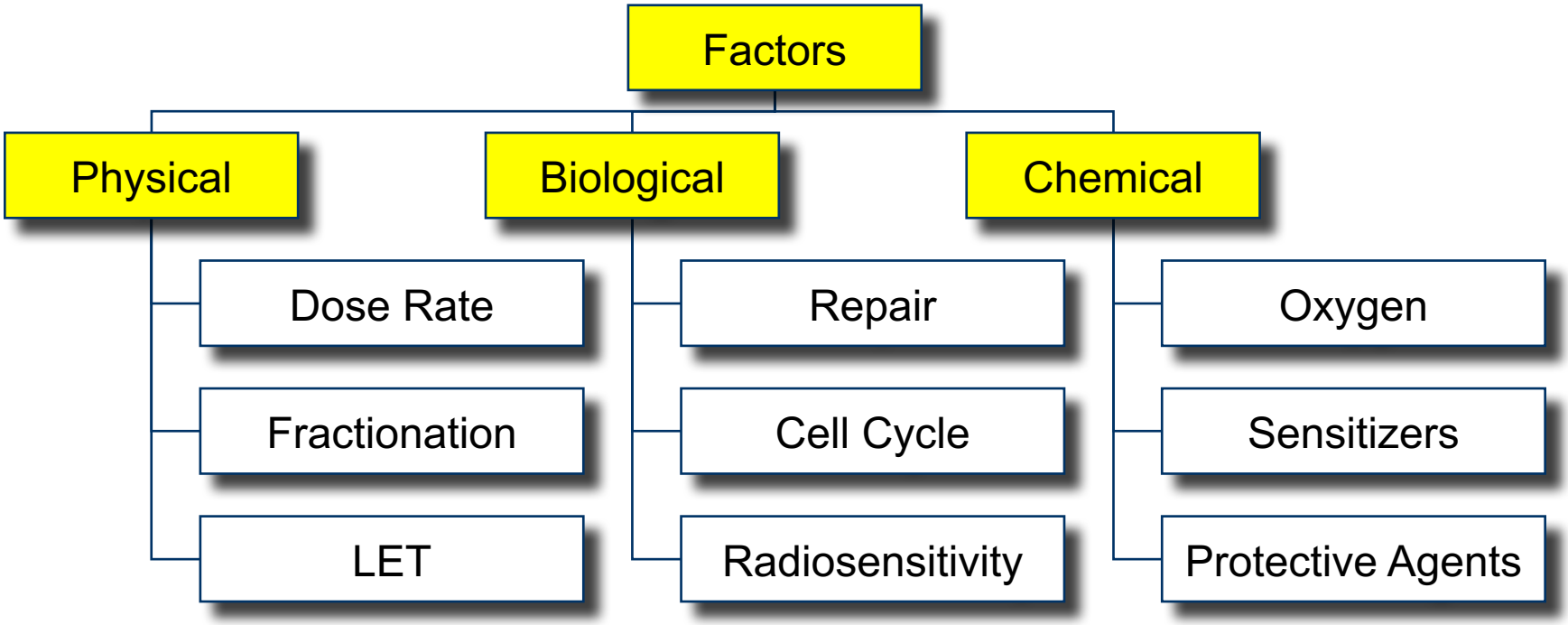
MPHY3892 Radiobiology

Lecture 3. Biological and Chemical Factors Affecting Radiation Effects



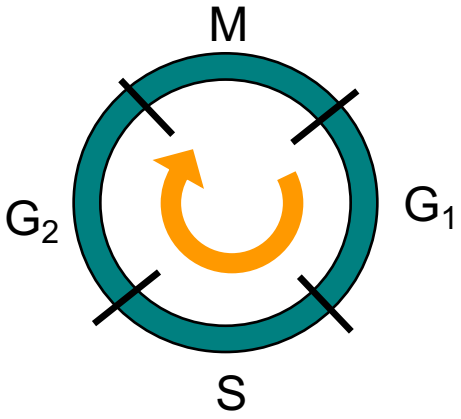
Biological Factors: Repair

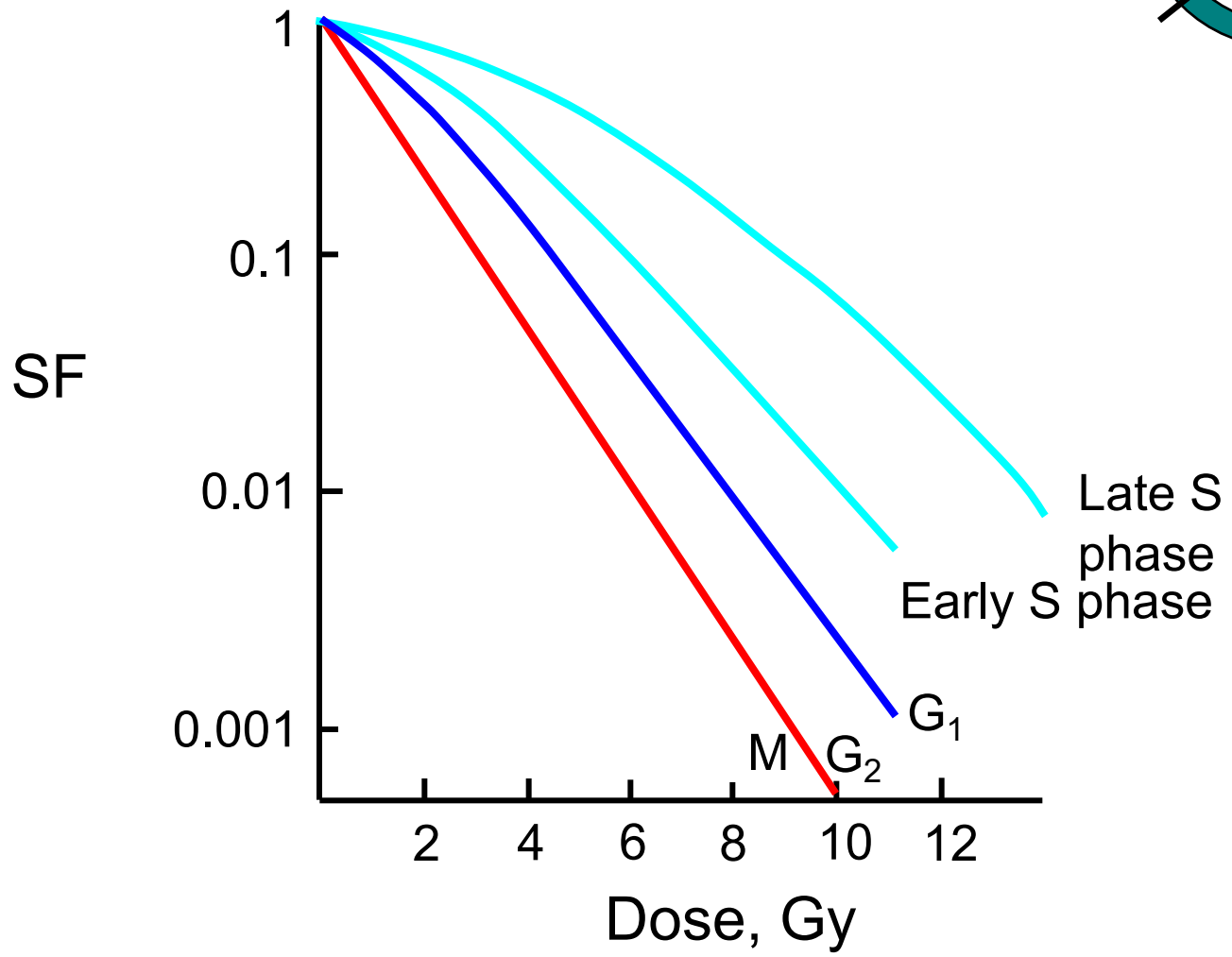
- Lethal damage - irreversible, irreparable $\Rightarrow$ cell death
- Sub-lethal damage - can be repaired given time. Repair of sub-lethal damage is the repair of double-strand breaks
- Potentially lethal damage - component of damage that can be modified by post irradiation environmental conditions (e.g. if post irradiation conditions are sub-optimal for growth, cells do not have to attempt mitosis while their chromosomes are damaged)
- In mammalian cells the mechanisms of repair are not fully understood at the molecular level

Cell Cycle

- Cell populations are normally asynchronous (divide at different times)
- Can be synchronised artificially *in-vitro*
- Methods of synchronisation of cells:
  - shake-off method ("mitotic harvest"): seeded cells that normally adhere to surface in monolayers become rounded and less firmly attached to surface when they approach mitosis $\Rightarrow$ shake of the vessel can detach mitotic cells. You can thus separate mitotic cells from others.
  - other methods of synchronisation make use of certain drugs

Cell Cycle

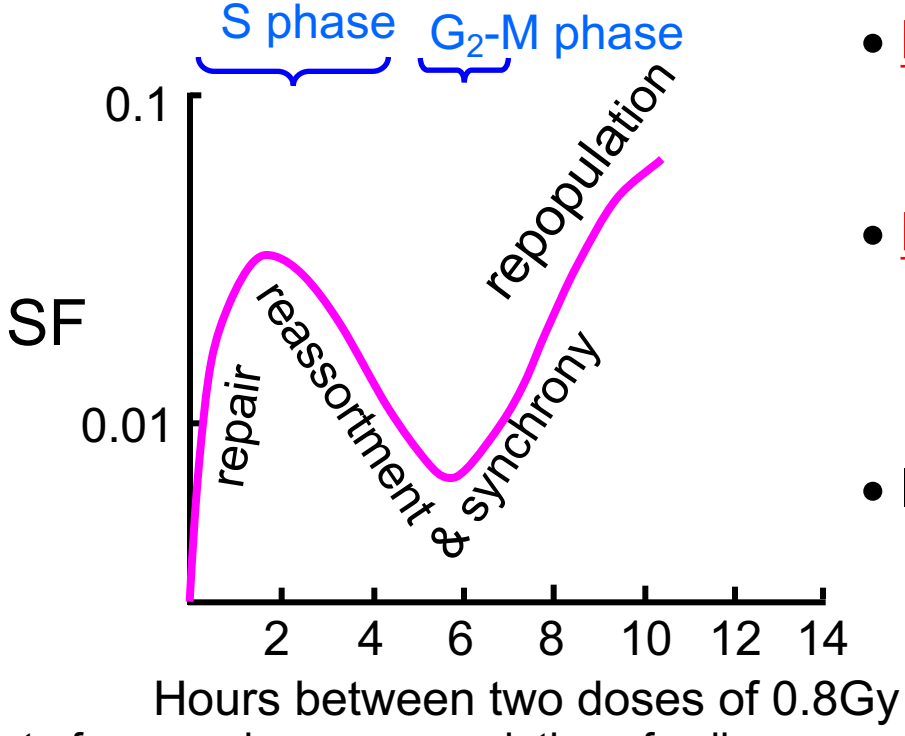




Data for Chinese hamster cells *in-vitro*

Cell Cycle

- Cells are most radiosensitive at, or close to, mitosis (G_2-M phase). During G_2 phase the chromosome integrity is checked and the repair is slower and less effective
- Radio-resistance is usually greatest in late S phase during DNA synthesis because the repair processes are most effective then



Data for asynchronous population of cells

- Repair - prompt repair of sub-lethal radiation damage
- Reassortment - progression of cells through the cell cycle during the interval between split doses
- Repopulation - increase of surviving fraction resulting from cell division, if the interval between the split doses exceeds the length of cell cycle
- In dose fractionation the increased G_2-M sensitivity can lead to synchronisation of survivors (more cells are killed during sensitive phases)

Radiosensitivity

- Tissue radiosensitivity is directly proportional to mitotic activity and inversely proportional to degree of differentiation of its cells
- Implies actively dividing tissues are sensitive compared to non-dividing tissues
- A whole-body dose to a mammal results in more damage to tissues with a high rate of cell division
- Survival curves of malignant cells do NOT show any differences from those of normal cells
- Radiosensitivity of tissues (tumours and normal tissues) differs considerably from patient to patient. Taking this into account is the goal of radiobiology of the future.

Dividing Tissues:

bone marrow
gonadal germinal cells
intestinal epithelium
skin: D_0 = 1.3Gy

} Mean lethal dose D_0 = 80cGy

Non-Dividing Tissues:

liver
kidney
muscle
brain
bone

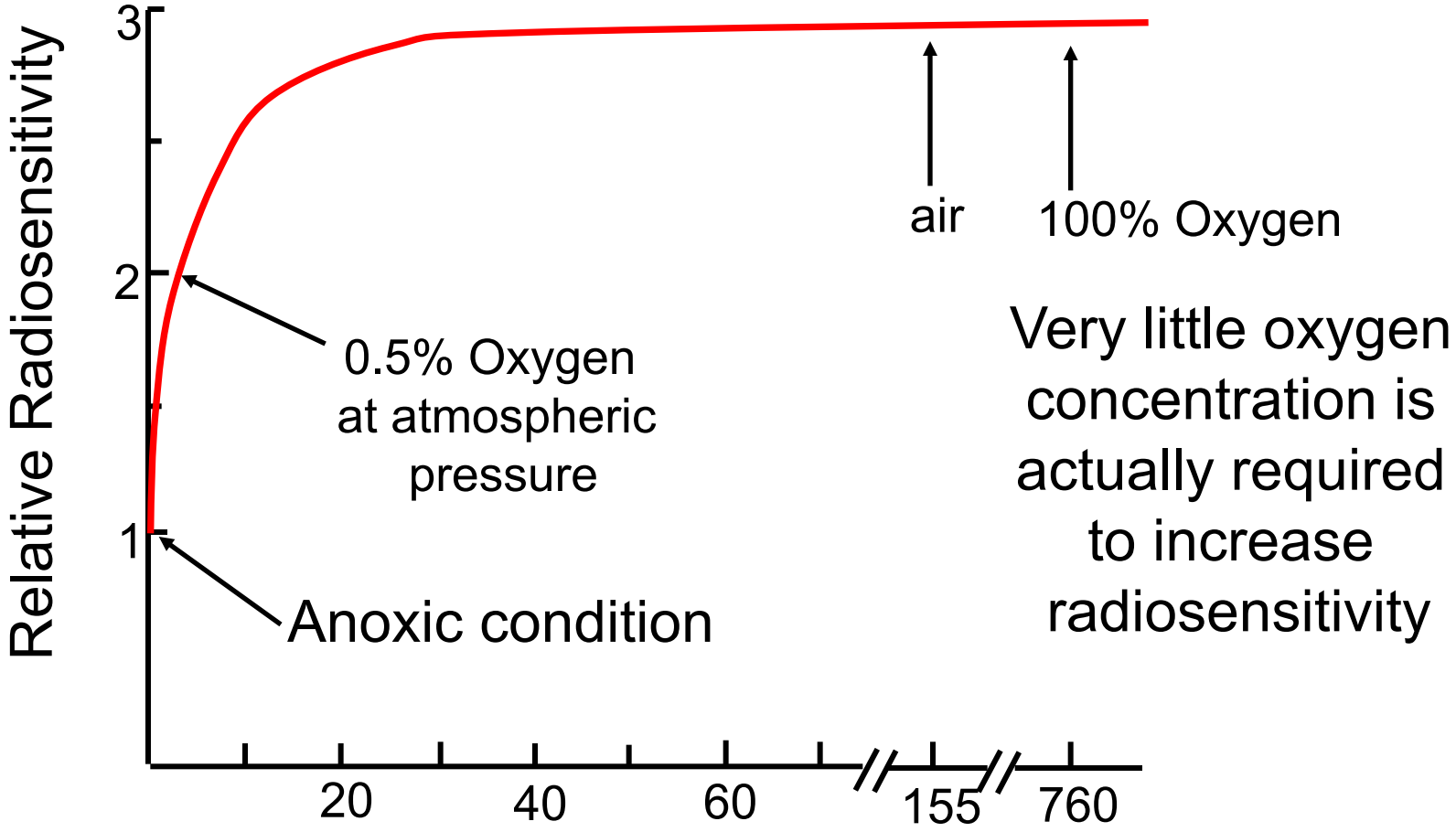
Chemical factors: Oxygen

- Oxygen fixes (makes permanent) radiation lesions
- If oxygen is present during the lifespan of free radicals $R^{\bullet}$, it reacts with them

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$$

- This reaction
  - produces organic peroxides that are non-restorable form of target molecules
  - results in change of chemical composition of the material
- In the absence of oxygen many of the ionised target molecules can repair themselves
- Oxygen tension of most normal tissues is similar to that of venous blood (20 - 40 mm Hg)
- Normal tissues can be all considered as well oxygenated (with the few exceptions such as cartilage - tough elastic tissue on the articulating ends of bones)

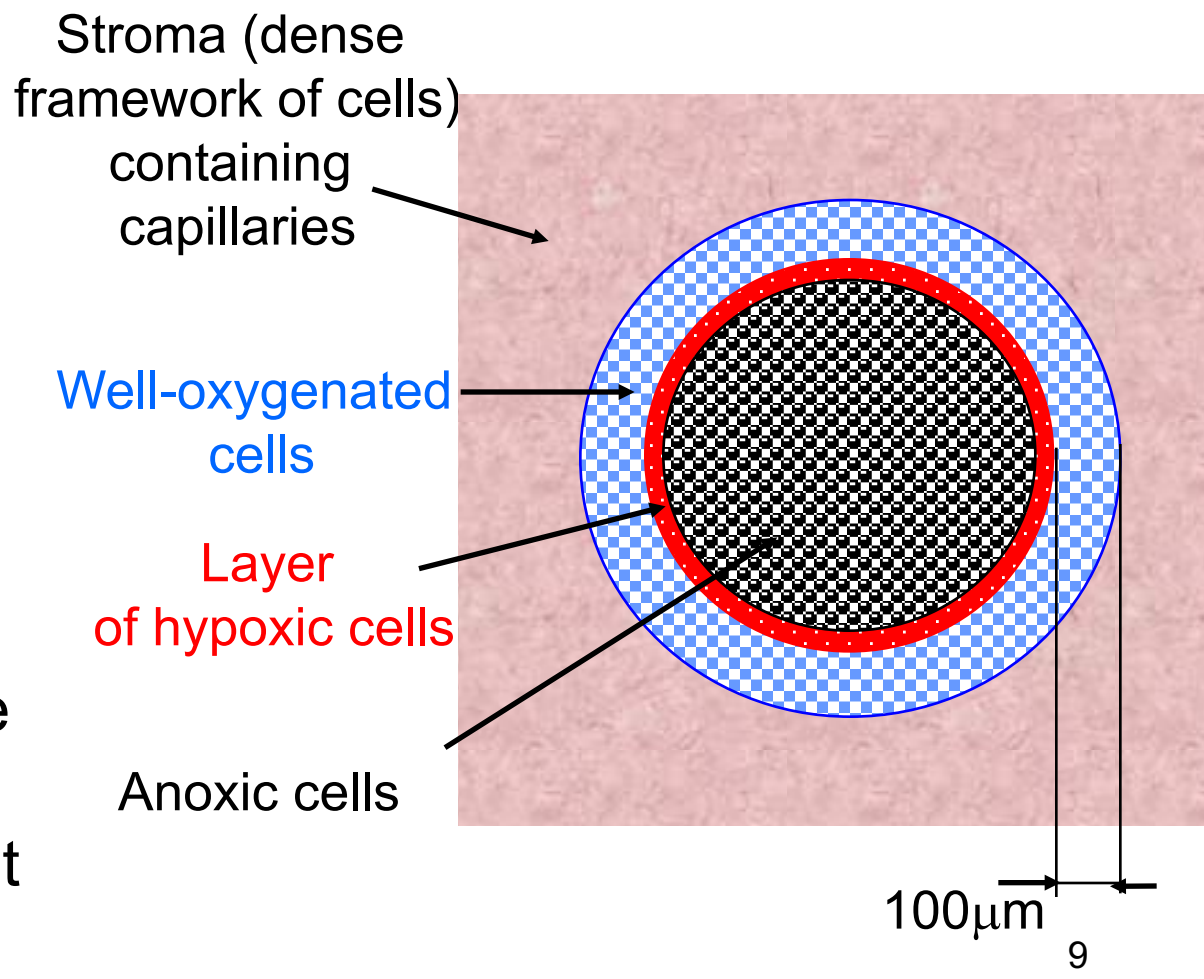
Oxygen



Data for x-rays

Hypoxia (Oxygen Deficiency) in Small Malignant Tumours

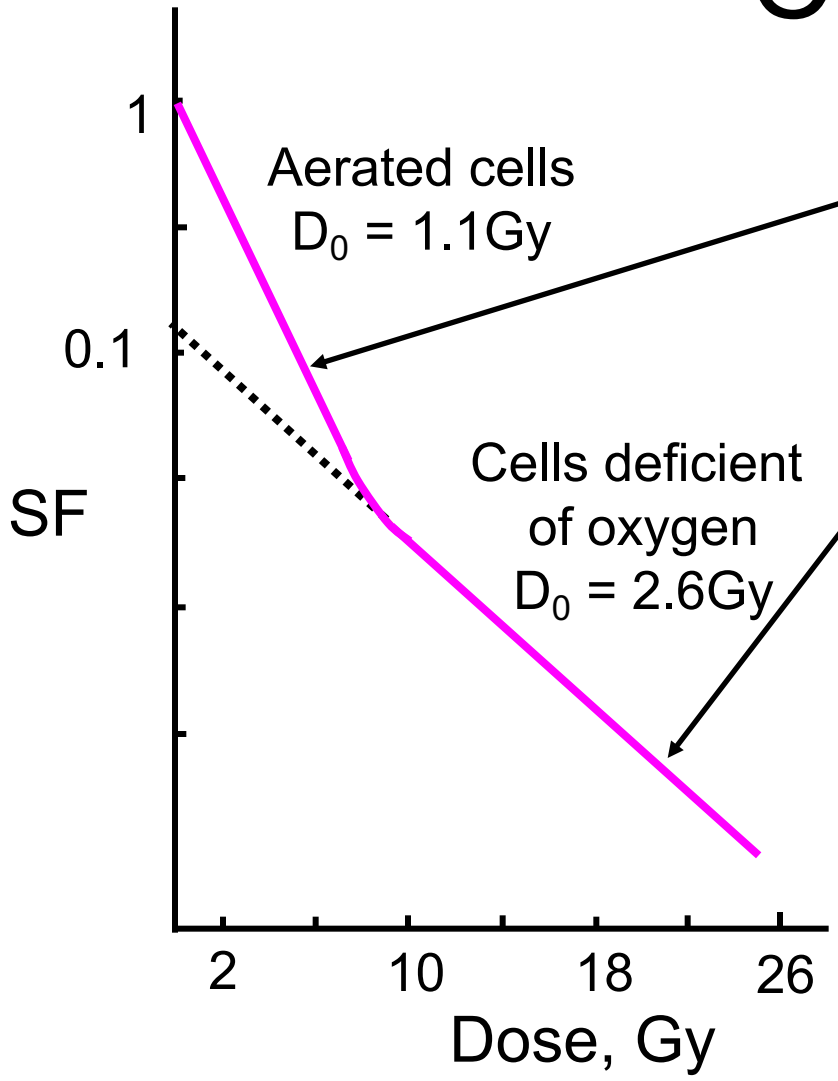
- The distance to which oxygen can diffuse is limited by the rapid rate at which it is metabolised by respiring tumour cells
- This distance is about 100μm at the arterial end of capillary and less at the venous end



Hypoxia in Large Tumours

- Tumour vascularity is growing with the tumour, but it is less efficient in supplying the tumour with blood than the vascularity of normal tissues
- Acute hypoxia of tumour cells is caused by temporary closing or blockage of a tumour blood vessel
- Chronic hypoxia results from limited diffusion distance (~100μm) of oxygen through respiring tissue
- Anoxic cells are those that are more than 100 - 200μm away from blood vessels

Oxygen: Biphasic Survival Curve



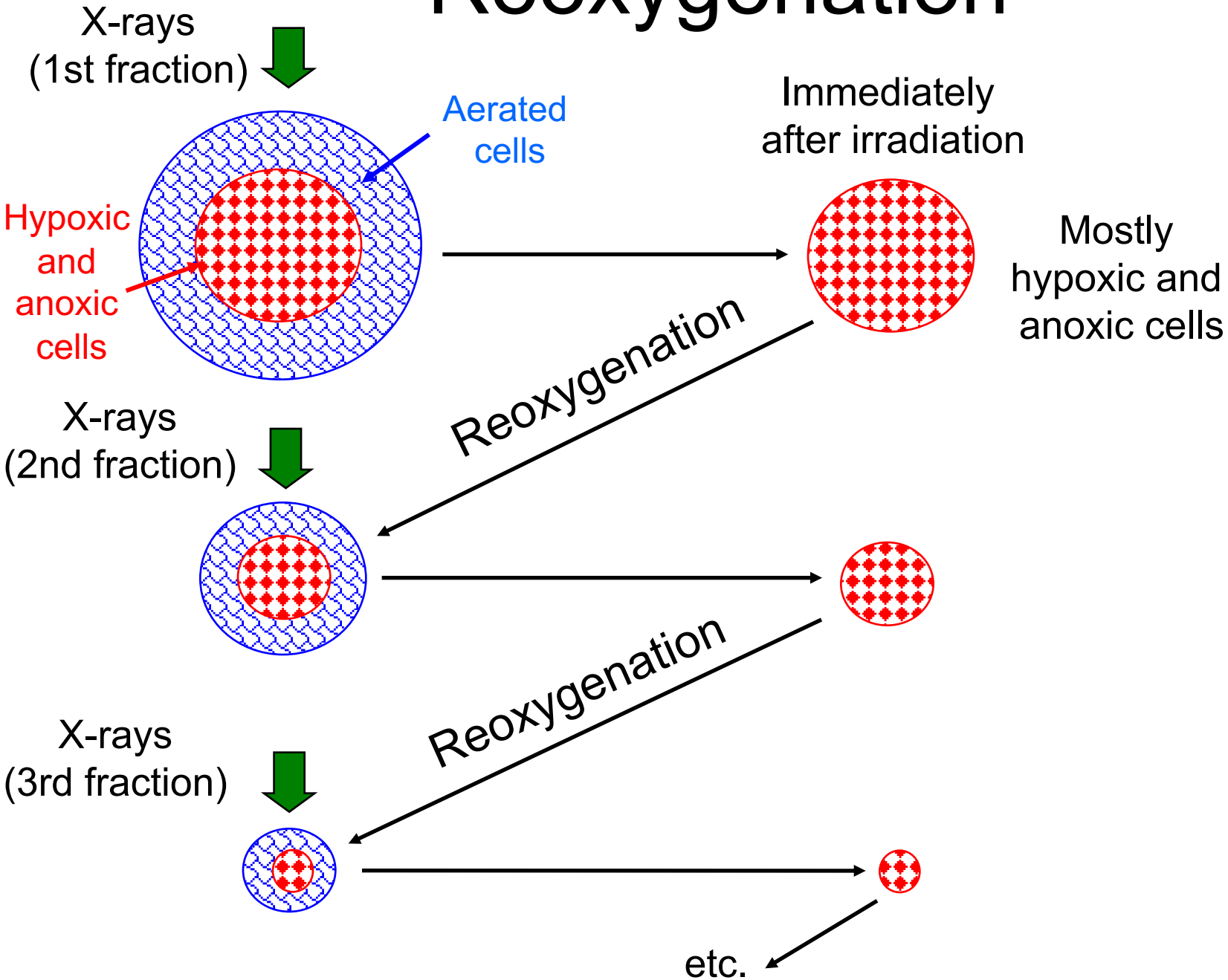
Response to lower doses is dominated by killing of well-oxygenated cells

Response to high doses is characteristic of killing cells deficient of oxygen

Extrapolation of the curve for cells deficient of oxygen gives % of hypoxic and anoxic cells at intercept: here 15% of cells are deficient of oxygen which is typical for animal tumours

Data for solid lymphosarcoma

Reoxygenation



Reoxygenation

- In radiotherapy: a dose of x-rays kills a greater proportion of aerated than hypoxic cells $\Rightarrow$ immediately after irradiation most cells in the tumour are hypoxic or anoxic
- Restructuring or revascularisation of the tumour happens as the cells killed by radiation are broken down and removed
- As tumour shrinks, surviving cells find themselves closer to a blood supply and so reoxygenate
- Hypoxic cells become oxygenated if sufficient time is allowed before the next radiation dose fraction is delivered
- Time sequence of reoxygenation: the proportion of hypoxic cells returns to its original pre-treatment level by 6 hours - 3 days depending on the type of tumour
- In radiotherapy doses of 60 Gy are usually delivered in 30 fractions
- It is suggested that some human tumours that do not respond to radiotherapy quickly are those that do not reoxygenate effectively

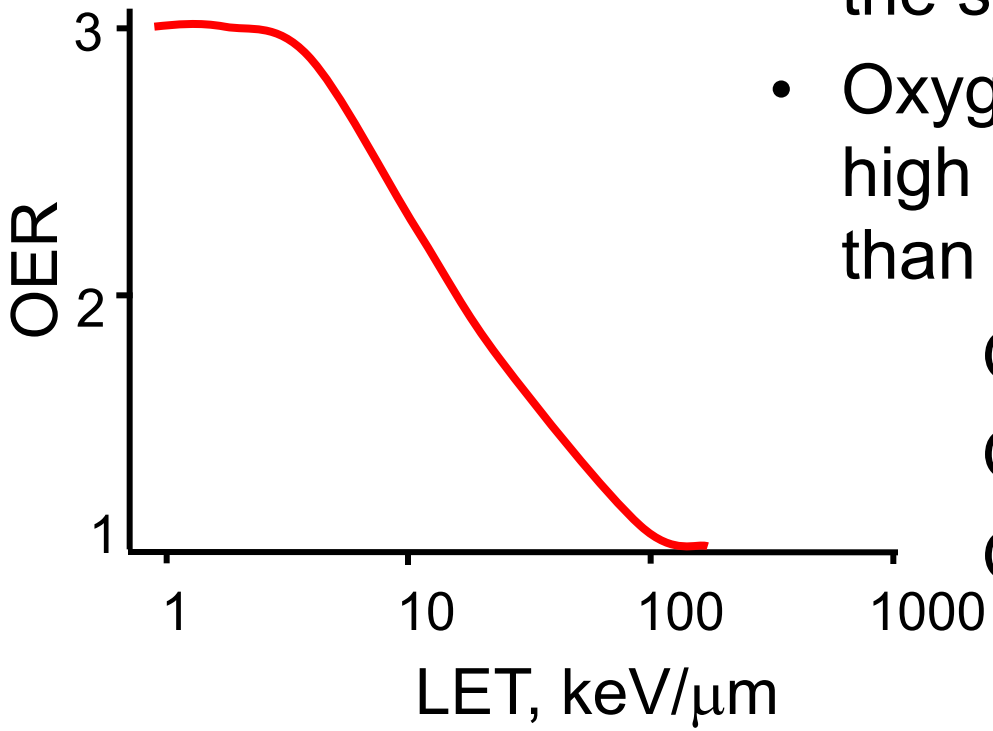
Oxygen Enhancement Ratio

- In the presence of Oxygen almost all cells are more radiosensitive
- Oxygen Enhancement Ratio (OER): the ratio of hypoxic to aerated doses needed to produce the same effect
- Oxygen Enhancement Ratio for high LET radiations is smaller than for γ-rays:

  OER = 3 for γ-rays

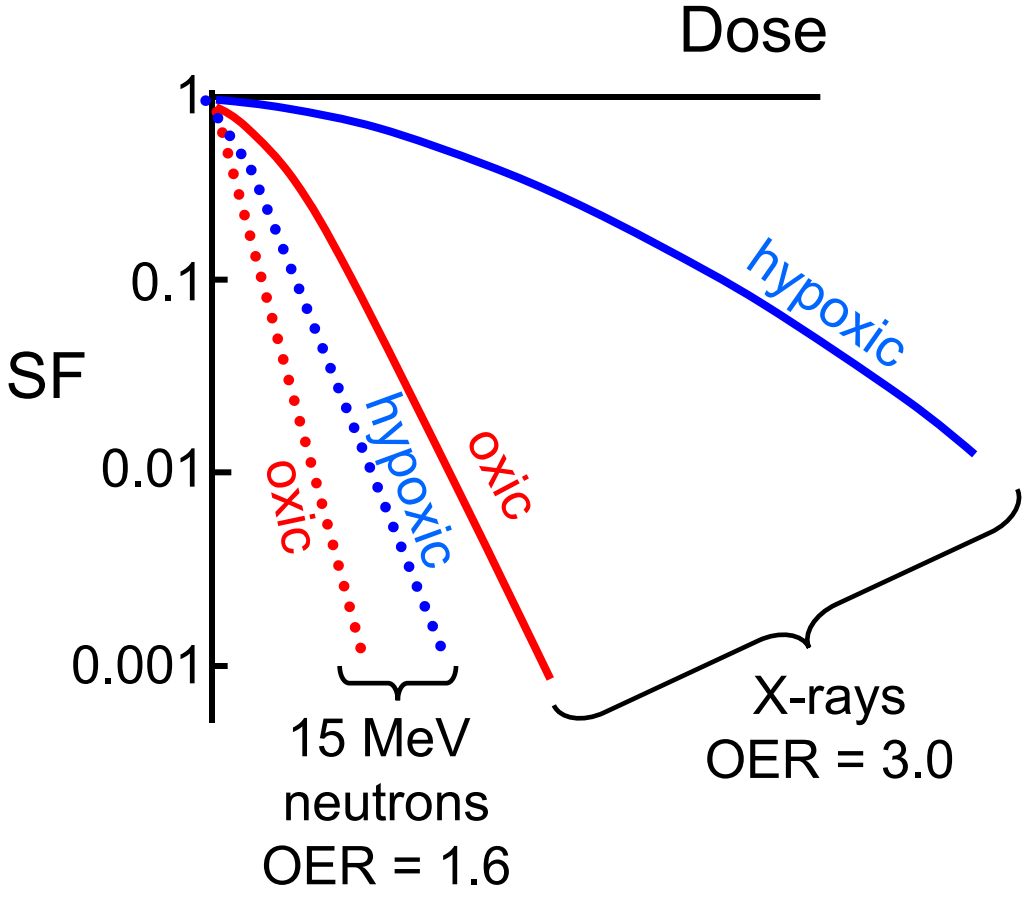
  OER = 1.6 for fast neutrons

  OER = 1 for α-particles - (no oxygen effect)



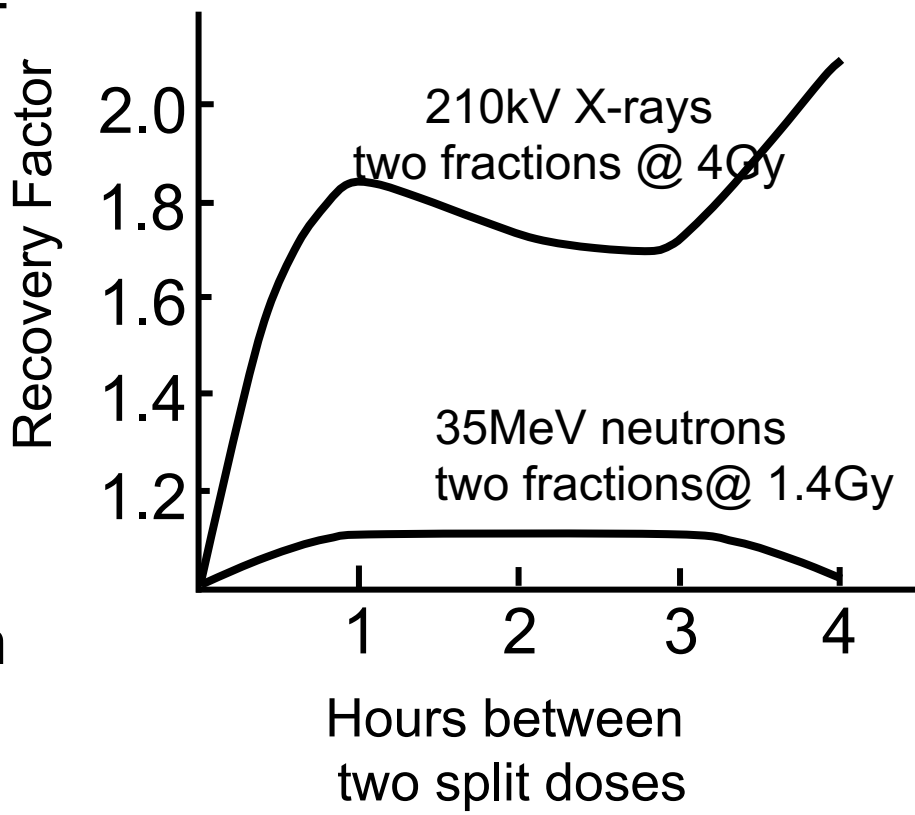
Oxygen Enhancement Ratio

- High-LET radiations kill hypoxic cells better than low-LET radiations
- This is one of the rationales for therapeutic use of fast neutrons and other high-LET radiations



Hypoxic Gain Factor (HGF)

- This is the ratio of the Oxygen Enhancement Ratio values for γ-rays and high-LET radiations
- For fast neutrons: HGF = 3/1.6 = 1.9
- Hypoxic Gain Factor represents therapeutic gain if the hypoxic cells are the determining factor in tumour resistance
- In practice, the therapeutic gain is less than the Hypoxic Gain Factor as tumour reoxygenation during fractionated irradiation reduces the population of hypoxic cells



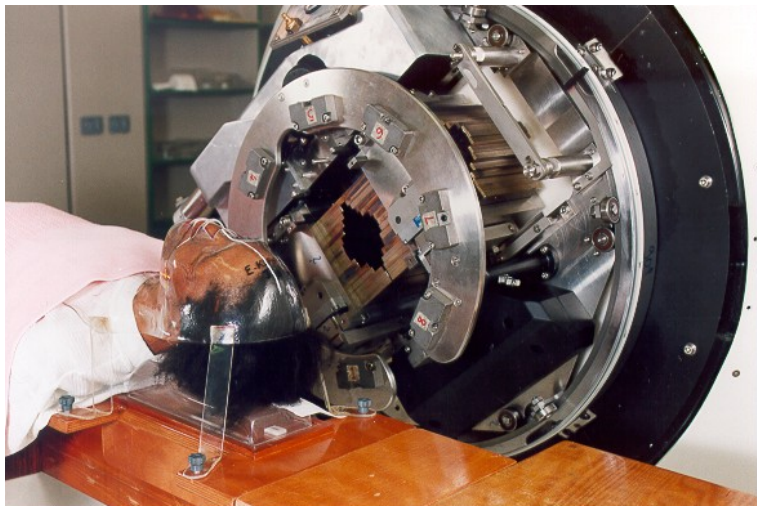
Studies *in-vitro*

Use of Fast Neutrons in Radiotherapy

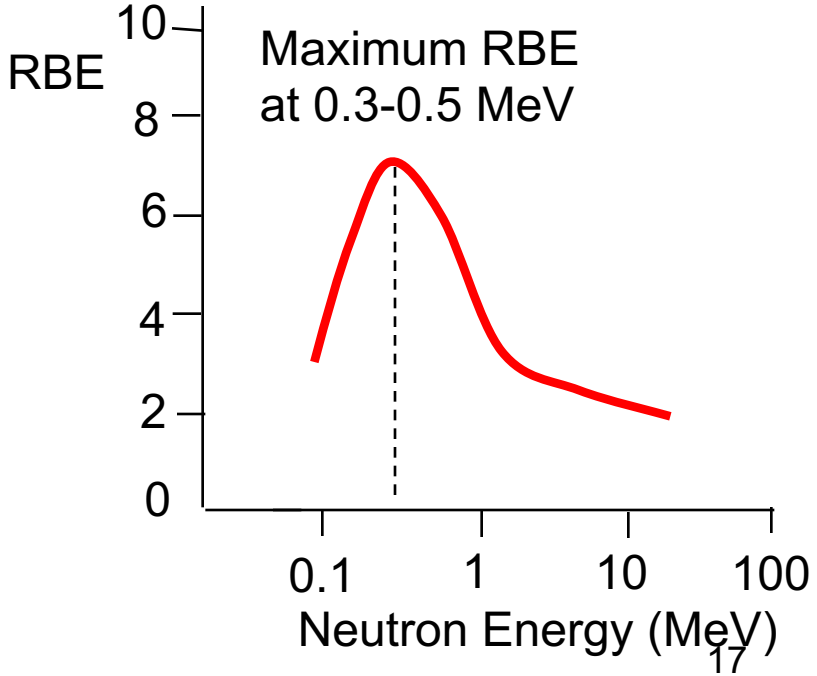
- Fast neutrons can be produced by accelerating deuterons $^{2}H_{1}$ with the help of a cyclotron and colliding them with a Beryllium foil:

$$^{2}H_{1} + {}^{9}Be_{4} \rightarrow {}^{10}B_{5} + n + \gamma$$

- 15 MeV neutrons are frequently used for radiotherapy
- The rationale for fast neutron therapy is now based on a higher neutron Relative Biological Efficiency (RBE) for slowly growing tumours
- Neutron therapy is very costly and is used at a few medical centres only



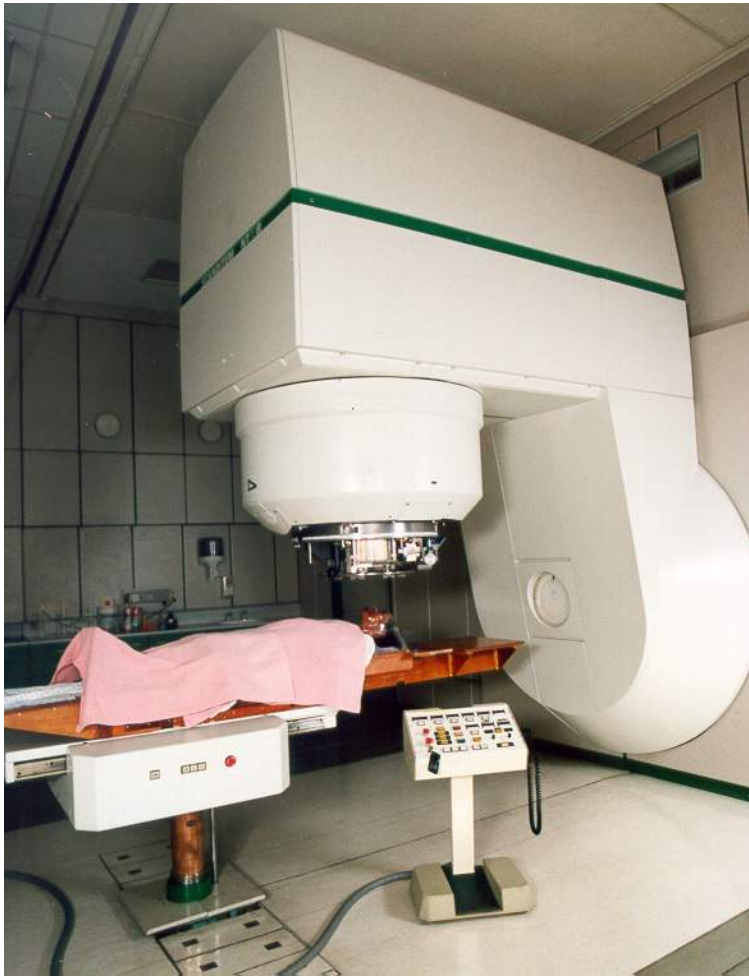
Fast neutron therapy



Data for hamster cells

Patient Selection for Fast Neutron Therapy

- Salivary-glands tumours: local control rates are 67% after neutron treatment compared to 24% after low-LET treatment with x-rays or γ-rays
- Soft tissue sarcomas that have lower rate of reoxygenation
- Prostate tumours
- Certain inoperative melanomes (malignant tumours of skin). Failure of low-LET radiotherapy (with x-rays or γ-rays) is connected with the large shoulder of the cell survival curve



Neutron therapy gantry

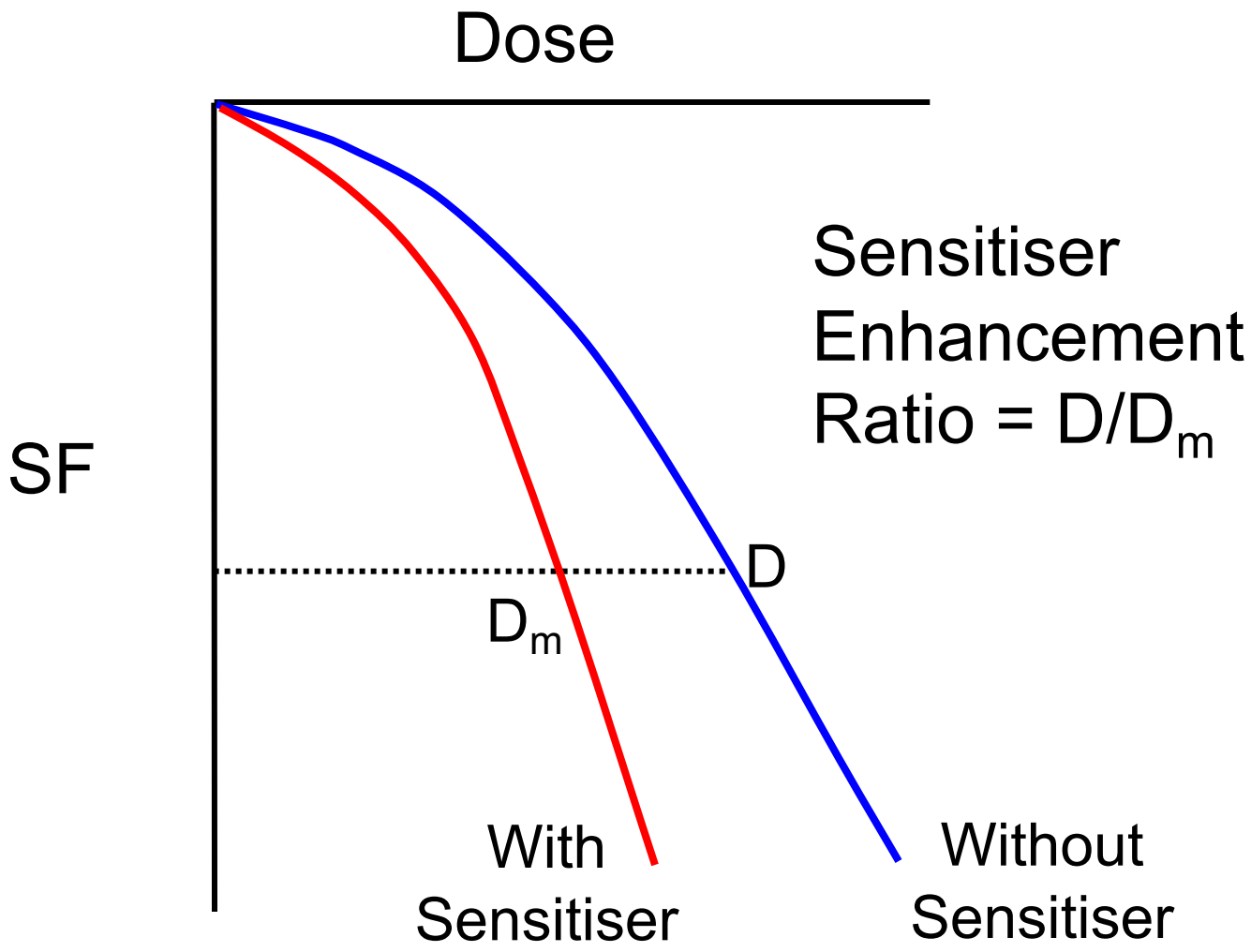
Radiosensitisers

Effect of radiation can be increased with a radiosensitiser that:

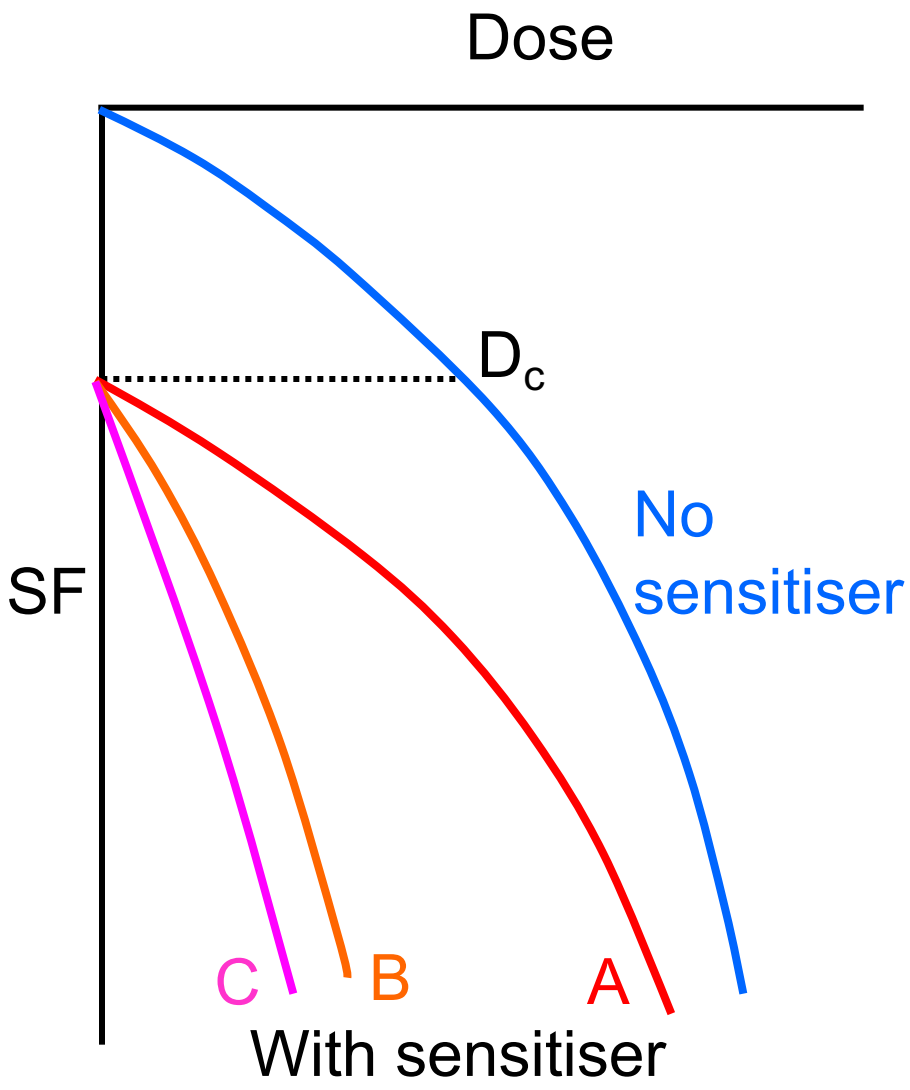
- mimics oxygen by fixing damage produced by free radicals
- is a substance with high oxidising potential
- should be present during the lifespan of free radicals
- ideally should have no lethal effect of its own, but at a given dose reduces the Surviving Fraction
- must show differential effect between tumours and normal tissues

- Sensitiser Enhancement Ratio (SER) = ratio of doses needed to obtain a given effect in the absence and presence of sensitiser
- Sensitiser may have selective lethal effect on cell sub-populations (e.g. S phase cells that are most radio-resistant in the cell cycle) because of synchronisation
- Oxygen is the most significant "true" sensitiser

Radiosensitisers



Radiosensitisers



A: vertical shift in SF during chemical pre-treatment due to toxicity of radiosensitiser: no true sensitisation

B: corresponds to SF after dose D_c - i.e. effect is the same as having dose D_c. Effect of dose D = that of $D+D_c$. $\Rightarrow$ simple ADDITION of chemical and radiation effects

C: true sensitisation

Radiosensitisers

- Attempts to overcome the problem of hypoxia:
  - Hyperbaric oxygen: the patient is placed in a chamber filled with oxygen at 3 atm
  - Breathing Carbogen (5% of carbon dioxide added to pure oxygen at atmospheric pressure) to avoid vasoconstriction caused by breathing pure oxygen
  - Blood transfusions
  - Chemical sensitisers
- Most sensitisers have properties similar to oxygen. Differential effect between tumours and normal tissues is based on the presence of hypoxic cells in tumours and not in normal tissues
- Other sensitisers act on DNA. Differential effect here is based on the assumption that tumour cells cycle faster and incorporate more of the drug than the surrounding tissue

Radiosensitisers Acting on DNA

3 types of radiosensitisers:

- Modifying DNA structure (e.g. by incorporation of a halogenated pyrimidine, which is a thymine analog). This weakens the DNA chain and cells become more susceptible to damage by radiation. The effect increases with the number of cell cycles
- Inhibiting repair of sub-lethal damage which eliminates the shoulder of Survival Fraction curve and lesions that are normally sub-lethal become lethal (examples are: *caffeine*; also *topotecan* that prevents repair of DNA single-strand breaks)
- Inhibiting DNA synthesis (not “true” sensitisers)

Clinical Trials

Analysis of 84 clinical trials of different radiosensitisers showed:

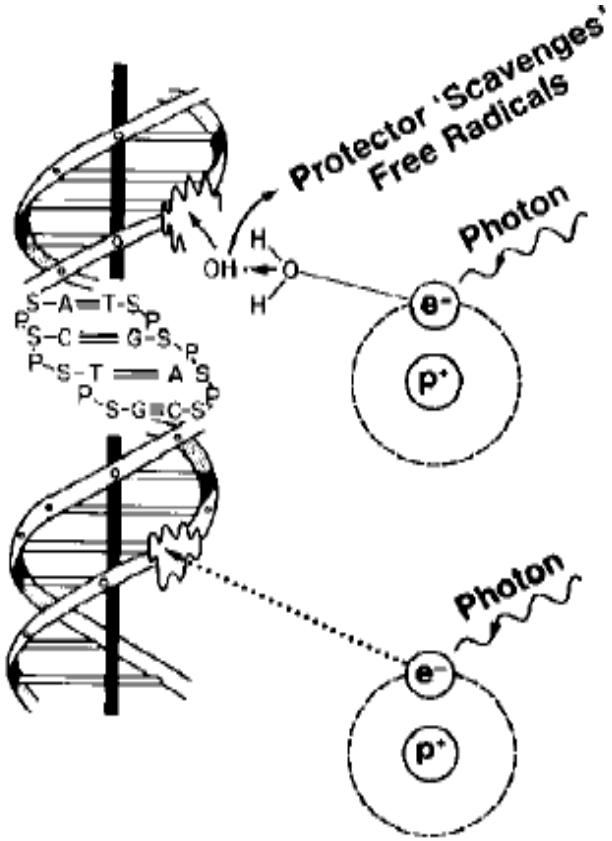
- 4.6% gain in local tumour control
- 2.8% increase in survival rate
- 0.8% increase in complication rate

Radioprotectors

- Some substances, though not directly affecting the radiosensitivity of cells, may protect the whole body because:
  - they cause vasoconstriction (narrowing of blood vessels)
  - or in some other way upset normal processes of metabolism
- This leads to reduction of oxygen concentration in organs $\Rightarrow$ cells become less sensitive to x-rays under hypoxic conditions
- Examples of these substances: carbon monoxide, histamine etc.
- But “true” radioprotectors are sulphydryl compounds (discovered in 1946)

“True” Radioprotectors

- Protective action of “true” radioprotectors is attributed to two mechanisms:
  - capture of $OH^{\bullet}$ radicals by sulphydryl groups (-SH) that protects against oxygen-based free-radical generation by ionising radiations
  - hydrogen-atom donation to facilitate direct chemical repair at sites of DNA damage
- Agent must have low toxicity
- Effect is reduced in presence of oxygen
- Effect is reduced as LET increases because the amount of local damage is so great



Radioprotectors

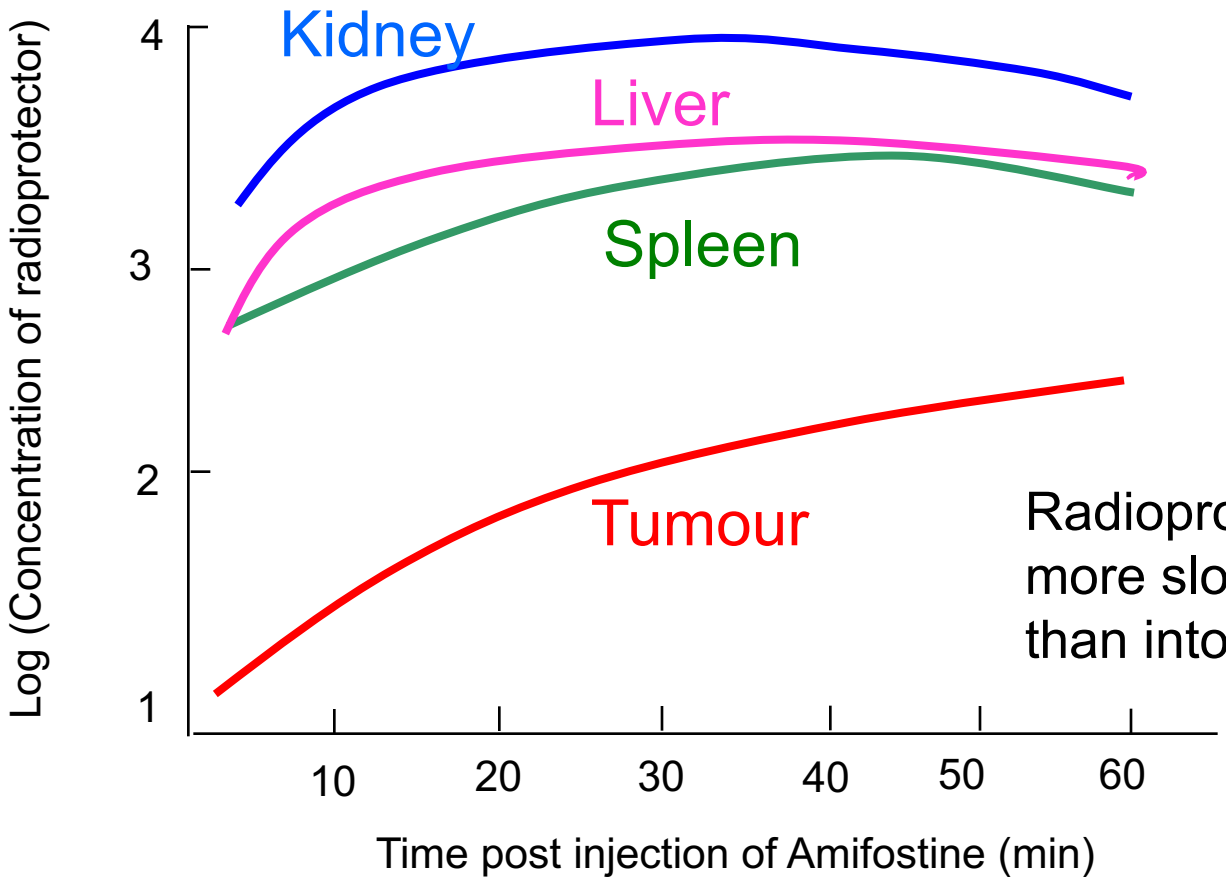
- Dose Reduction Factor (DRF) is the factor by which a dose must be multiplied to produce a given effect after the administration of a protective agent
- Radioprotectors can counter the effect of oxygen by effective scavenging of nearly all free radicals and so the largest possible Dose Reduction Factor would be equal the Oxygen Enhancement Ratio, with a value of 2.5 to 3.0
- DRF of 3 for bone marrow can be achieved
- DRF of 1.8 was achieved for mortality rate in animal experiments
- Duration of protection after administration of “true” protector is 15 min - 3 hours, depending on protector
- Problems
  - toxicity (leading to nausea, vomiting, hypotension, diarrhoea etc.)
  - must be present at time of exposure
  - might interfere with consciousness

Radioprotectors: Amifostine

Amifostine - the best radioprotector for radiotherapy so far

- Is chosen from over 4000 compounds of radioprotectors synthesised for the US Army for military and space applications when irradiation can be foreseen (e.g. in space missions to protect against solar flares)
- Is still tested in clinical trials
- In radiotherapy, protection of normal tissues is achieved through differential uptake of protector in tumours:
  - because of active transport of amifostine into normal tissues with passive diffusion into tumours
  - also because of better vascularity in normal tissues
- Is useful as protector in chemotherapy as well

Radioprotectors: Amifostine



Radioprotector penetrates more slowly into the tumour, than into normal tissues

Radioprotectors: Amifostine

- If radiation dose is given within minutes after administration of radioprotector, there is a differential sparing of normal tissue compared with tumour cells
- Good protection by amifostine of the haematopoietic system and the lining of the gut. No protection of the brain (because amifostine does not cross the blood-brain barrier) and little protection of the lung
- Dose-limiting toxicity of amifostine is hypotension (abnormally low blood pressure) $\Rightarrow$ the amount of drug has to be limited to levels lower than those that are necessary to achieve maximum protection