Chapter 6 – Oxygen Effect and Reoxygenation

9/30/2024

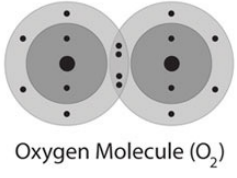
Outline

- **The Oxygen Effect**
- Chronic and Acute Hypoxia
- Experimental Evidence
- Techniques to Measure Tumor Oxygenation
- Reoxygenation
- Hypoxia and Chemoresistance
- Hypoxia and Tumor Progression

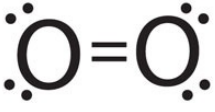
The Oxygen Effect

- Many chemical and pharmacologic agents can modify the biological effect of ionizing radiation

- **Oxygen** is a power radiation sensitizer

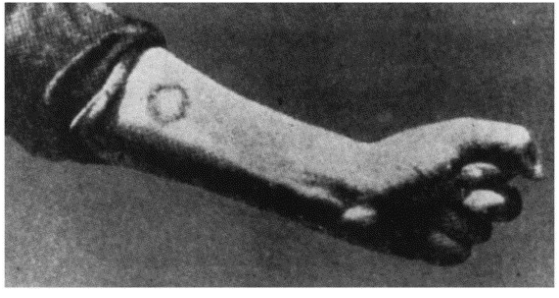


Oxygen Molecule (O_2)



The Oxygen Effect

- The oxygen effect was observed as early as 1912

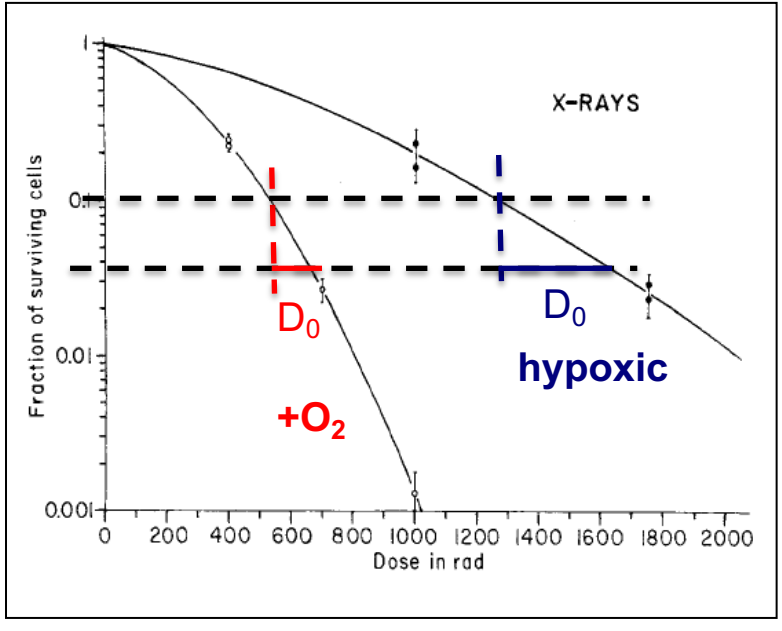


Swartz noted that the skin reaction produced on his forearm by a radium applicator was reduced if the applicator was **pressed hard** onto the skin

- Work by Thomlinson and Gray in 1955 generated tremendous interest in role of oxygen in radiation therapy

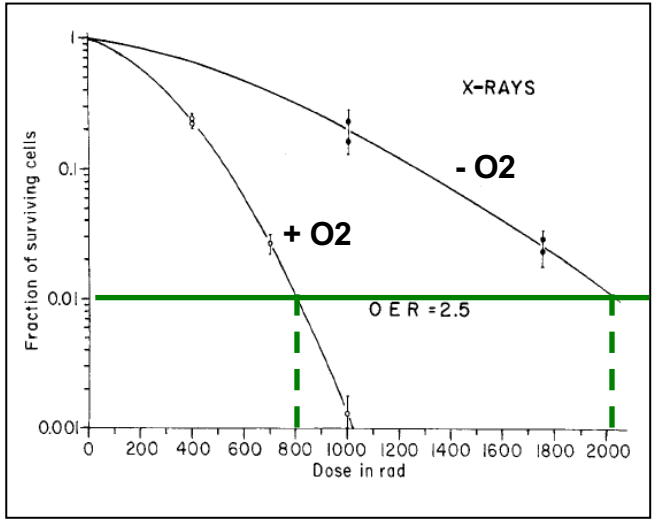
The Oxygen Effect

Survival Curves $\pm$ O_2



Note the broader shoulder and larger D_0 for the hypoxic curve

Oxygen Enhancement Ratio (OER)



$$OER = \frac{\text{dose under hypoxic condition}}{\text{dose under aerobic condition}}$$

to produce the **same biological effect**

SF = 0.01
OER = 2000/800 = 2.5

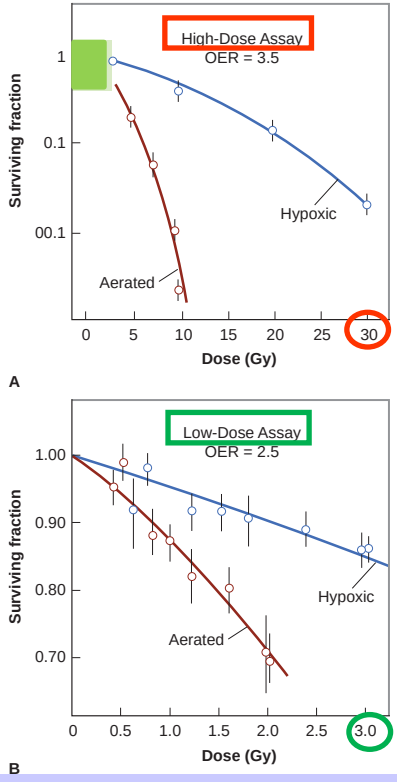
The degree of sensitization is expressed in terms of **Oxygen Enhancement Ratio (OER)**

OER as a Function of Cell Cycle

- OER = 2.5 – 3.5 for sparsely IR (X-ray, γ-rays)
- OER varies through the cell cycle, at least for fast-growing proliferating cells *in vitro*
- OER = **2.8-2.9** for **S** phase; OER = **2.3-2.4** for **G2** phase; G1 falls in between

See chapter 4 (p63)

OER as a Function of Dose (& Dose Rate)



Little significance in clinical radiation therapy

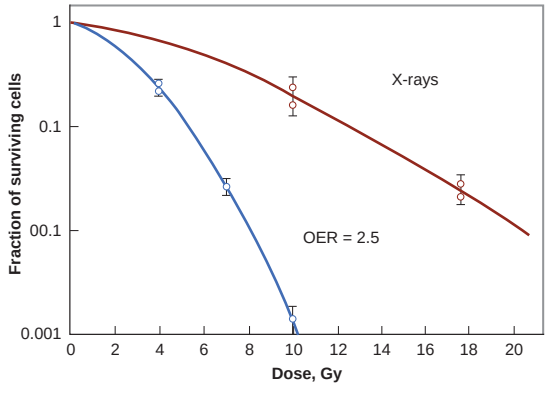
At high dose, cell killing is dominated by cells in radioresistant phase, therefore OER has a larger value **(OER for S phase = 2.8-2.9)**

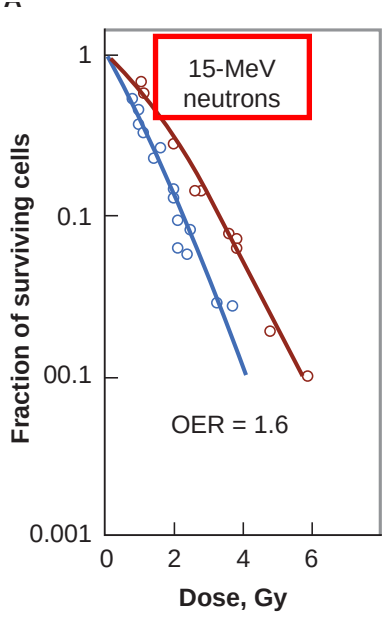
At low dose, cell killing is dominated by cells in radiosensitive phase, therefore OER has a smaller value **(OER for G2 phase = 2.3-2.4)**

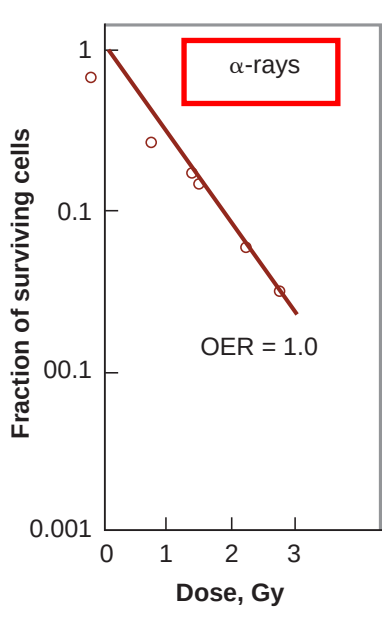
For rapidly growing cells, OER at low doses (& dose rates) tends to be lower than the OER at high doses (& dose rates)

OER as a Function of Radiation Quality

Do you expect OER for neutron and α-rays to be larger or smaller than that for X-ray?







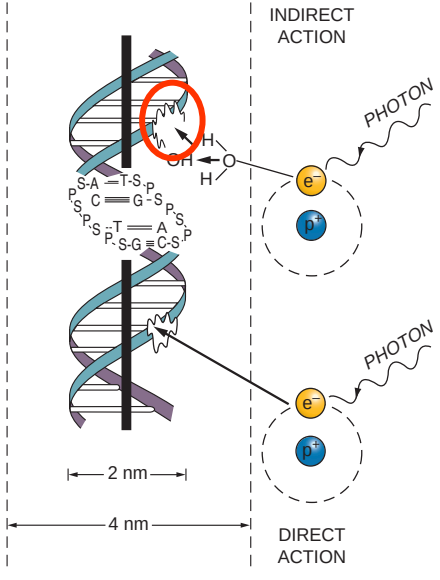
OER ↓ as LET ↑

Summary

The oxygen effect is large and important for sparsely ionizing radiations, absent for *densely ionizing radiations*, and has an intermediate value for fast neutrons

Mechanism of the Oxygen Effect

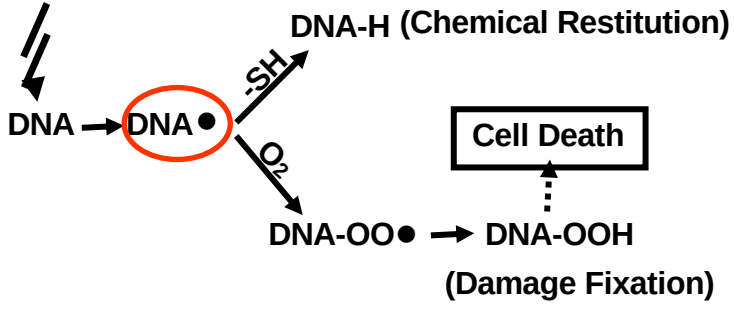
- Experiments have shown that O_2 needs to be present either *during* or *within a few microseconds* **after** exposure in order to act as a sensitizer
- This is said to imply that O_2 acts at the free radical levels



Recall the mechanism of indirect action

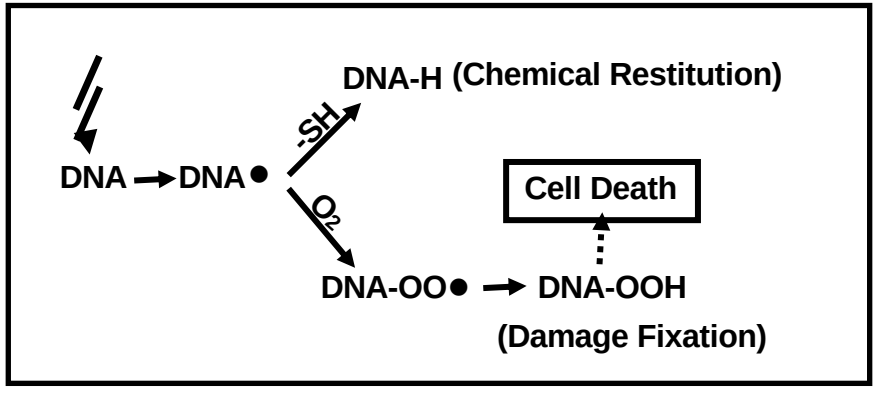
Radiation interacts first with other atoms or molecules in the cell (usually H_2O) to produce free radicals, which in turn diffuse and damage DNA

Mechanism of the Oxygen Effect



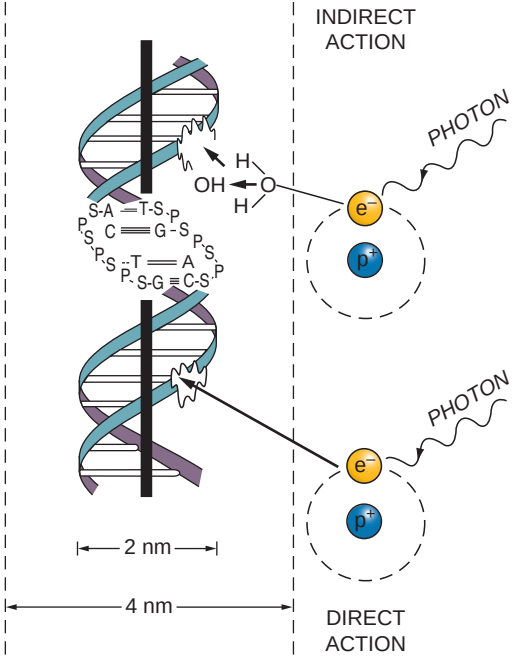
- Apparently, when O_2 is present, it reacts with a broken end (free radical) of a DNA strand, R●, to form the **peroxide RO_2●**
- This prevents chemical restitution of the DNA radical, and thus "fixes" the radiation lesion
- This is known as the **"oxygen fixation hypothesis"**

The Oxygen Fixation Hypothesis



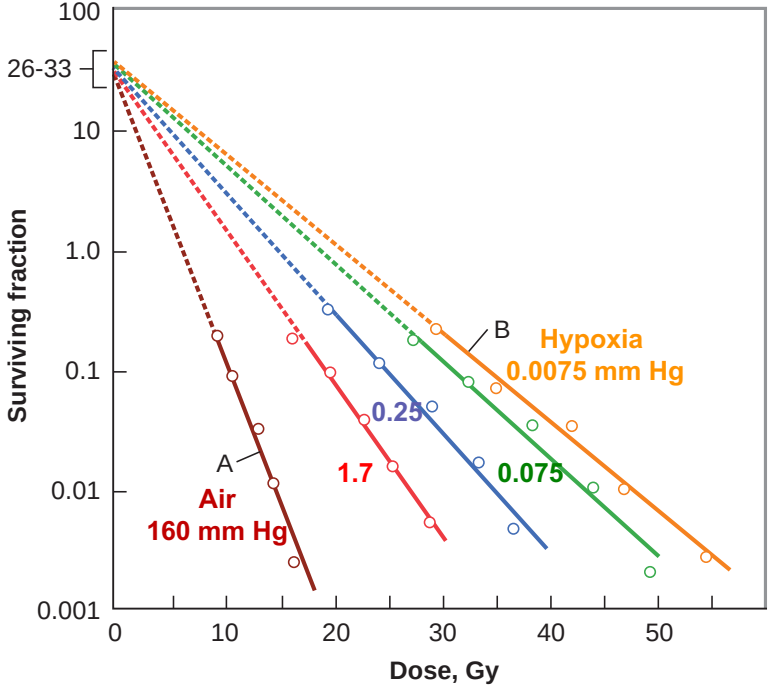
Free radicals have a $t_{1/2}$ of 10^{-5} sec, therefore, O_2 must be present during or shortly afterwards

For sparsely ionizing radiation, ~ 2/3 of the damage produced is mediated by which may be 'fixed' by oxygen



For densely ionizing radiation, direct action dominates, thus no appreciable oxygen effect

Concentration of O_2 Required



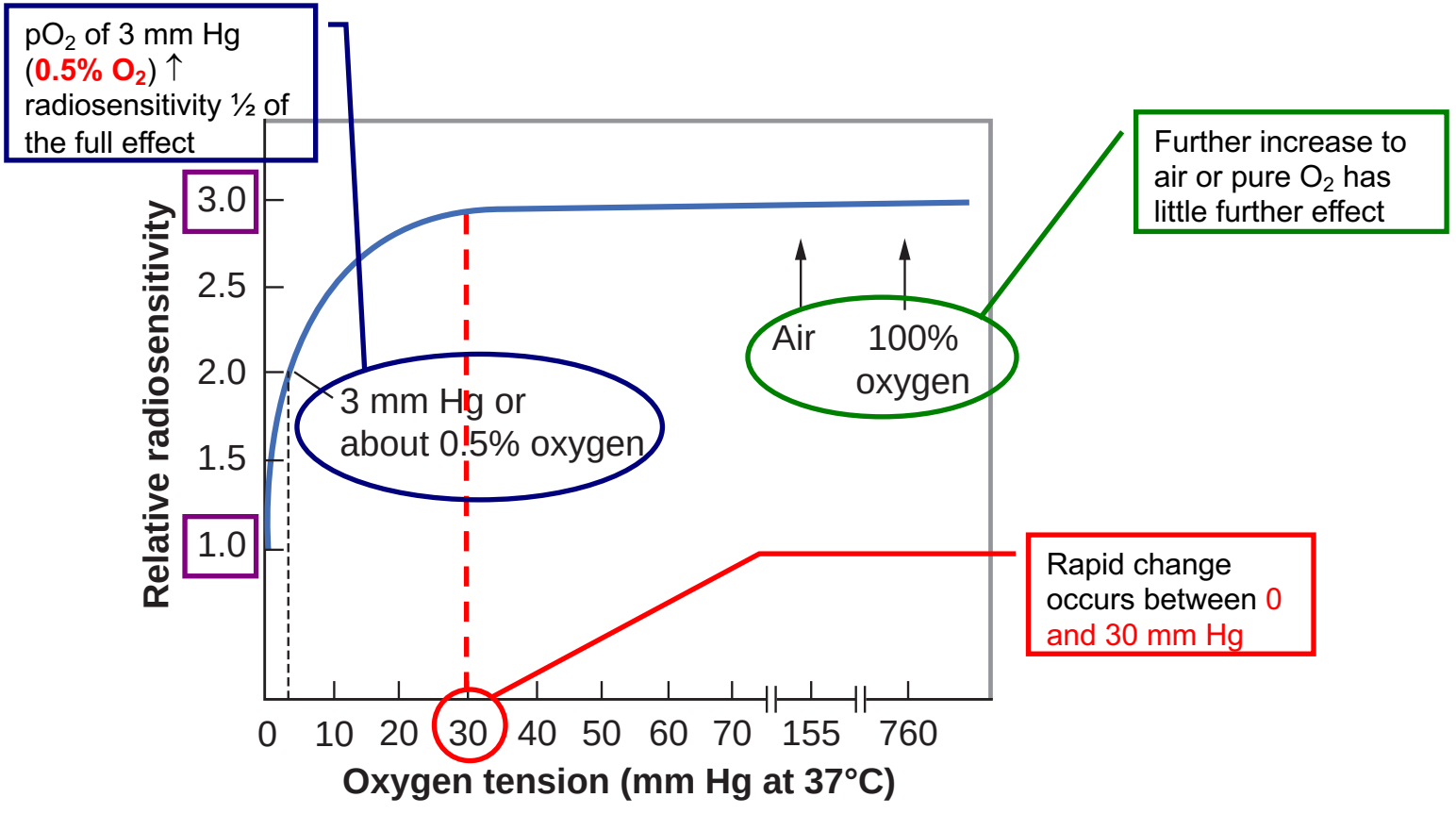
Survival curve of CHO cells exposed to X-rays at various concentrations of O_2

O_2 Concentration in dry air = 21%
O_2 tension pO_2 = 760 x 0.21 = 160 mm Hg (Torr)

Introduction of even a very small amount of O_2 leads to a dramatic increase in radiosensitivity

At pO_2 of 1.7 mm Hg (= 0.22%), the survival curve is ~ halfway toward the fully aerated condition

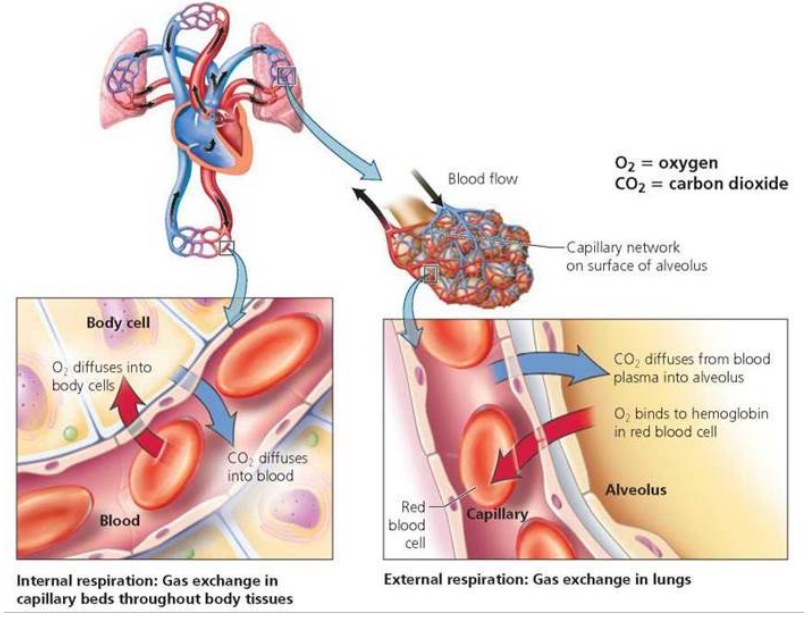
Dependence of Radiosensitivity on O_2 Concentration



Outline

- The Oxygen Effect
- **Chronic and Acute Hypoxia**
- Experimental Evidence
- Techniques to Measure Tumor Oxygenation
- Reoxygenation
- Hypoxia and Chemoresistance
- Hypoxia and Tumor Progression

What Happens in Tissue?

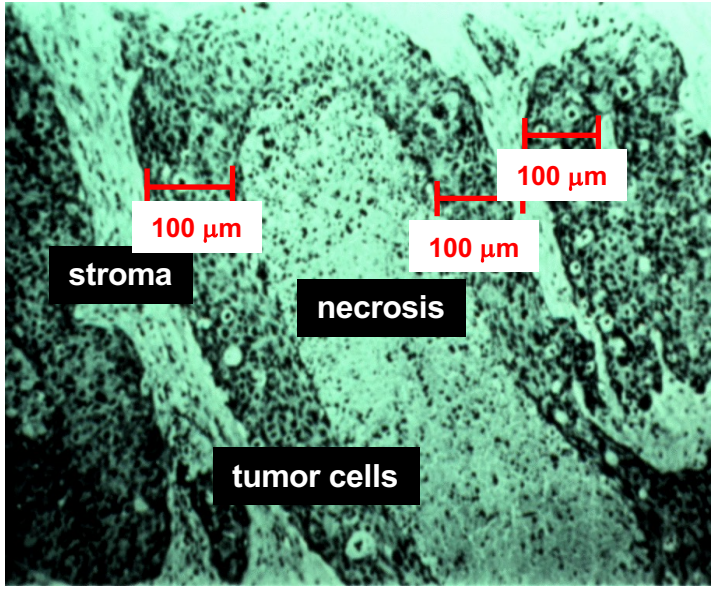


- It is usually assumed that the O_2 tension of most normal tissues is similar to that of venous blood (20-40 mm Hg)
- In reality, O_2 tensions may vary from 1 to 100 mm Hg
- Thus, many tissues are borderline hypoxic and contain a small proportion of cells that are radiologically hypoxic (e.g., liver, skeletal muscle)

Chronic vs. Acute Hypoxia

- Hypoxia in tumors can result from 2 different mechanisms
- **Chronic Hypoxia**
  - Due to limited diffusion distance of O_2 through tissue
  - Cells may remain hypoxic for extended periods
- **Acute Hypoxia**
  - Due to temporary closing of a blood vessel

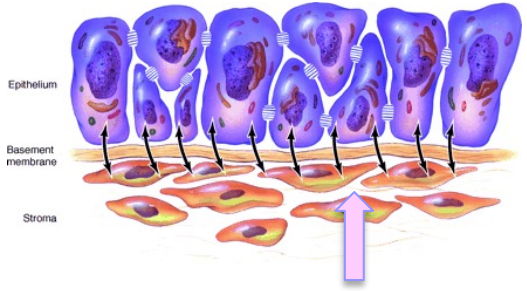
Chronic Hypoxia



Histologic sections of bronchial carcinoma

(Thomlinson & Gray 1955)

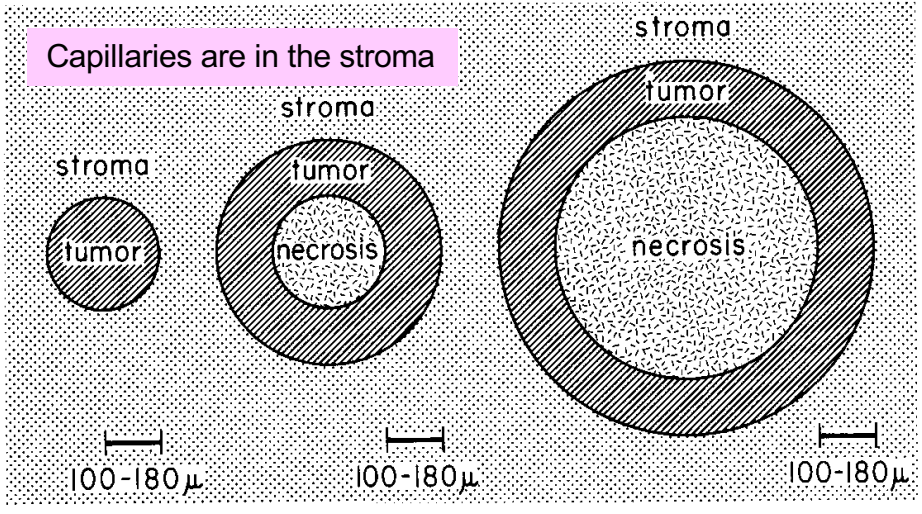
Epithelium



Capillaries are in the stroma

Observation – large areas of necrosis are separated from the stroma by a band of tumor cells of **100 μm** wide

Chronic Hypoxia

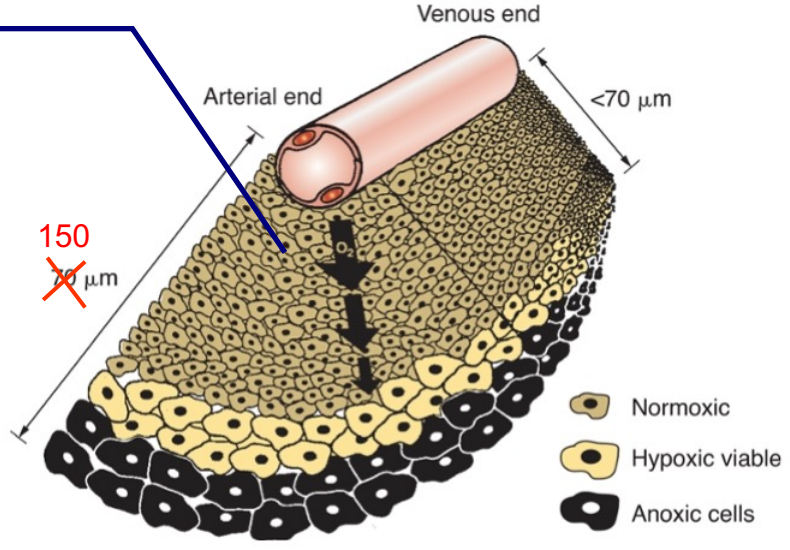


As tumors grows larger, the necrotic center also enlarges, so that the thickness of the sheath of viable tumor cells remains essentially constant at 100 – 180 μm

Conclusion – tumor cells could proliferate and grow actively only if they were close to a supply of oxygen or nutrients from the vessel

O_2 Diffusion Distance

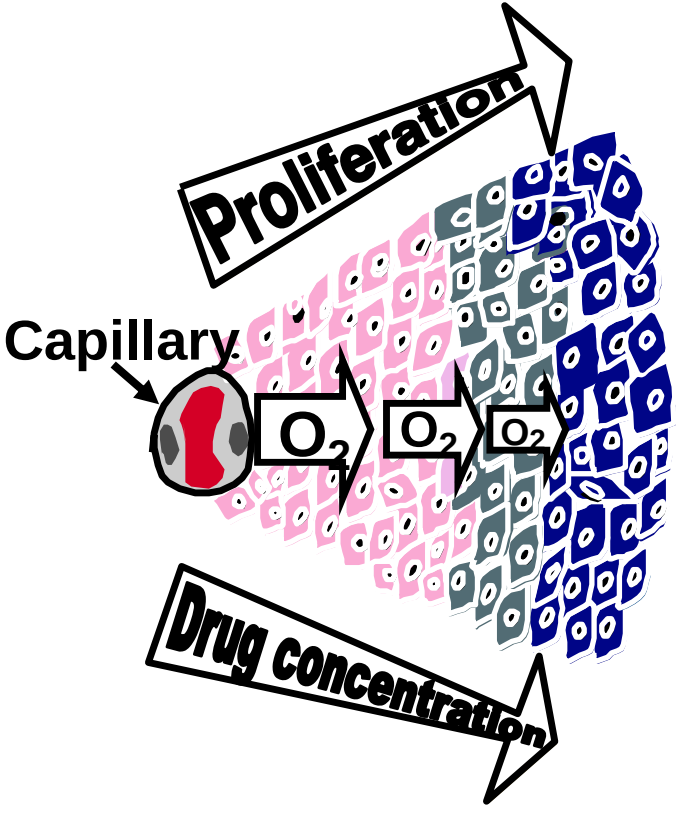
As O_2 diffuses through the tissue, it is rapidly metabolized by respiring tumor cells → O_2 concentration decreases steadily over a distance

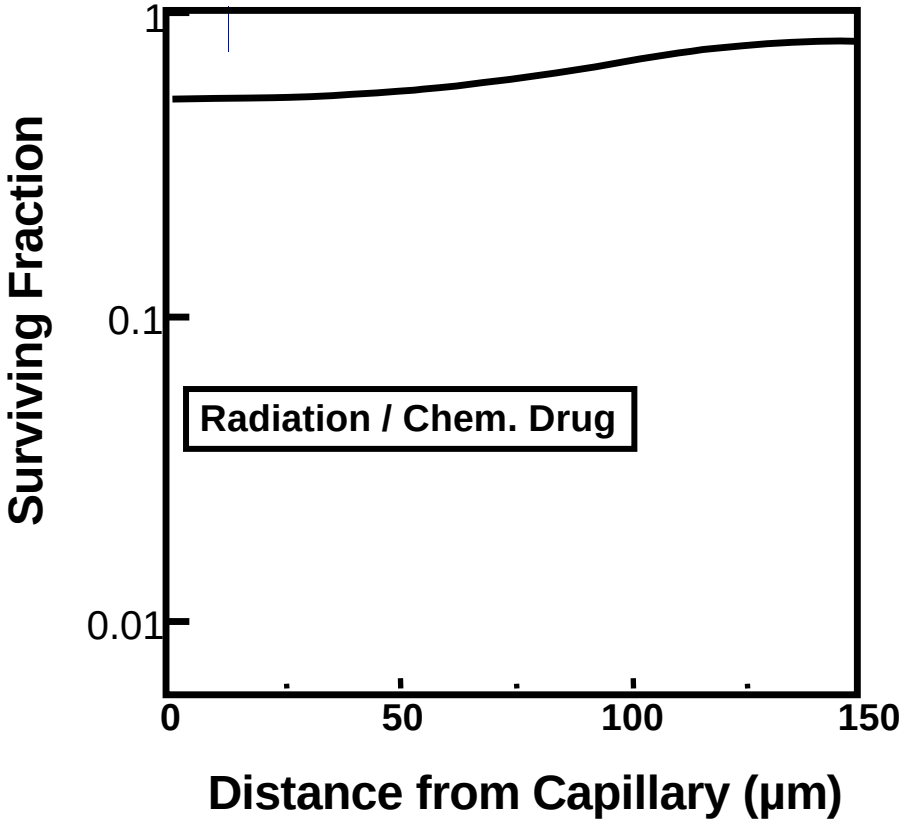


Using more appropriate values of O_2 diffusion coefficients and consumption values, a better estimate of the distance of O_2 diffusion in respiring tissue ranges from **70 μm** to **200 μm**

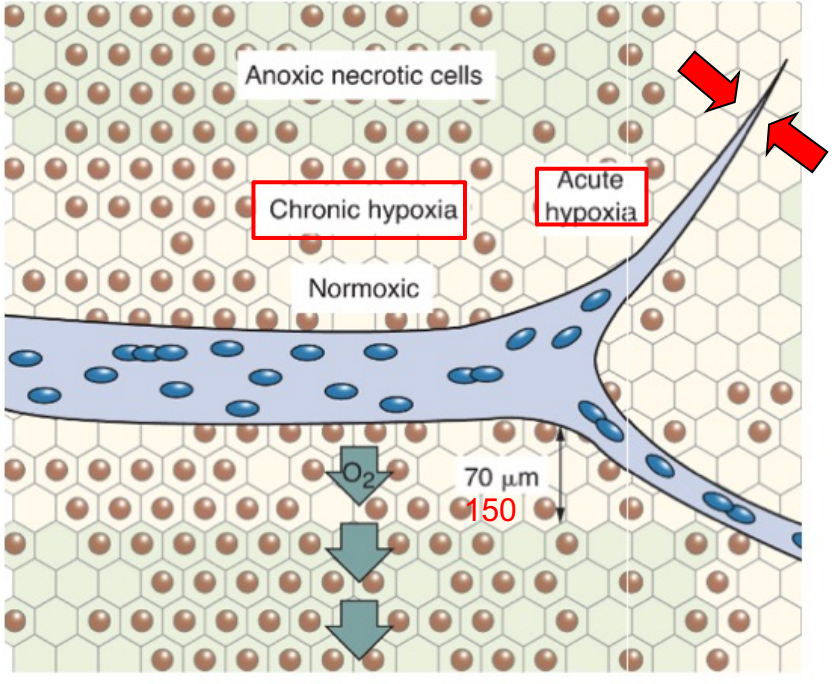
Hypoxic cells are radioresistant but fully viable and clonogenic

Clinical Implications



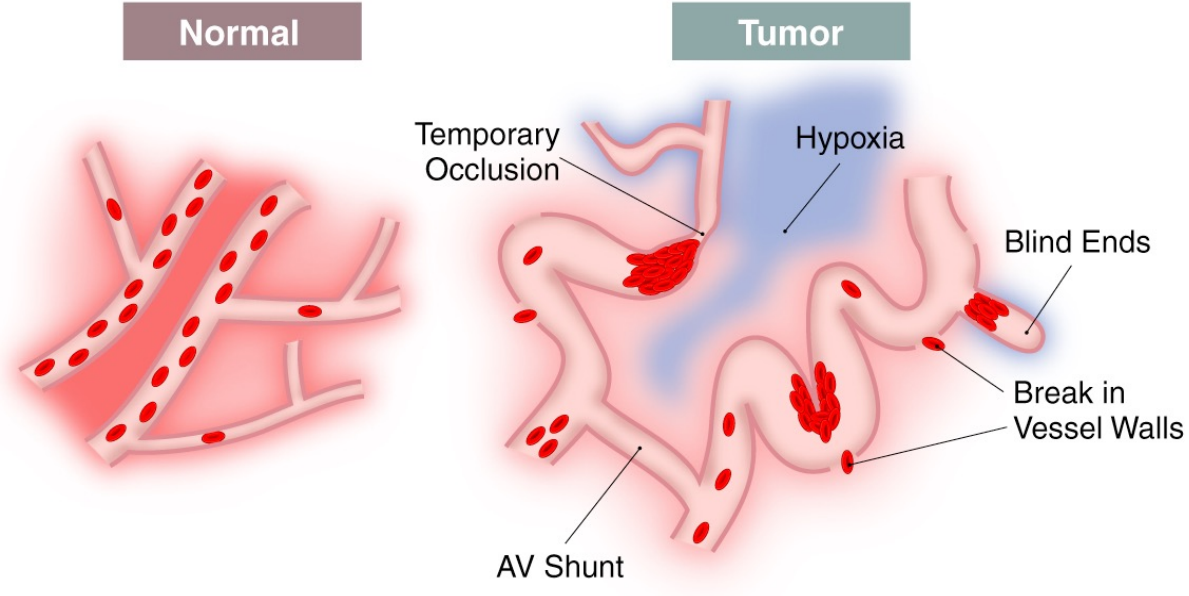


Acute Hypoxia



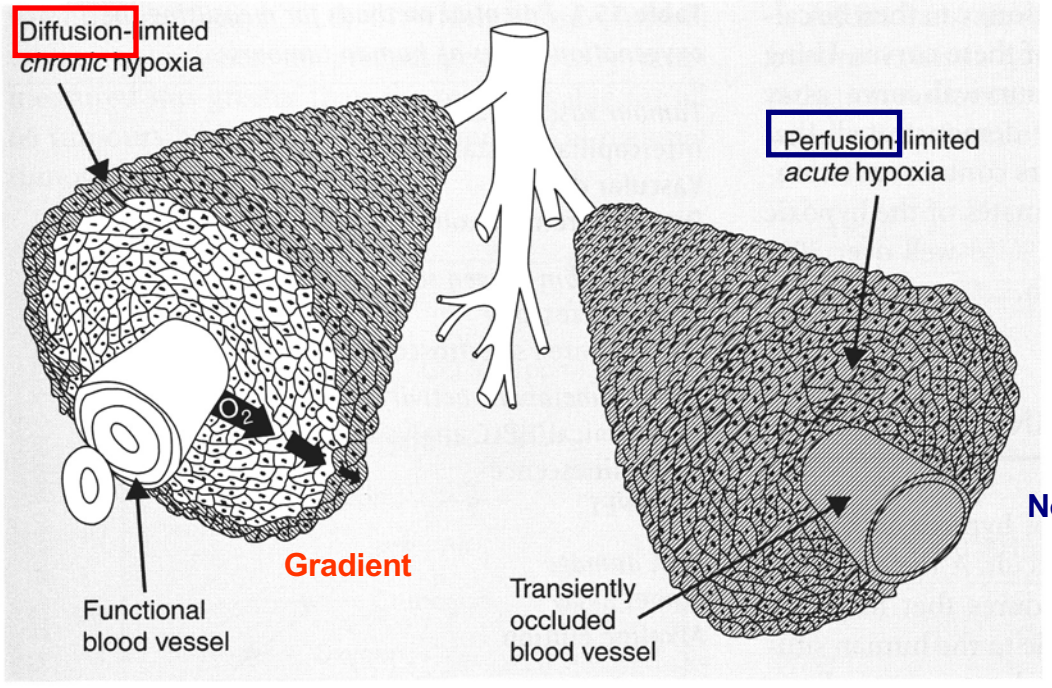
- Tumor vessels are malformed
- These vessels open and close, resulting in transient fluctuation of blood flow, hence O_2 concentration
- Different cells may become hypoxic **intermittently** during the course of the treatment
- Acutely hypoxic cells are more likely to be reoxygenated than chronically hypoxic cells

Difference in Vasculature in Normal vs. Malignant Tissues



Vasculature of the tumor lacks smooth muscle and often has an incomplete endothelial lining and basement membrane

Chronic vs. Acute Hypoxia



Hypoxic cells less likely to become re-oxygenated

Hypoxic cells more likely to become re-oxygenated

Outline

- The Oxygen Effect
- Chronic and Acute Hypoxia
- **Experimental Demonstration of Hypoxia in Tumor**
- Techniques to Measure Tumor Oxygenation
- Reoxygenation
- Hypoxia and Chemoresistance
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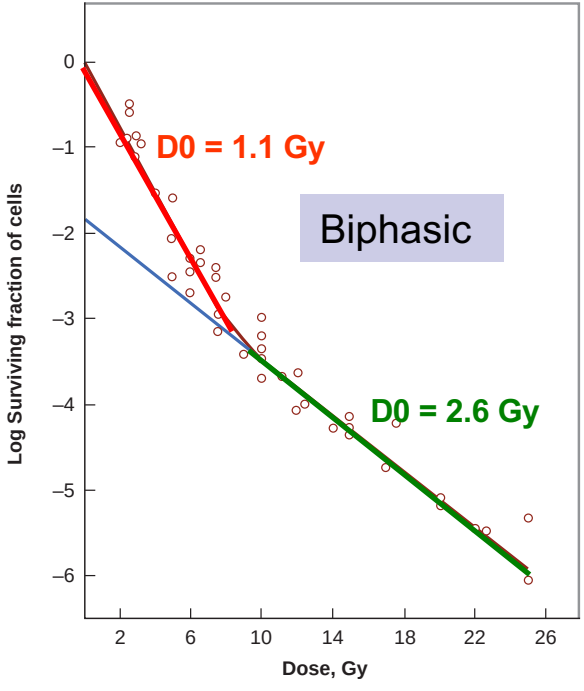
First Demonstration of Hypoxic Cells in a Tumor



Subcutaneous lymphosarcoma

Powers & Tolmach 1963

in vivo irradiation

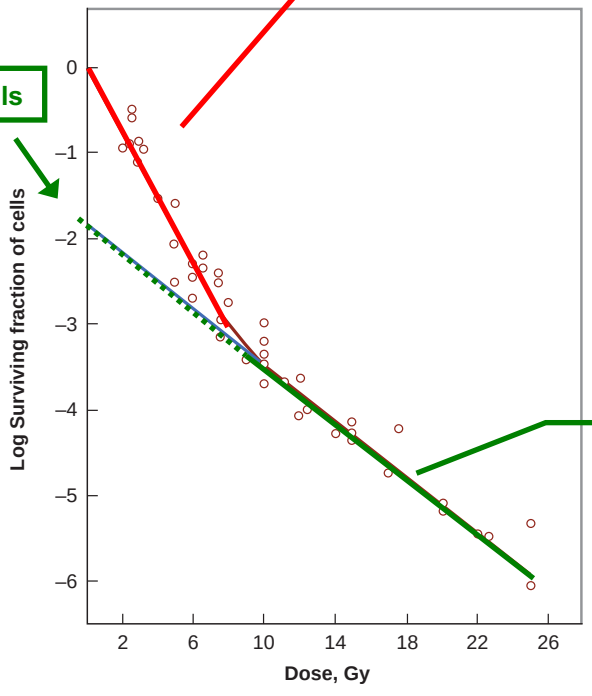
Recall that OER for X-ray is 2.5

⇩

Tumor consists of 2 separate group of cells, one **oxygenated,** and the other **hypoxic**

This is the first unequivocal demonstration that a solid tumor could contain cells sufficiently hypoxic to be protected from cell killing by x-rays but still clonogenic

Biphasic Survival Curves



Lower dose – dominated by killing of well-oxygenated cells

99% well oxygenated cells
1% hypoxic cells

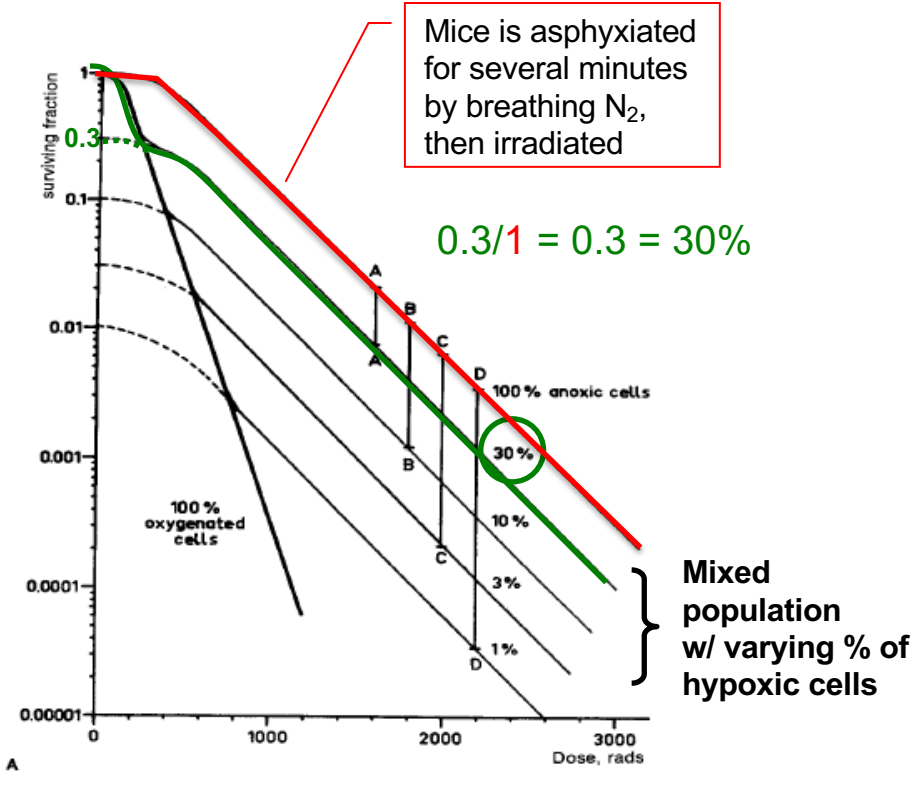
High dose – response characteristic of hypoxic cells

Extrapolation of high dose region to the cell survival axis gives the fraction of cells that were hypoxic at the time of irradiation (though this is not straightforward due to curvature of cell survival curves)

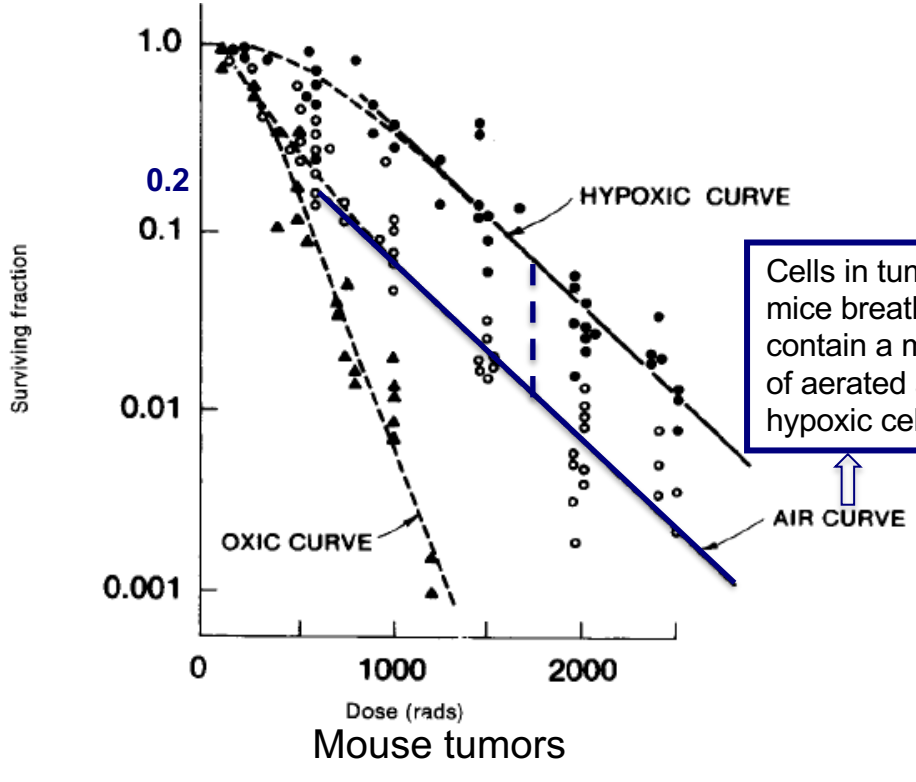
Practical Measurement of the Hypoxic Fraction – Paired Survival Curves

Paired Curves – At high doses, the number of surviving oxgenated cells is negligible compare with the number of anoxic cells → the curves for the mixed population is parallel to the curve for the hypoxic population

The hypoxic fraction is determined by the vertical separation of the cell survival curves, i.e., the ratio of survival of the completely and partially hypoxic populations



Practical Measurement of the Hypoxic Fraction – Experimental Data



Mouse tumors

The vertical shift b/w the hypoxic and air lines on the surviving fraction axis is from 1.0 down to ~ 0.2

Cells in tumor of mice breathing air contain a mixture of aerated and hypoxic cells

The hypoxic fraction is **~ 20%**

Obviously, such experiments cannot be done with human tumors!!!

Summary of Experimental Evidence on Animal Tumors

- A survey of all published studies on hypoxic fractions of transplantable tumors in animals
- Of the 42 tumor types studied, 37 were found to contain hypoxic cells in at least one study
- Hypoxic fractions range from 0% to 50%, with a tendency for many results to average about **15%**

Moulder & Rockwell

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- **Techniques to Measure Tumor Oxygenation**
- Reoxygenation
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Evidence for Hypoxic Cells in Human Tumors

- Oxygen probe measurements correlate with local control
- Pretreatment Hgb levels correlate with local control
- Binding of radioactively-labeled nitroimidazoles (hypoxic-cell sensitizers) occurs
- Histological appearance suggests hypoxia
- Analogy with animal experiments

Hypoxia is a common feature of human solid tumors

Malignant Progression

Response to Therapy

Techniques to Measure Hypoxia in Human Tumors

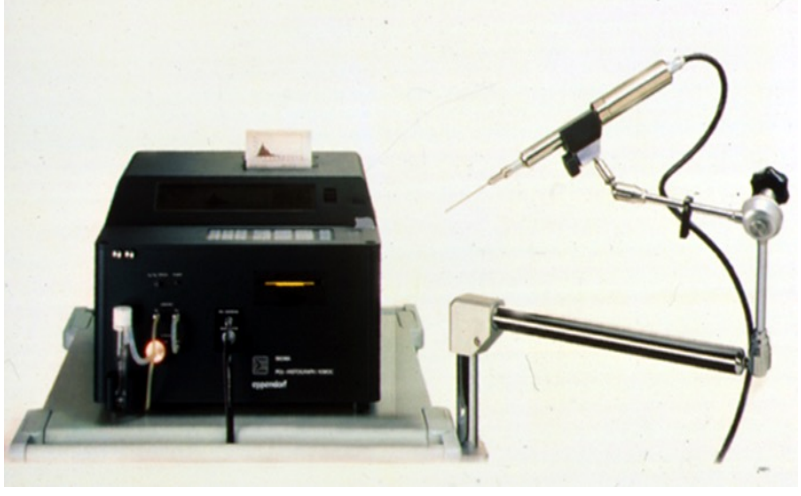
Techniques

- O_2 Probe Measurement
- Exogenous Hypoxia Markers
  - Nitroimidazole
  - EF5
- Endogenous Markers
  - Hypoxia-inducible Factor (HIF-1α)
  - Carbonic anhydrase IX (CA9)
- Comet Assay
- Noninvasive Hypoxia Imaging

Hypoxia is a common feature of human tumors

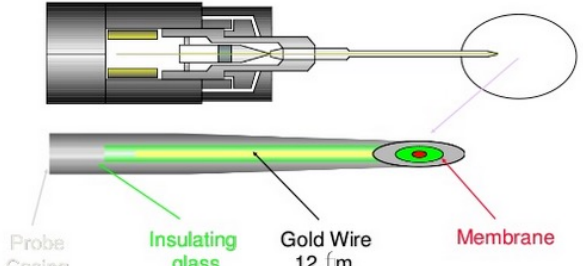
Hypoxia influences *tumor aggressiveness* and the *response to therapy*

Oxygen Probe Measurements



Electrodes implanted directed into tumors

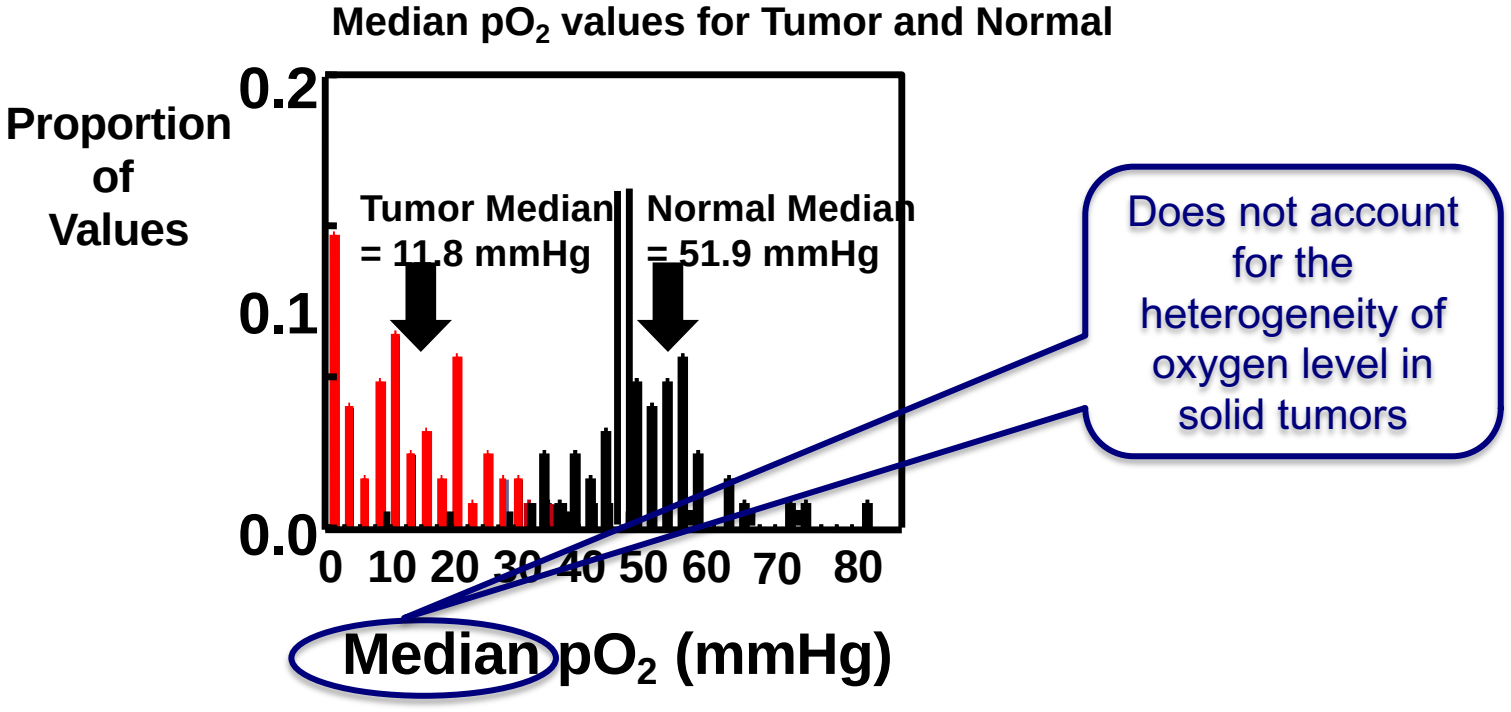
O_2 concentration measured by polarographic technique – measured current is proportional to local O_2 tension



Eppendorf Probe

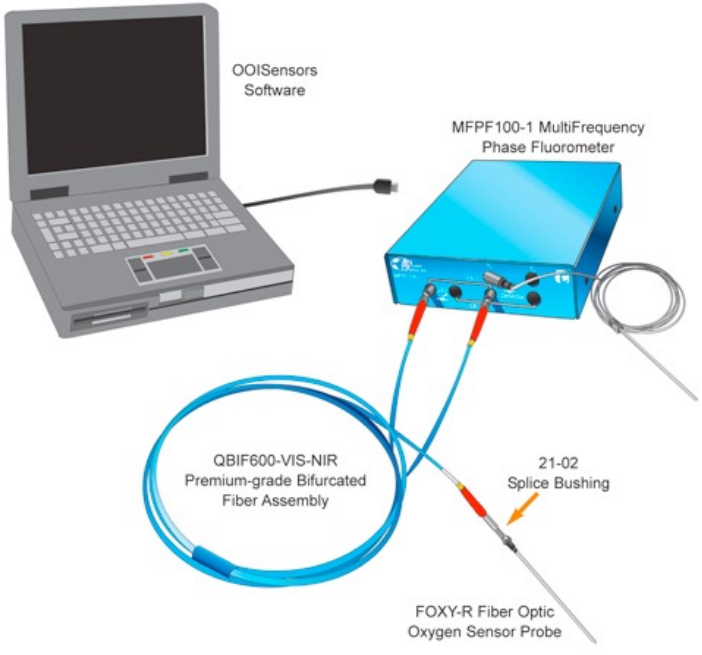
Eppendorf probe has a very fast response time and can be moved quickly through a tumor to obtain large # of O_2 measurements along multiple tracks

Median pO_2 Values in H&N Cancer Patients



Le et al,2005

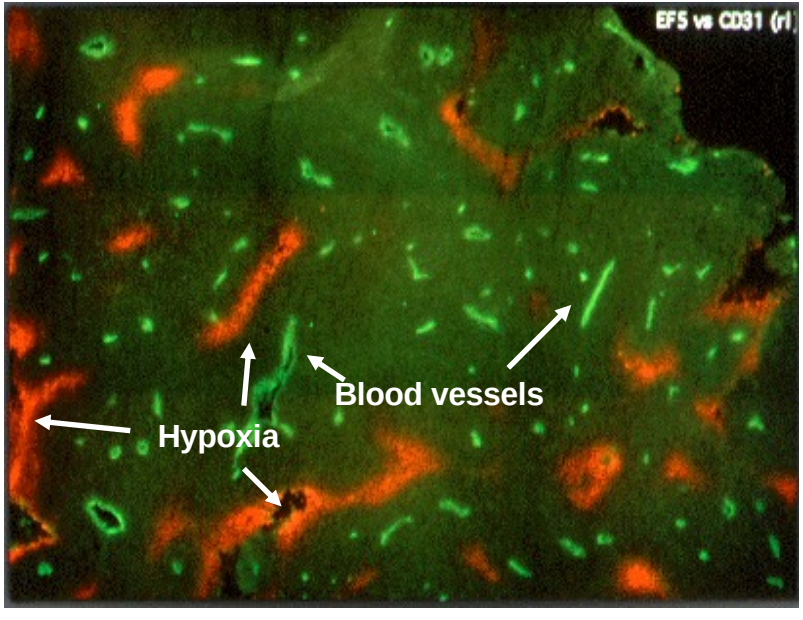
Fiber-Optic Oxygen Sensor

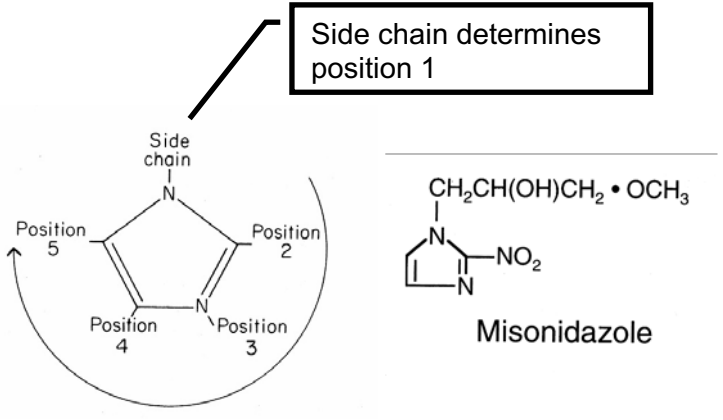


Oxygen is sensed by measuring the decrease in fluorescence intensity of a fluorophore bound to the tip of an optical fiber.

The sensor responds to the partial pressure of oxygen in gases, liquids and even viscous samples.

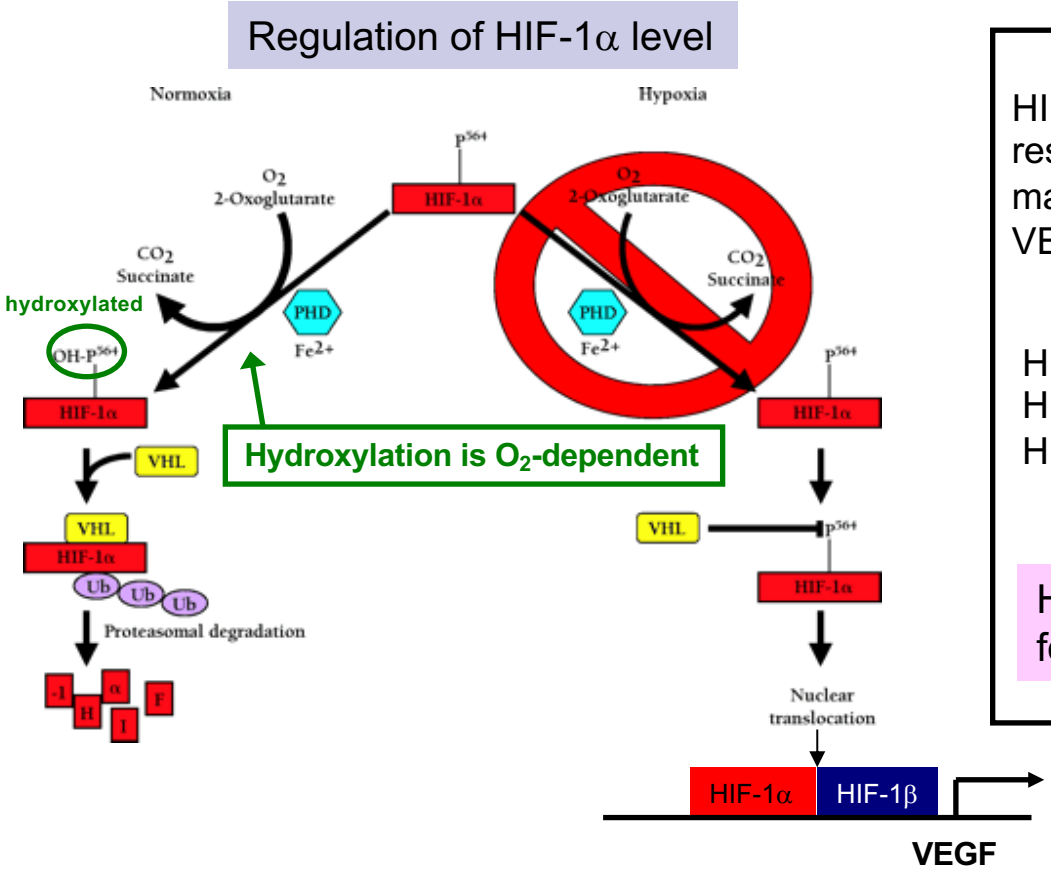
2-Nitroimidazole Hypoxia Markers





2-nitroimidazoles (e.g., pimonidazole, EF5) are only metabolized to form adducts under hypoxic condition, therefore can be used as a hypoxia marker

Endogenous Hypoxia Markers – Hypoxia-Inducible Factor 1 α (HIF-1α)

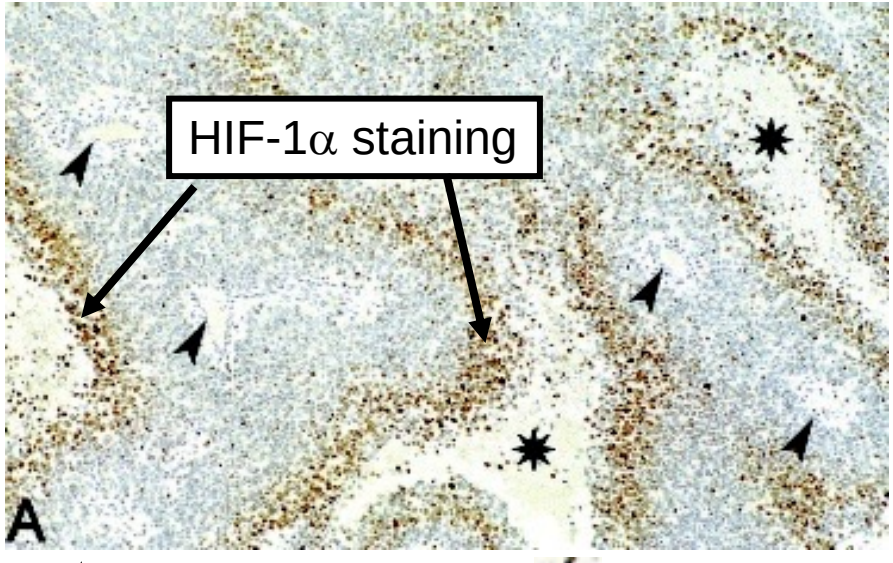


HIF-1 is a transcription factor responsible for the expression of many hypoxia-inducible genes (e.g. VEGF)

HIF-1 has 2 components
HIF-1α – level tightly regulated
HIF-1β – constitutively expressed

HIF-1α is a cellular marker for hypoxia

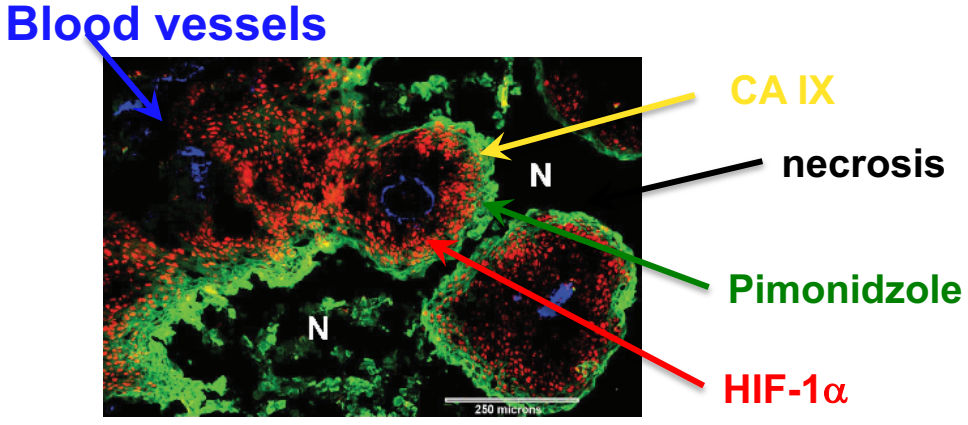
HIF1-α Staining in H&N Cancer



Expression of endogenous marker, however, can be regulated by factors other than oxygen, thereby complicating their use in quantifying tumor hypoxia

HIF1-α & Pimonidazole Staining

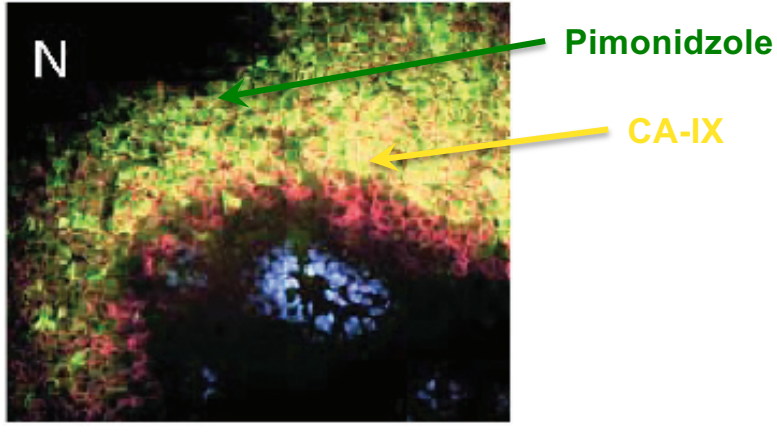
Pts with cervical cancer were given pimonidazole IV → cervical bx → frozen section were analyzed for distribution of pimonidazole (green), HIF-1α (red), carbonic anhydrase IX (yellow)



Expression of HIF-1α was reduced in the most hypoxic regions that border necrosis

Sobhanifar 2005; Cancer Res 65: (16) 7259

CA IX & Pimonidazole Staining

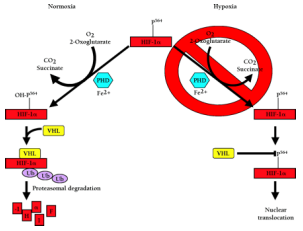


However, CA-IX was present in these perinecrotic cells, co-localizing with pimonidazole

Nutrient deprivation seems to be largely responsible for the lack of HIF-1α expression in perinecrotic regions (HIF-1α was degraded)
The half-life of CA-IX was sufficiently long

These results have implications for the use of HIF-1α as an indicator of tumor hypoxia and aggressiveness as well as development of hypoxia-directed antitumor therapies based on the expression of HIF-1α

HIF1-α as a Hypoxia Biomarker



- The reduction in O_2 concentration required to stabilize and activate HIF is much less than that necessary to induce radioresistance
- Consequently, the fraction of cells expressing HIF or HIF-dependent genes in a tumor can be significantly greater than the fraction of radiation-resistant cells
- This is an important consideration in clinical studies investigating "endogenous" hypoxia markers

Using HIF as an indicator of radioresistance can be MISLEADING !

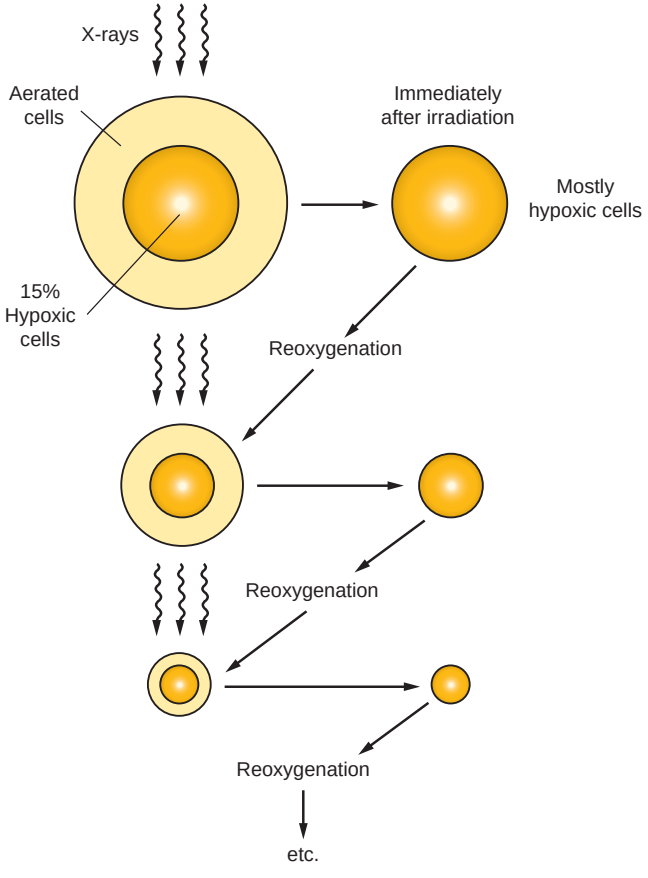
Outline

- The Oxygen Effect
- Chronic and Acute Hypoxia
- Experimental Evidence
- Techniques to Measure Tumor Oxygenation
- **Reoxygenation**
- Hypoxia and Chemoresistance
- Hypoxia and Tumor Progression

Early Evidence

- Van Putten and Kallman (1968) studied the proportion of hypoxic cells in mouse sarcomas at various times during fractionated radiotherapy
- They found that the proportion of hypoxic cells remained constant even though it would be expected that it would increase sharply as well-oxygenated cells would be killed by the first few fractions
- They interpreted this as re-oxygenation

The Process of Re-oxygenation

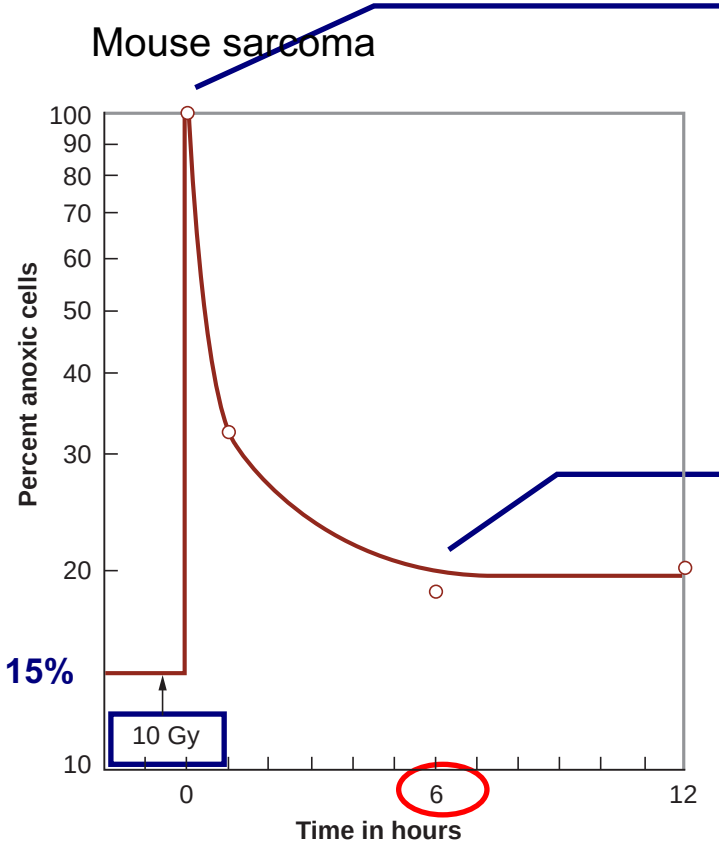


Reoxygenation is the process by which cells that are hypoxic at the time of irradiation become oxygenated afterward

Clinical Implication

The presence of hypoxic cells does not greatly influence the response of the tumor as long as there is sufficient time for re-oxygenation between fractions

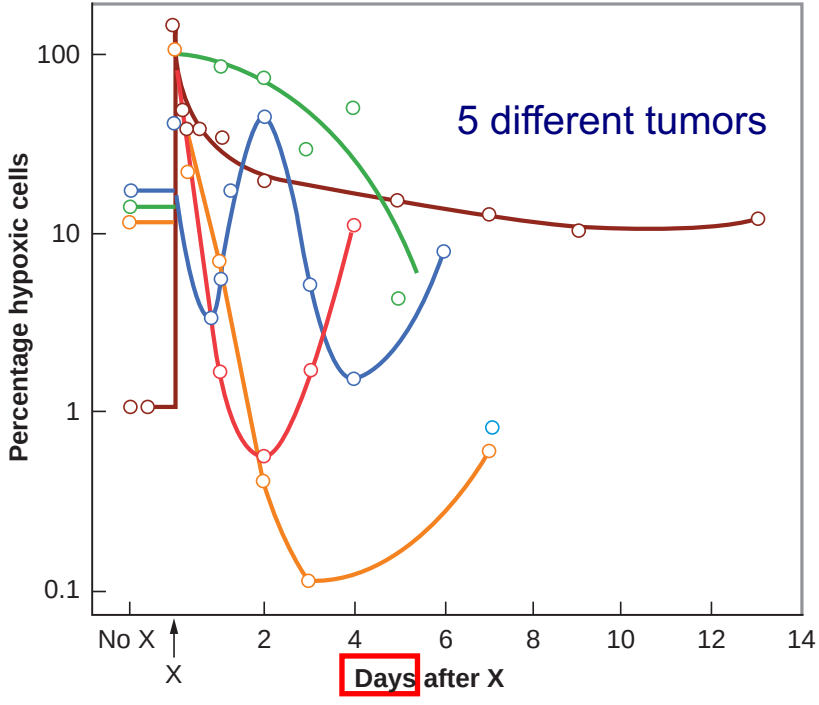
Time Sequence of Re-oxygenation



Immediately after radiation, almost all viable cells are hypoxic, because oxygenated cells are preferentially killed

By 6 hours, the proportion of cells that are hypoxic has been restored to close to pre-irradiation level

Reoxygenation Variability



Proportion of hypoxic cells as a function of time

- Some cells reoxygenate readily
- Some hardly reoxygenate at all
- Others reoxygenate well but very slowly

The differences of time scale reflect the different types of hypoxia being reversed

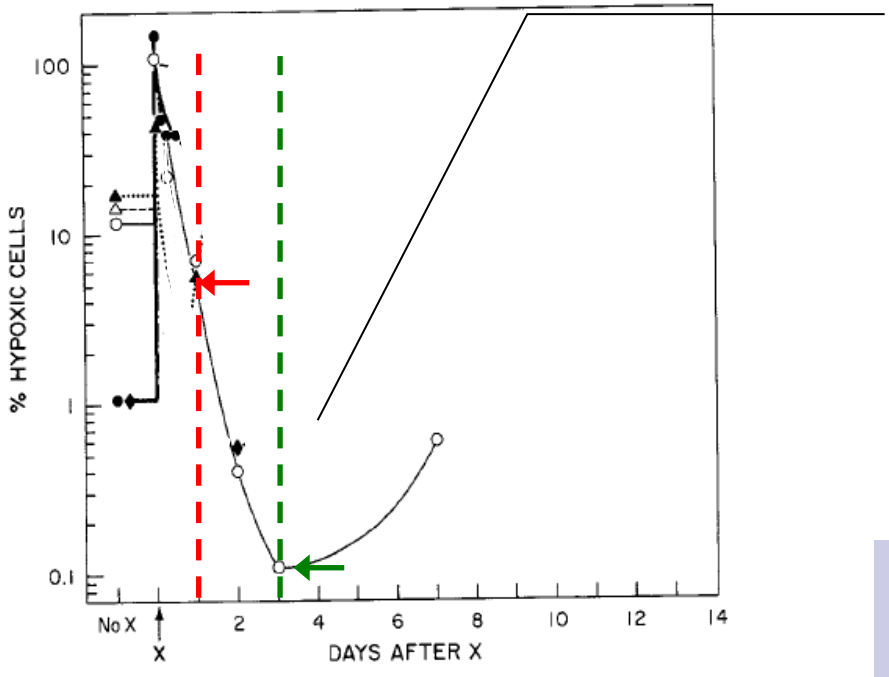
Mechanism of Reoxygenation

	Time frame	**Mechanism**	
"Fast Component"	Hours	Reoxygenation of ***acutely hypoxic*** cells	Blood vessel reopening
"Slow Component"	Days	Re-oxygenation of ***chronically hypoxic*** cells	Re-structuring – as well oxygenated cells are killed, cells previously beyond the range of O_2 diffusion find themselves closer to blood vessels Revascularization

Clinical Implications

- If the reoxygenation is efficient between dose fractions, the presence of hypoxic cells does not have a significant impact on the outcome of a multifraction regimen
- The fact that 60 Gy given in 30 fractions eradicate many tumors argues strongly that reoxygenation does occur in human humors
- Conversely, some tumors that do not respond to conventional radiation therapy may lack a timely and efficient reoxygenation process

Individualized Fractionation



Mouse Mammary Sarcoma

Based on the kinetics of reoxygenation, an ideal regimen would be several large doses given at 2-3 days interval

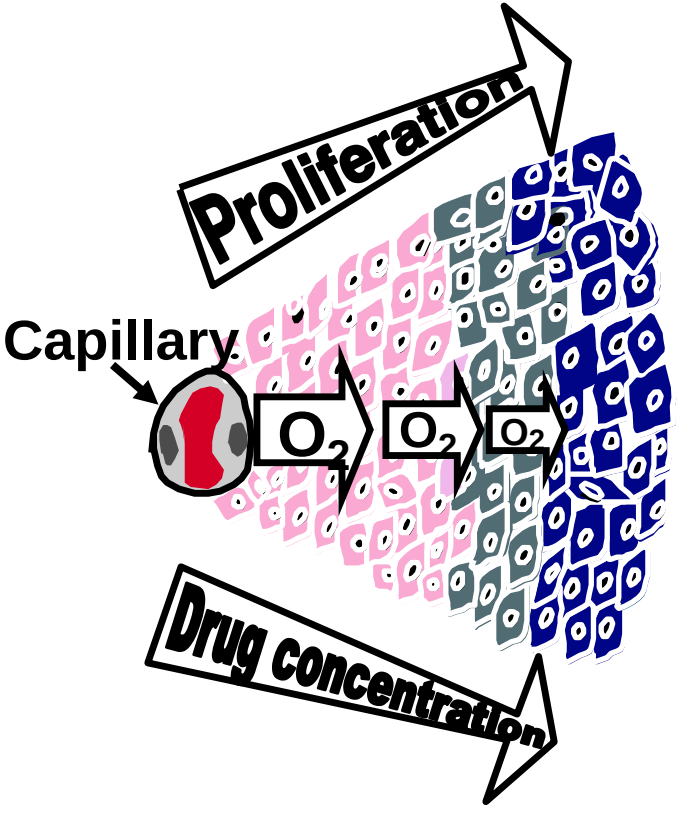
Indeed, Fowler showed that the preferred schedule for the eradication of this tumor would be 5 large doses in 9 days

In reality, it is impossible and impractical to obtain the time course of reoxygenation for individual human tumors

Outline

- The Oxygen Effect
- Chronic and Acute Hypoxia
- Experimental Evidence
- Techniques to Measure Tumor Oxygenation
- Reoxygenation
- **Hypoxia and Chemoresistance**
- Hypoxia and Tumor Progression
- Summary

Hypoxia and Chemoresistance



- Decreased proliferation (e.g., 5-FU, methotrexate)
- Decreased free-radical generation (e.g. bleomycin, doxorubicin)
- Low pH

More on this in Chapter 27

Outline

- The Oxygen Effect
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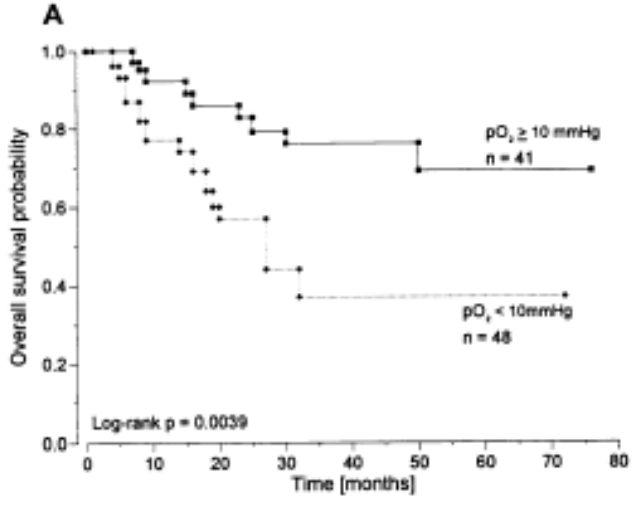
- **Hypoxia and Tumor Progression**

Hypoxia and Tumor Aggressiveness

- Hypoxia can limit radiocurability
- Evidence suggests that hypoxia itself, is a source of malignant progression
- This is separate from its role in reducing radiosensitivity

Cervical Cancer – Degree of Hypoxia Correlates with Survival



103 patients with locally advanced cervical cancer

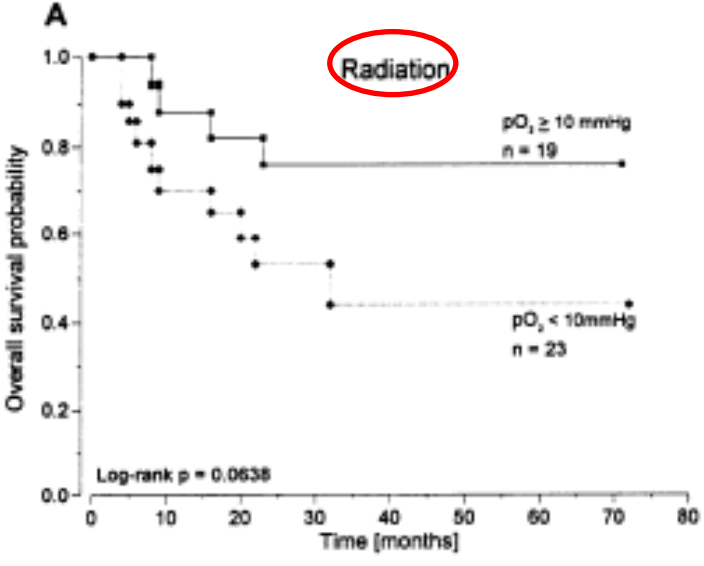
Tumor Oxygenation was measured with polarographic oxygen probe

50% of pts were found to have tumors with $pO_2 < 10$ mm Hgb

Observation – pts with hypoxic tumors have significantly worse survival

Hoeckel et al. 1996

Cervical Cancer – Treated with Radiation



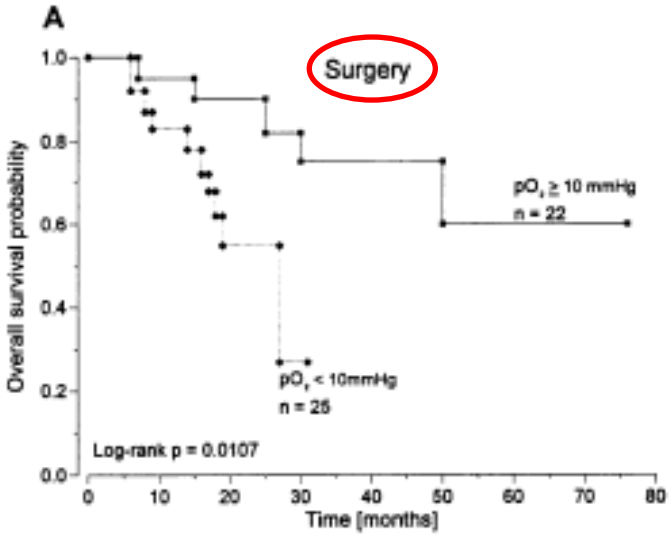
42 pts were treated with primary radiation therapy

Observation – pts with hypoxic tumors had significantly worse outcome

Interpretation – hypoxia conferred radioresistance, therefore, less chance of tumor eradication

Cervical Cancer – Treated with Surgery

47 pts were treated with primary surgery

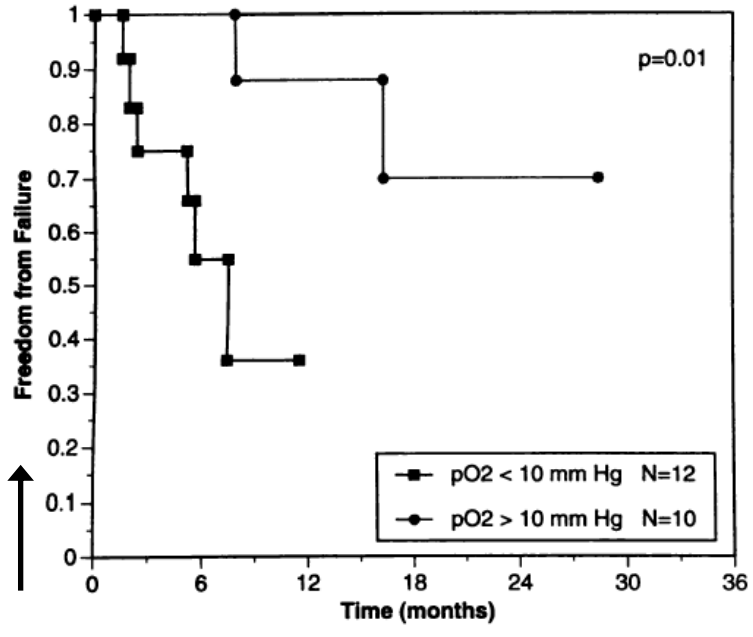


Observation 1 – hypoxic tumors had *larger tumor extensions*, *more frequent parametrial spread*, and *lymph-vascular space involvement* compared to the well-oxygenated tumors

Observation 2 – pts with hypoxic tumors had worse outcomes

Interpretation – **hypoxia is a general indicator of tumor aggressiveness in these patients**

Soft Tissue Sarcoma – Hypoxia Correlates with Distant Spread



7/8 failures are distant metastases

Brizel 1996

22 pts with high grade **soft tissue sarcoma**; pO_2 was measured prior to treatment

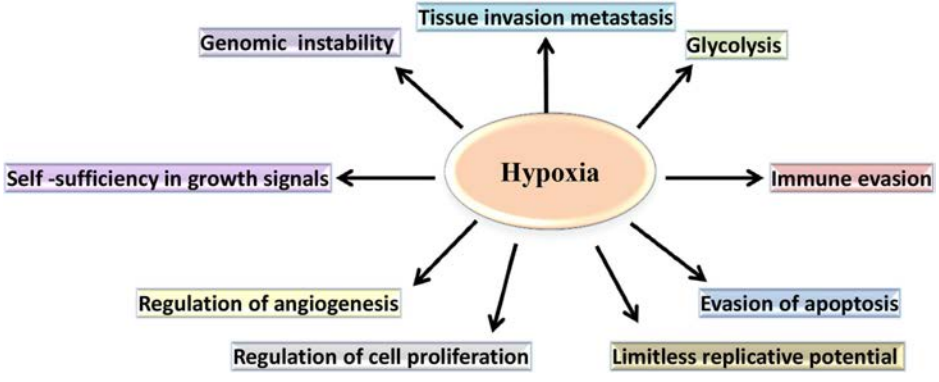
Observation 1 – pts with hypoxic tumors are more likely to fail the treatment

Observation 2 – Median pO_2 for those with distant recurrence was 7.5 mm Hg vs. 20 mm Hg for those without

Interpretation – hypoxic tumors have an **increased risk of metastatic spread**

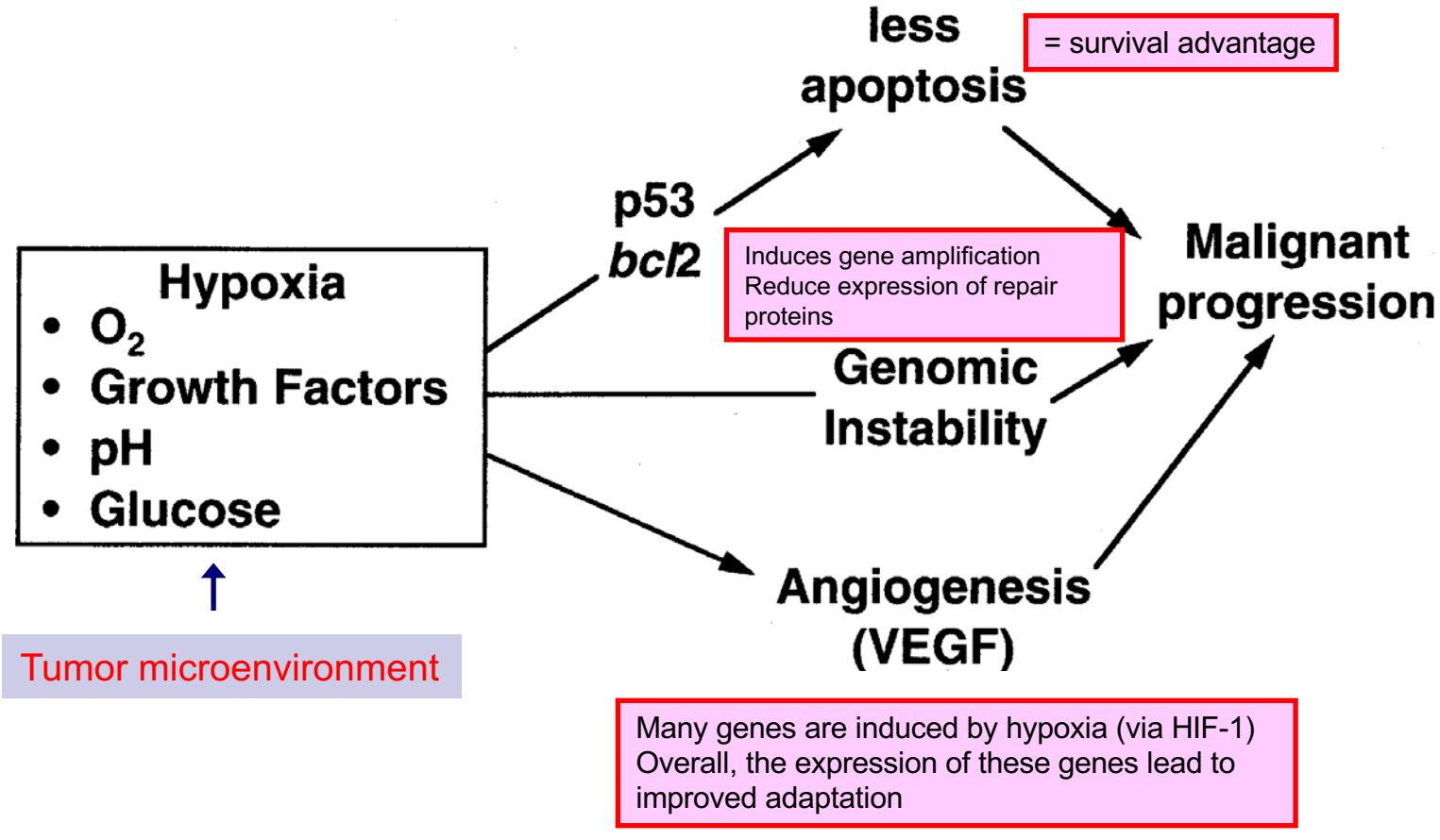
Hypoxia-Driven Biological Adaptation

- During evolution, organisms have developed a number of different pathways whose function is to allow adaptation to low oxygen availability
- Cancer cells utilize these same physiological response pathways to support the growth and spread of tumors



Front. Cell Dev. Biol., 29 January 2019

Tumor Microenvironment and Tumor Progression



Review Questions

Question 1

What is the approximate maximum diffusion distance of oxygen from a normally-oxygenated capillary through a typical respiring tissue?

A. 5 nm

B. 15 μm

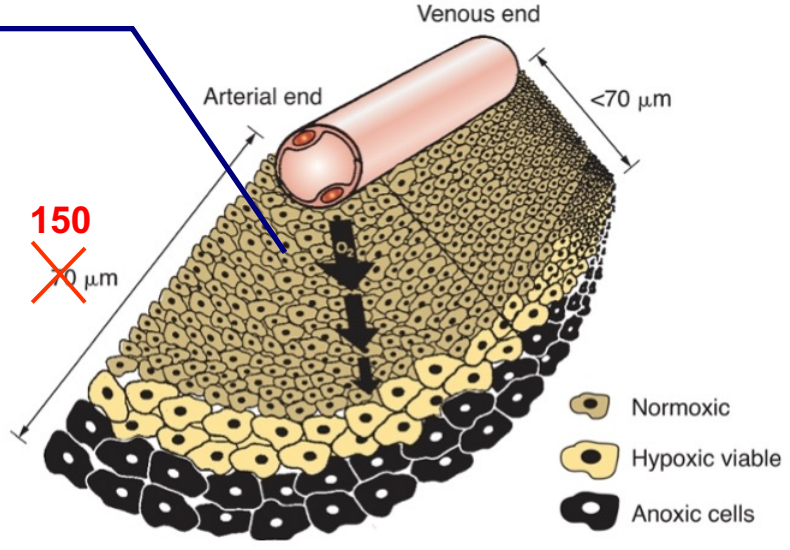
C. 200 μm

D. 900 μm

E. 2.6 mm

O_2 Diffusion Distance

As O_2 diffuses through the tissue, it is rapidly metabolized by respiring tumor cells → O_2 concentration decreases steadily over a distance



Using more appropriate values of O_2 diffusion coefficients and consumption values, a better estimate of the distance of O_2 diffusion in respiring tissue ranges from **70 μm** to **200 μm**

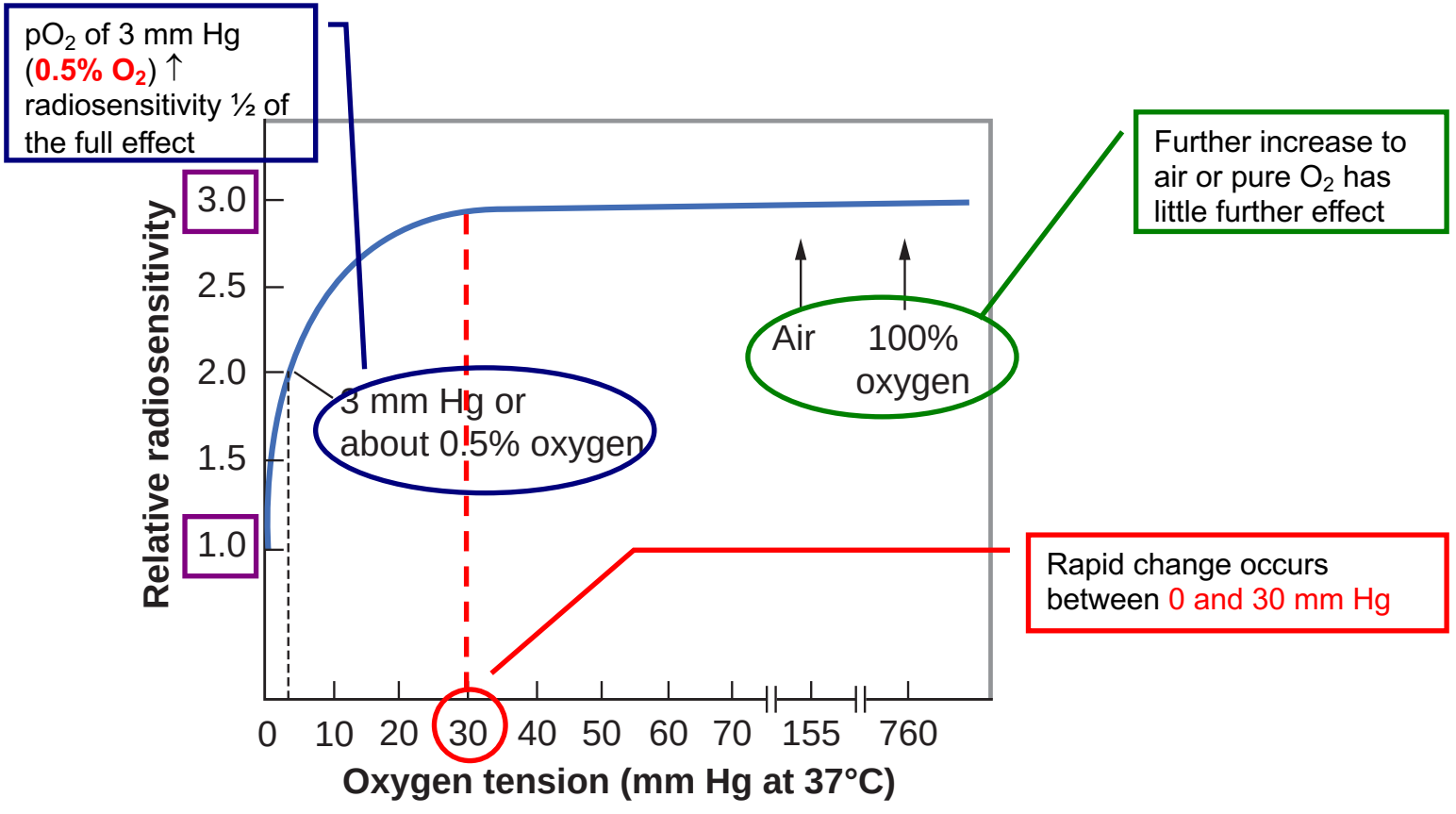
Hypoxic cells are radioresistant but fully viable and clonogenic

Question 2

The most dramatic change in radiation sensitivity occurs over which of the following ranges of oxygen tension (in units of mm Hg or Torr)?

A. 0-30
B. 30-60
C. 60-100
D. 100-250
E. 250-500

Dependence of Radiosensitivity on O_2 Concentration



Question 3

Oxygen enhancement ratio (OER) changes depending on the type of radiation. Which of the following combination is FALSE?

A. OER 3.0 for x-rays

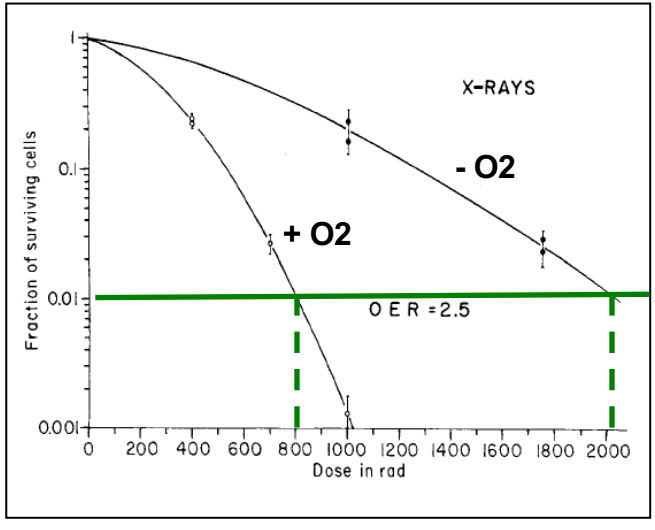
B. OER 1.6 for neutron

C. OER 3.0 for proton

D. OER 0.5 for energized ions

E. OER 1.0 for alpha-particle

Oxygen Enhancement Ratio (OER)



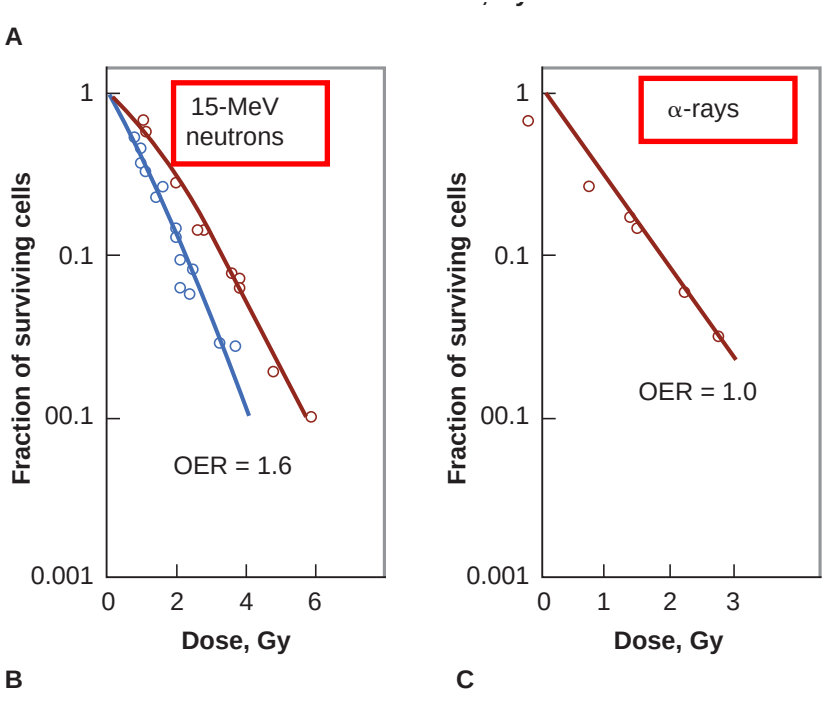
$$OER = \frac{\text{dose under hypoxic condition}}{\text{dose under aerobic condition}}$$

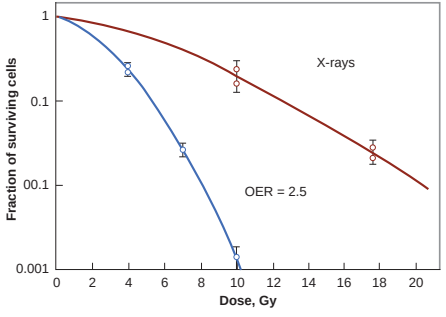
to produce the **same biological effect**

SF = 0.01
OER = 2000/800 = 2.5

The degree of sensitization is expressed in terms of **Oxygen Enhancement Ratio (OER)**

OER as a Function of Radiation Quality





OER ↓ as LET ↑

OER can never fall below 1.0

Summary

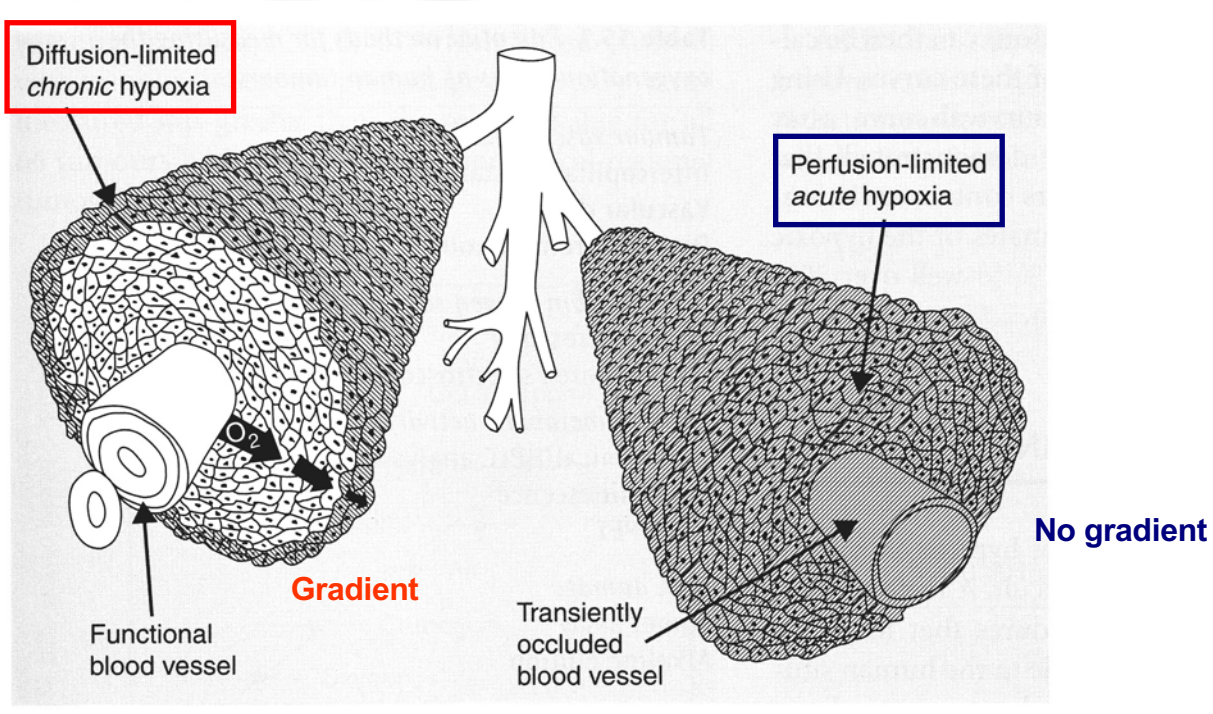
The oxygen effect is large and important for sparsely ionizing radiations, absent for *densely ionizing radiations*, and has an intermediate value for fast neutrons

Question 4

Reoxygenation

A. occurs in normal tissue cells nearest the tumor
B. makes some tumor tissues more resistant to neutrons
C. can occur as the result of fractionation of the radiation dose
D. makes cells more sensitive to alpha particles

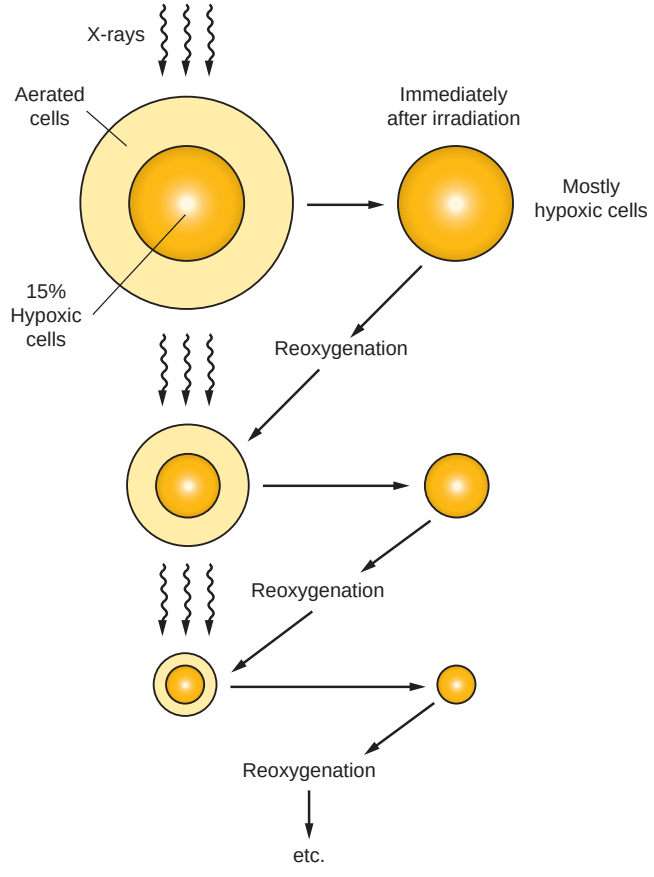
Chronic vs. Acute Hypoxia



Hypoxic cells less likely to become re-oxygenated

Hypoxic cells more likely to become re-oxygenated

The Process of Re-oxygenation



Reoxygenation is the process by which cells that are hypoxic at the time of irradiation become oxygenated afterward

Clinical Implication

The presence of hypoxic cells does not greatly influence the response of the tumor as long as there is sufficient time for re-oxygenation between fractions

Question 5

Which one of the following statements regarding radiation and hypoxia is TRUE?

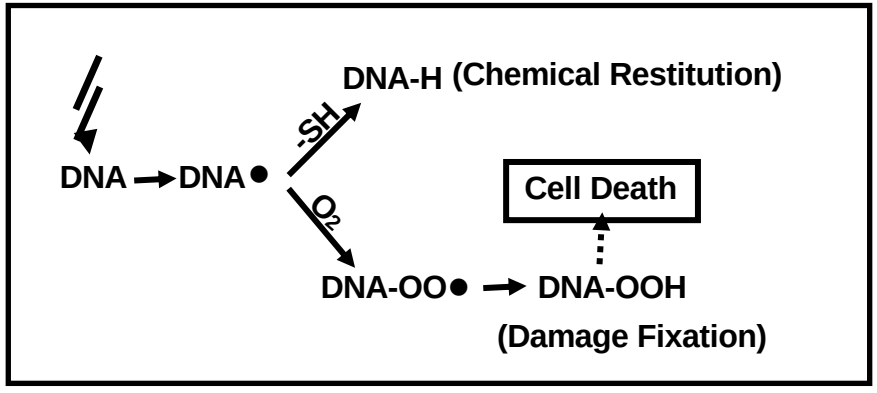
A. Irradiation under hypoxic conditions yields more DNA strand breaks than under aerated conditions

B. Irradiation under aerated conditions leads to less overall cellular damage than irradiation under hypoxic conditions

C. The presence of oxygen reduces radiation toxicity

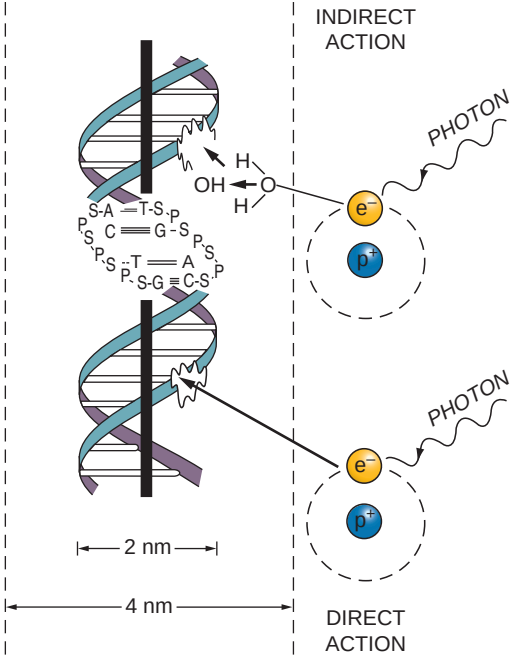
D. Oxygen must be present either during or within microseconds following irradiation to act as a radiosensitizer

The Oxygen Fixation Hypothesis



Free radicals have a $t_{1/2}$ of 10^{-5} sec, therefore, O_2 must be present during or shortly afterwards

For sparsely ionizing radiation, ~ 2/3 of the damage produced is mediated by which may be 'fixed' by oxygen



For densely ionizing radiation, direct action dominates, thus no appreciable oxygen effect

Question 6

The average radiobiological hypoxic fraction in experimental rodent tumors is approximately:

A. 1%

B. 4%

C. 15%

D. 50%

E. 90%

Summary of Experimental Evidence on Animal Tumors

- A survey of all published studies on hypoxic fractions of transplantable tumors in animals
- Of the 42 tumor types studied, 37 were found to contain hypoxic cells in at least one study
- Hypoxic fractions range from 0% to 50%, with a tendency for many results to average about **15%**

Moulder & Rockwell