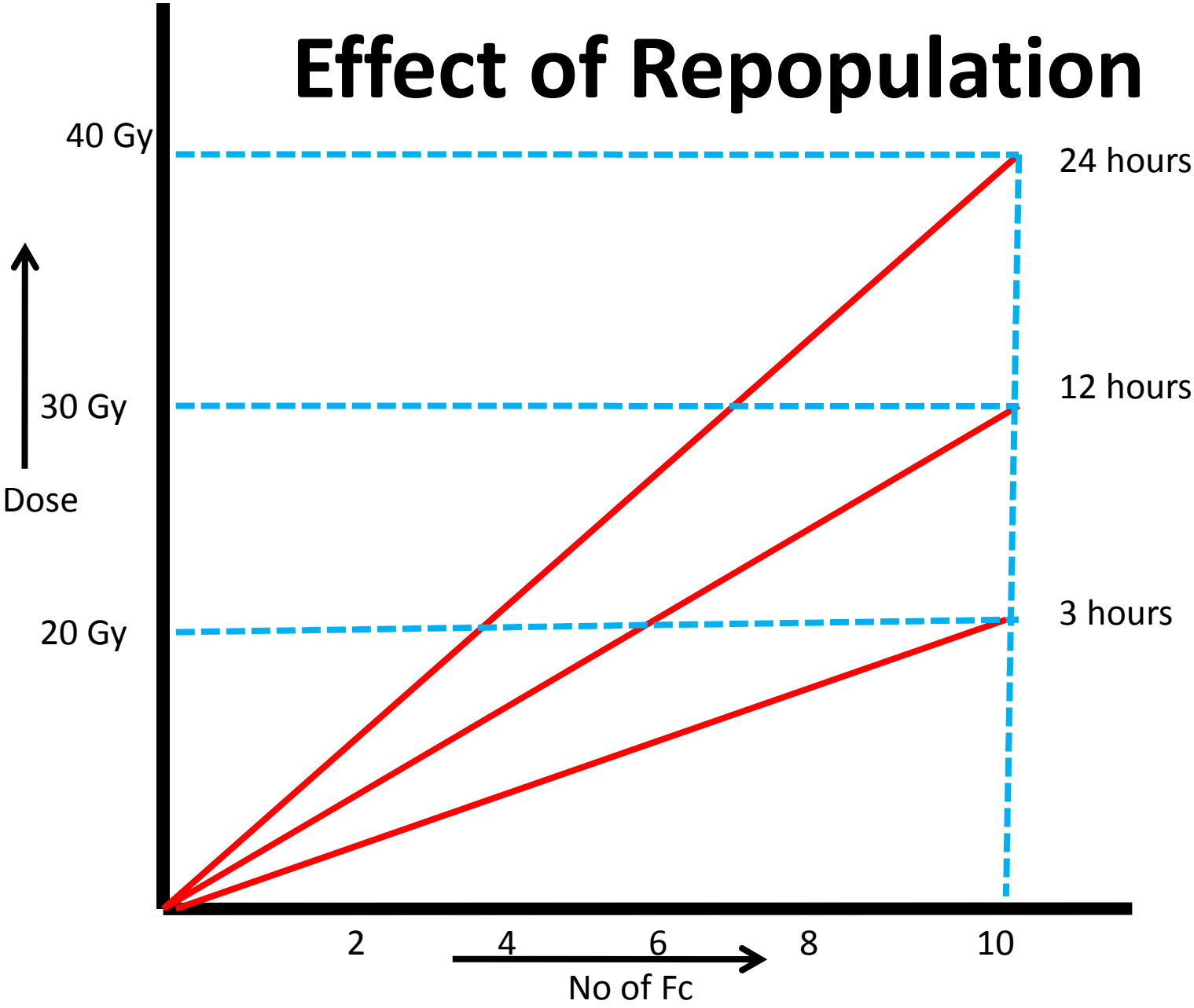
Repopulation (Accelerated)

- As a result of any injury to tissue which causes into depopulation of cells eg. Physical injury (Cut, burn etc.), the cells at the edge of the wound will start multiplying faster in order to replace the dead tissue.
- Same thing happens in injury due to Radiation or Cytotoxic drugs. This phenomenon is called

Accelerated Repopulation.


A
B
1
2
2'
Relative tumor volume
Fraction of clonogenic cells
Days after irradiation
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
-20
-16
-12
-8
-4
0
4
8
12
16
20
24
28
32

Effect of Repopulation



Mouse colon being irradiated by giving various time interval between fractions

- When 3 hours gap is given between fractions the total dose is 20 Gy/10 Fc to achieve a given biological effect.
- To get the same effect if time interval is increased to 12 hours and 24 hours, the total dose also increased to 30 Gy and 40 Gy respectively.
- This indicate that there is accelerated repopulation between fractions.
- The magnitude of regeneration also increases with each passing day.

- The regeneration depends upon the rate of growth.
- Slowly dividing tissues like CNS, Bone, Connective tissues etc. show little regenerative response.
- Rapidly dividing tissues like lining mucosa, bone marrow etc show quick regenerative response.
- Regeneration usually begins during 4^{th} week in head and neck region.
- Accelerated repopulation is also observed in head and neck tumors and usually start at about 28 days after the beginning of radiation.

Clinical Implications

- Side effects are reduced as normal tissue injury is healed by accelerated repopulation.
- Effect on tumor is negative as more dose of RT is required to compensate the accelerated repopulation.
- As the overall treatment time is increased, total dose to get the same effect will also be increased.
- Once the treatment is started (either by RT or CT), the treatment should be completed as early as possible.
- All forms of avoidable delay should be avoided.

Clinical Implications

- Since late reacting tissues do not show any significant repopulation, prolonging overall treatment time has little sparing effect on late reactions but a large sparing effect on early reactions.
- In head and neck cancers the repopulation starts at the end of 4^{th} week, so any treatment schedule longer than 4 week require extra dose to compensate the accelerated repopulation.
- A dose increment of about 0.6 Gy (60 rad) per day is required to compensate for this repopulation. Such a dose increment is consistent with a 5-day clonogen doubling rate, compared with a median of about 60 days for unperturbed growth

Take Home

- 4 Rs of Radiobiology forms the basis of fractionated radiotherapy
- Re-oxygenation is most important as only tumor enjoys hypoxia.
- Most of the process of 4 Rs complete within 24 hours.

2nd Teaching Course on "Basic Radiobiology for Radiation Oncologists"

- **Venue:**- Regional Cancer Center, Indira Gandhi Medical College, Shimla.
- **Date:-** 8th Sept. 2012 (one day)
- **Target Audience:-** 1st and 2nd year MD, DNB
- **Course Director:-** Dr Manoj Gupta
- **Email:-** mkgupta62@yahoo.co.in
- **Mobile:-** 09418470607
- **Registration:-** **FREE**
- **Number of seats** **30**