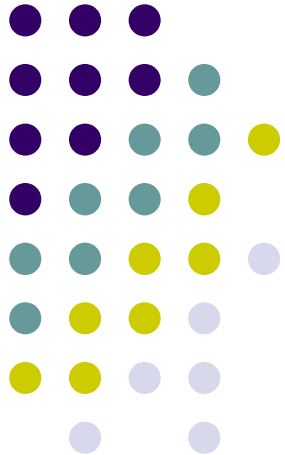
# Curso de Radiobiología
# UDELAR
# Facultad de Ciencias
# Unidad de Física Médica

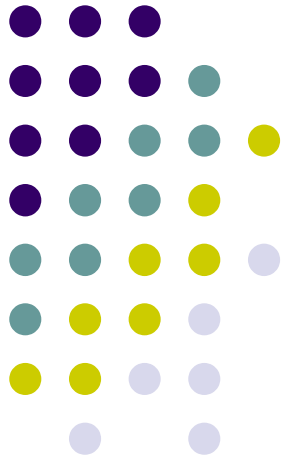
**Dr. Eduardo Francisco Larrinaga Cortina**



# 4R. Reoxigenación

**Créditos:**

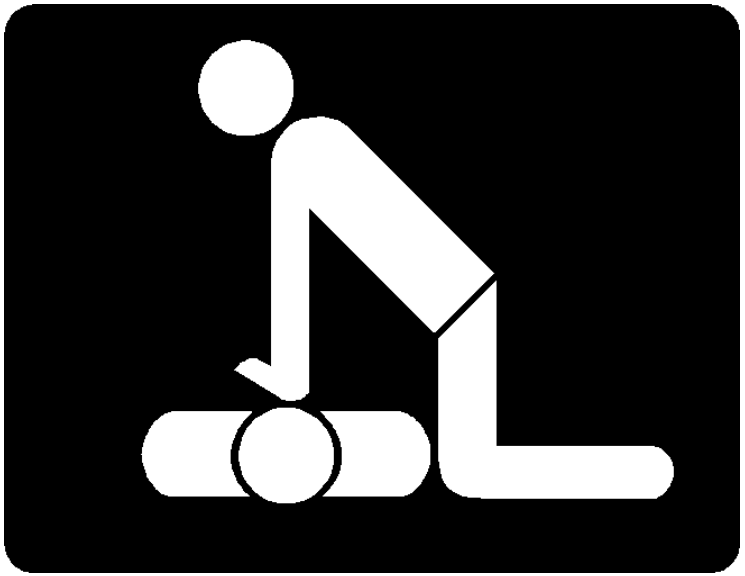
**Dr. Jerry Battista**



March 2011

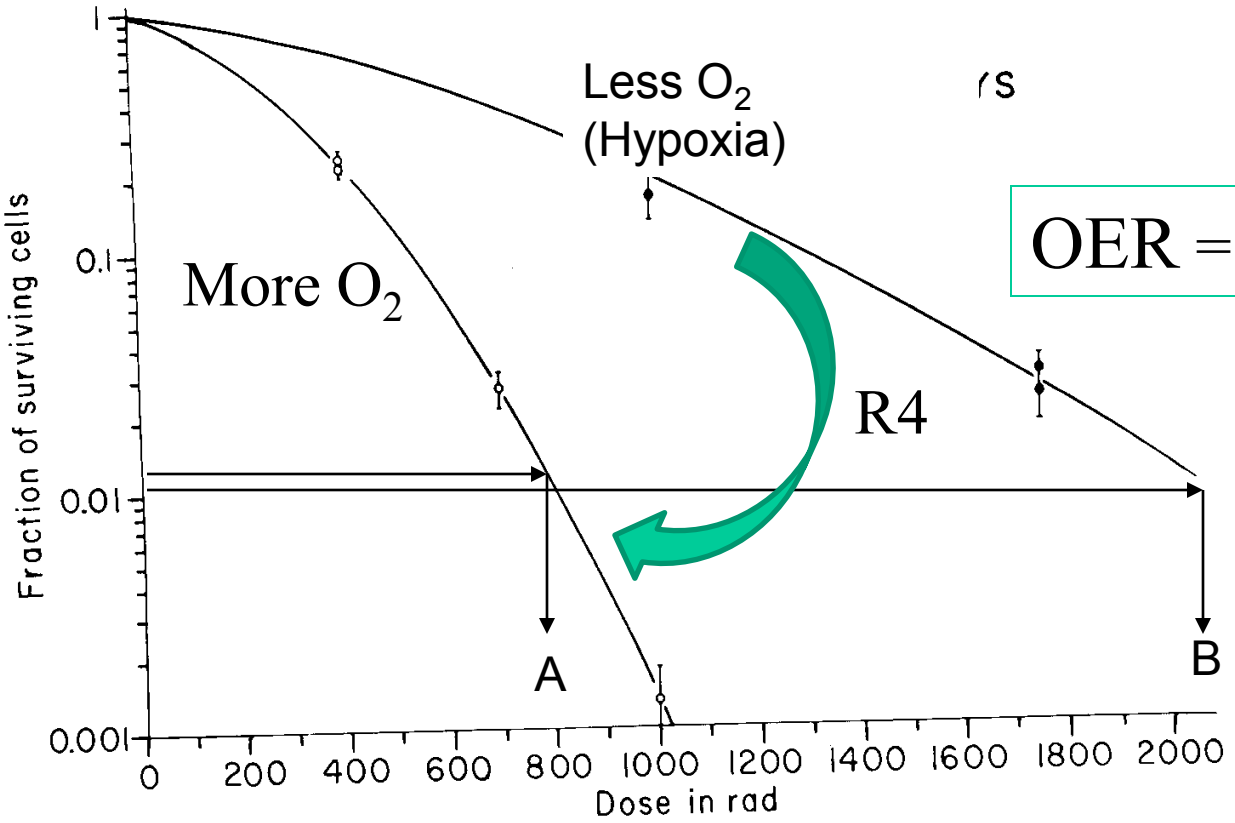
# Re-Oxygenation (R4)

J. Battista



Chapter 6,20

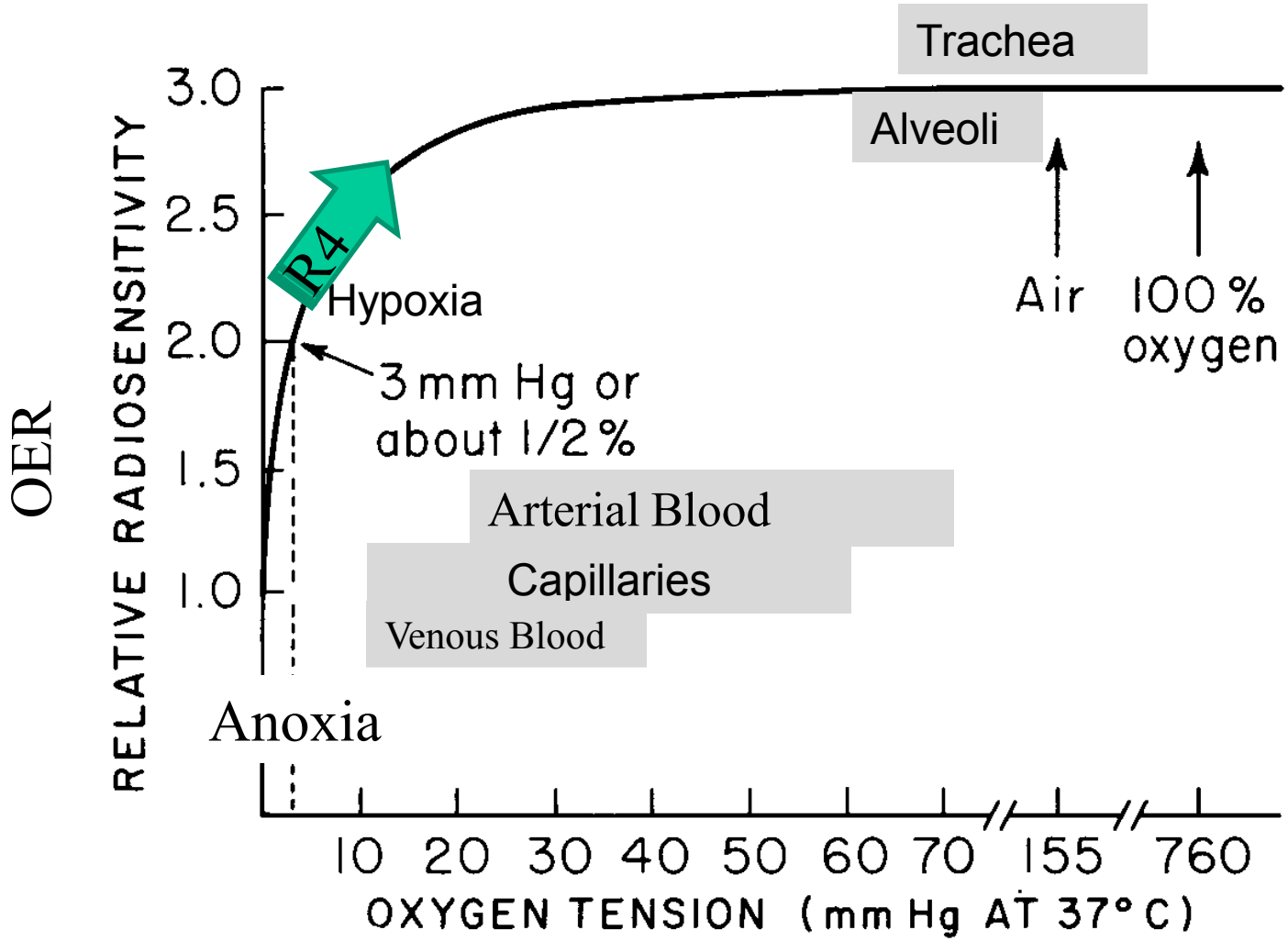
# Oxygen Enhancement Ratio




OER = Dose B / Dose A

Dose in cGy

# How Much Oxygen Pressure is needed ?



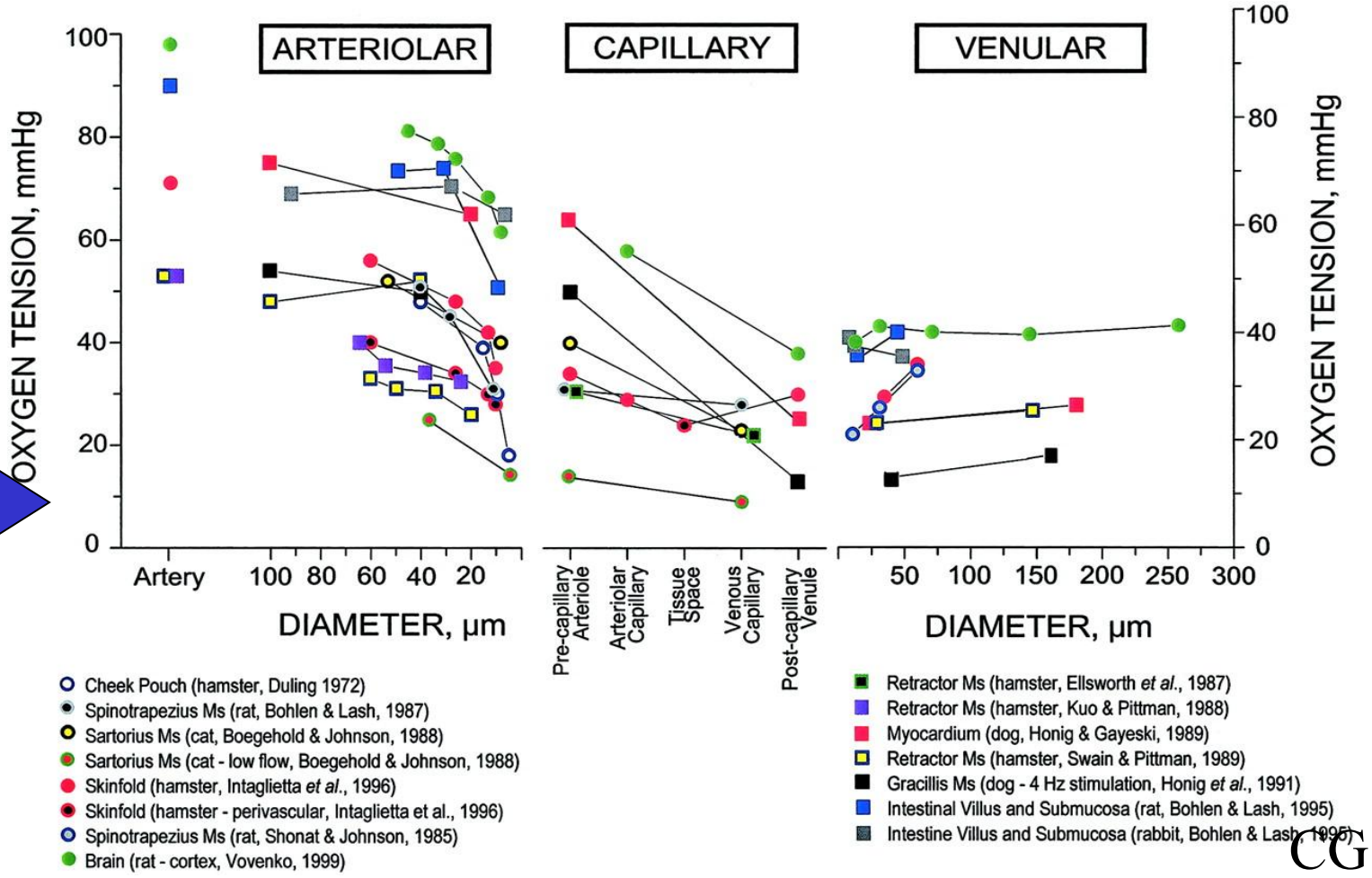

http://www.nda.ox.ac.uk/wfsa/html/u10/u1003_01.htm

# Oxygen Cascade in Vascular System

In early 70's Duling demonstrated that there was a significant "pre-capillary loss" of oxygen along the arteriolar tree.

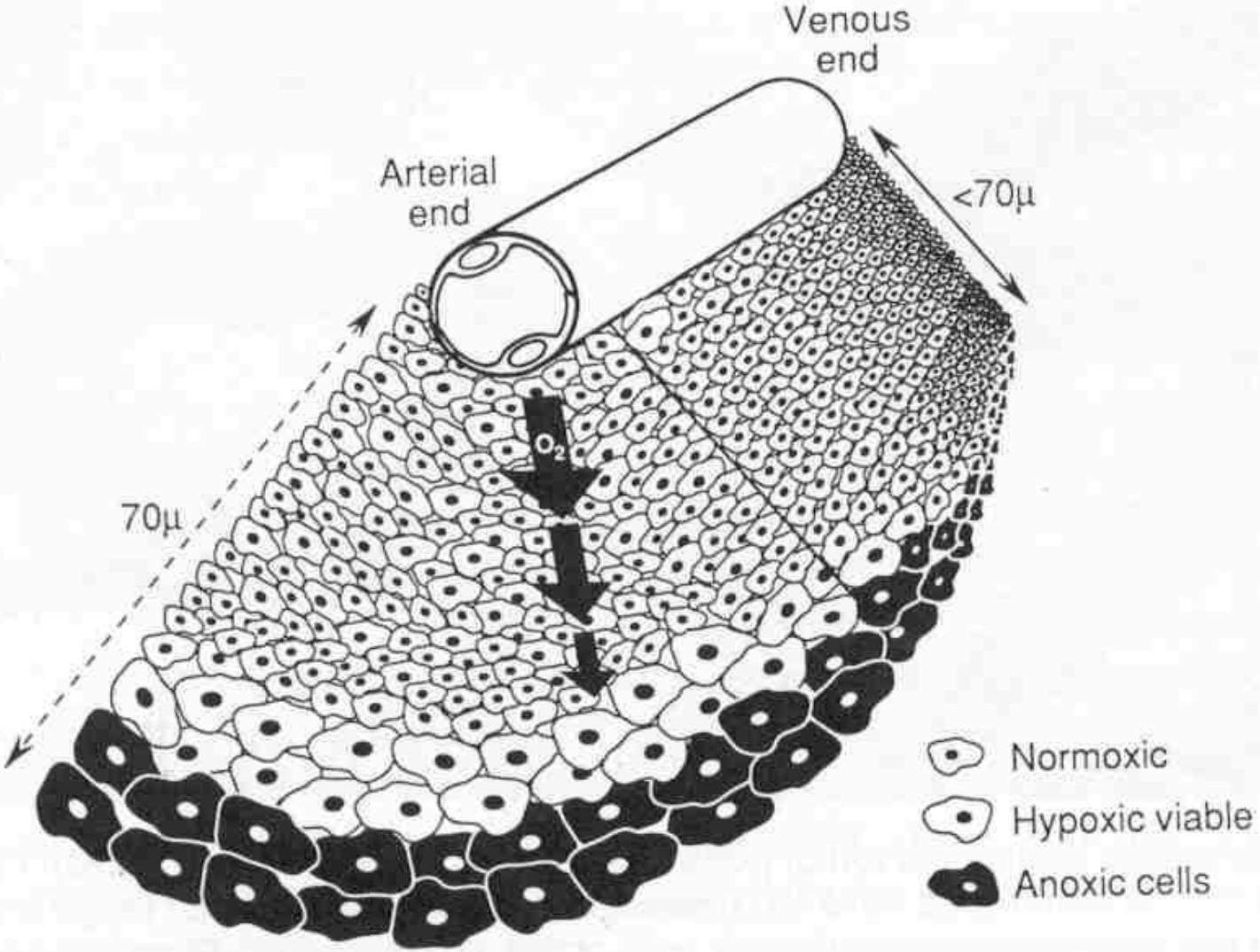
*Figure from Tsia et al., Physiological Reviews, 2003*

ypoxia

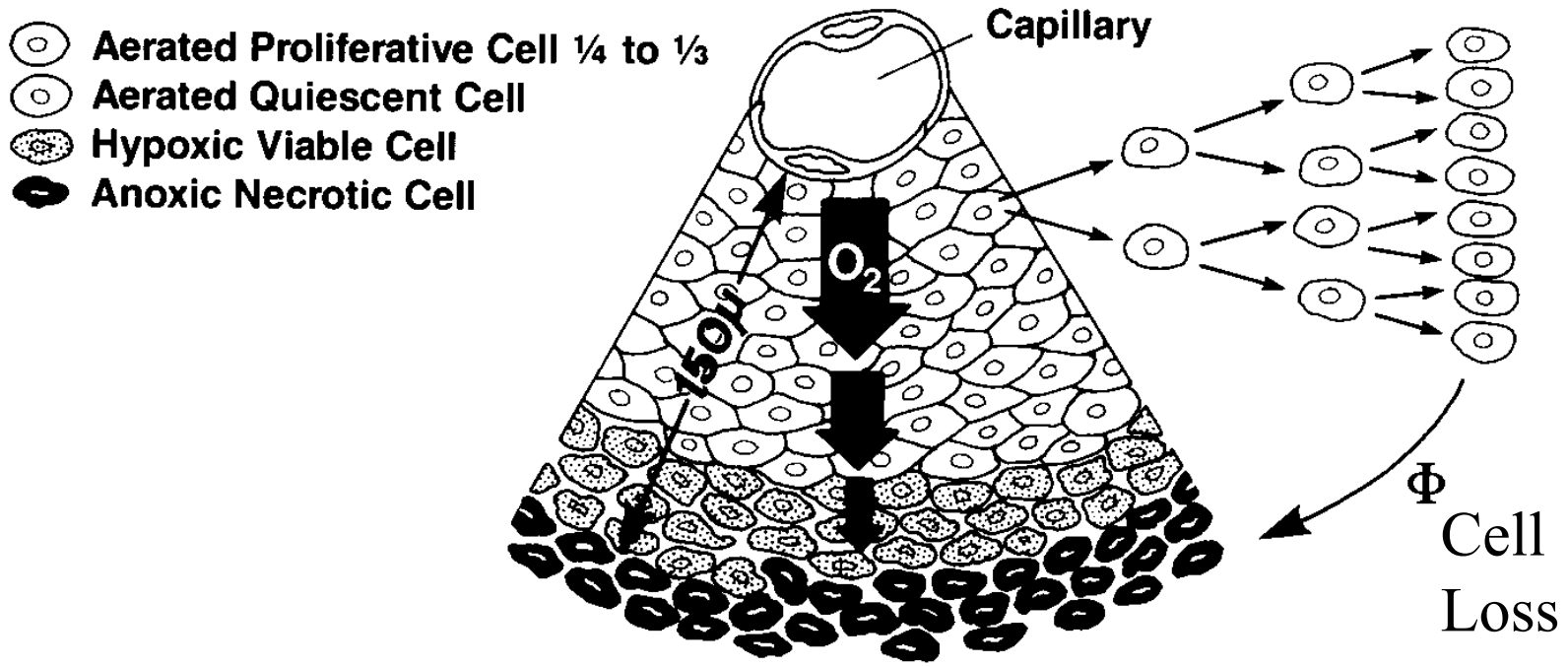



CG Ellis

# A 3D Problem


Venous end
Arterial end
<70μ
70μ
$O_2$
Normoxic
Hypoxic viable
Anoxic cells

# Cells in Tumours




The overall pattern of the growth of a tumor. Clonogenic cells consist of proliferative (P) and quiescent (Q) cells. Quiescent cells can be recruited into the cell cycle as the tumor shrinks after treatment with radiation or a cytotoxic drug. In animal tumors the growth fraction is frequently 30% to 50%. Of the cells produced by division, many are lost, principally into necrotic areas of the tumor remote from the vasculature. The cell loss factor (Φ) varies from 0% to 100% and dominates the pattern of tumor growth. As the tumor outgrows its blood supply, some cells become hypoxic. This accounts for some of the quiescent cells that are out of cycle.

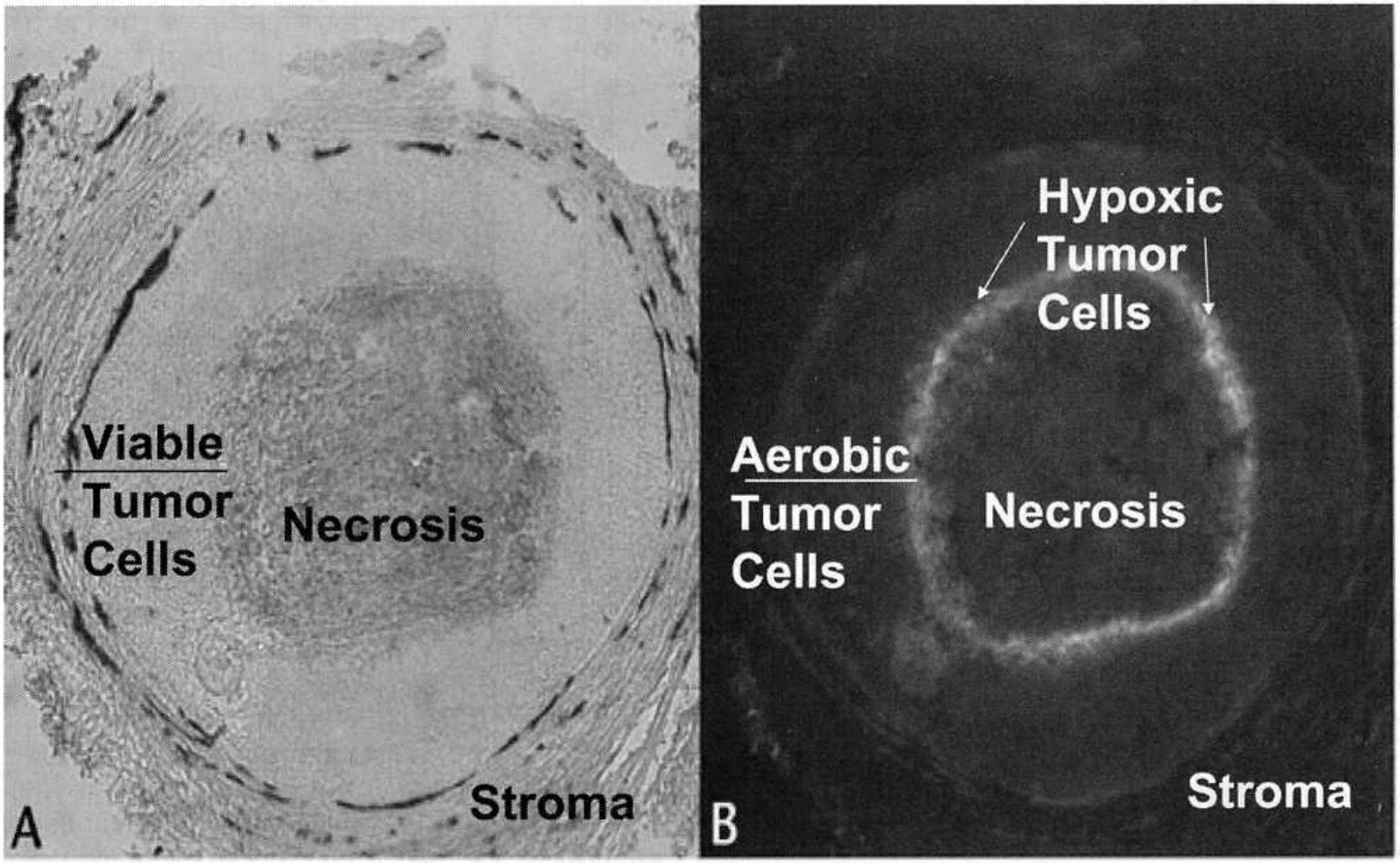
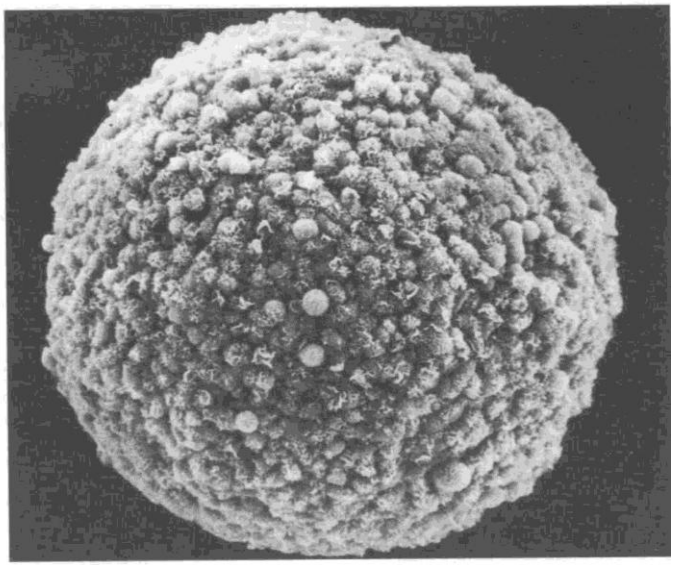



***FIGURE 6.12*** ● These images show a section from a rodent tumor illustrating chronic hypoxic cells. The animal was treated with the 2-nitroimidazole hypoxia detection agent EF5 24 hours preceding surgical removal of the tumor. **A:** Photomicrograph of the tumor section illustrating the tumor stroma, viable tumor cells, and necrotic tumor core. **B:** The same tumor section demonstrating the presence of chronically hypoxic tumor cells that stain positive with EF5 (white rim) adjacent to the necrotic core. (Courtesy of Dr. Sydney Evans.)

# Multi-Cellular Spheroid Tumor Model



**ure 20.9.** Photograph of an 800-μm spheroid containing about $8 \times 10^4$ cells. (Courtesy of Sutherland.)

R. Sutherland

R. Inch

A.C. Burton

Christopher G. Ellis

# Spheroid Tumour Model

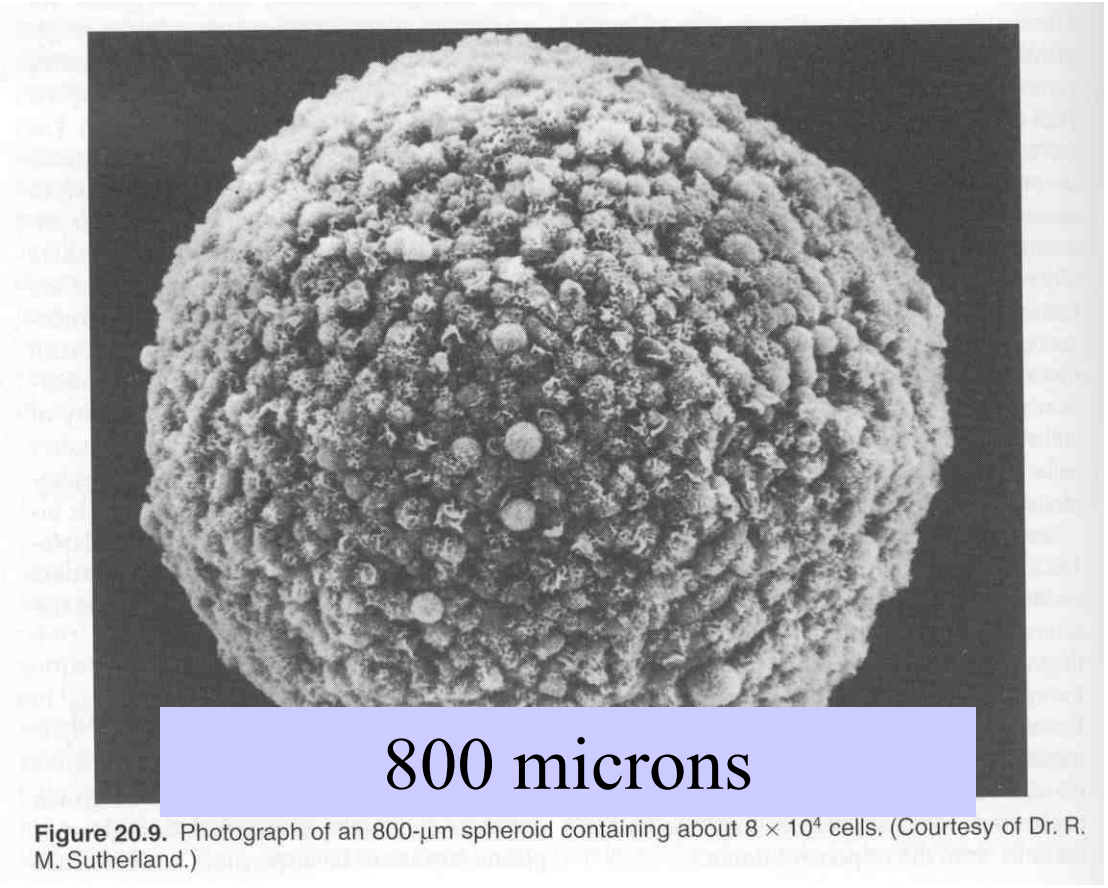



**Figure 20.9.** Photograph of an 800-μm spheroid containing about $8 \times 10^4$ cells. (Courtesy of Dr. R. M. Sutherland.)

Fig 20.9

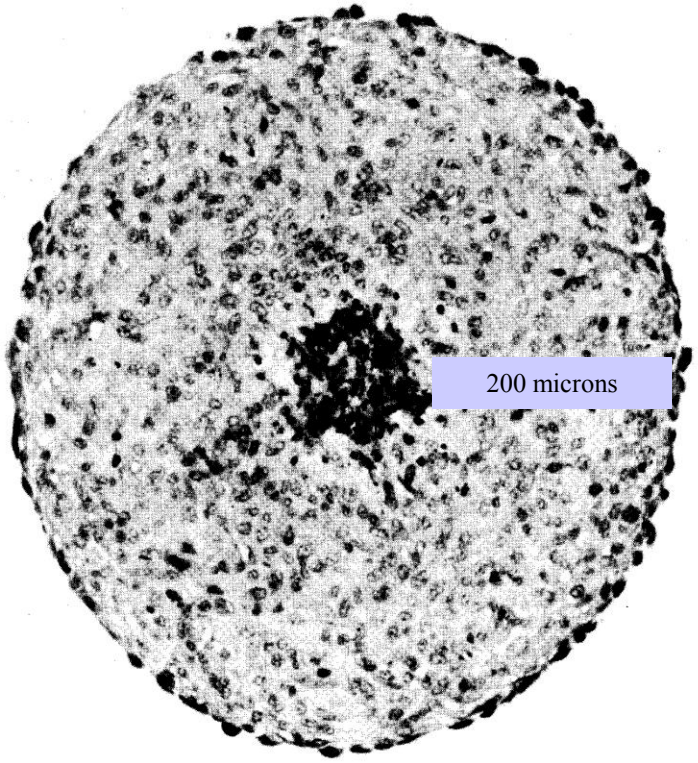



Fig 20.10

# Spheroid in Infinite Medium with Necrosis

$pO_2$ at tumor surface rapidly falls to near zero as the tumor grows beyond Rmax.

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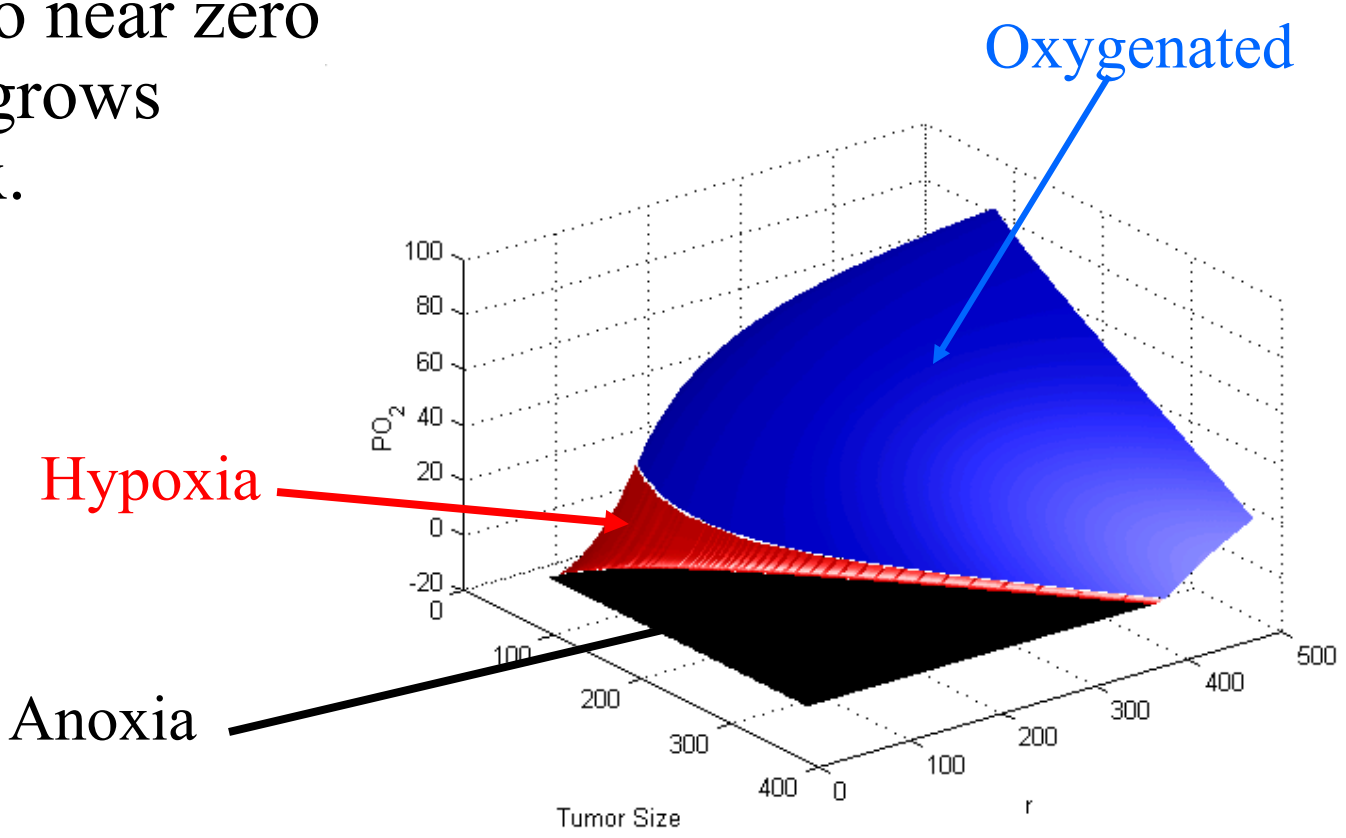
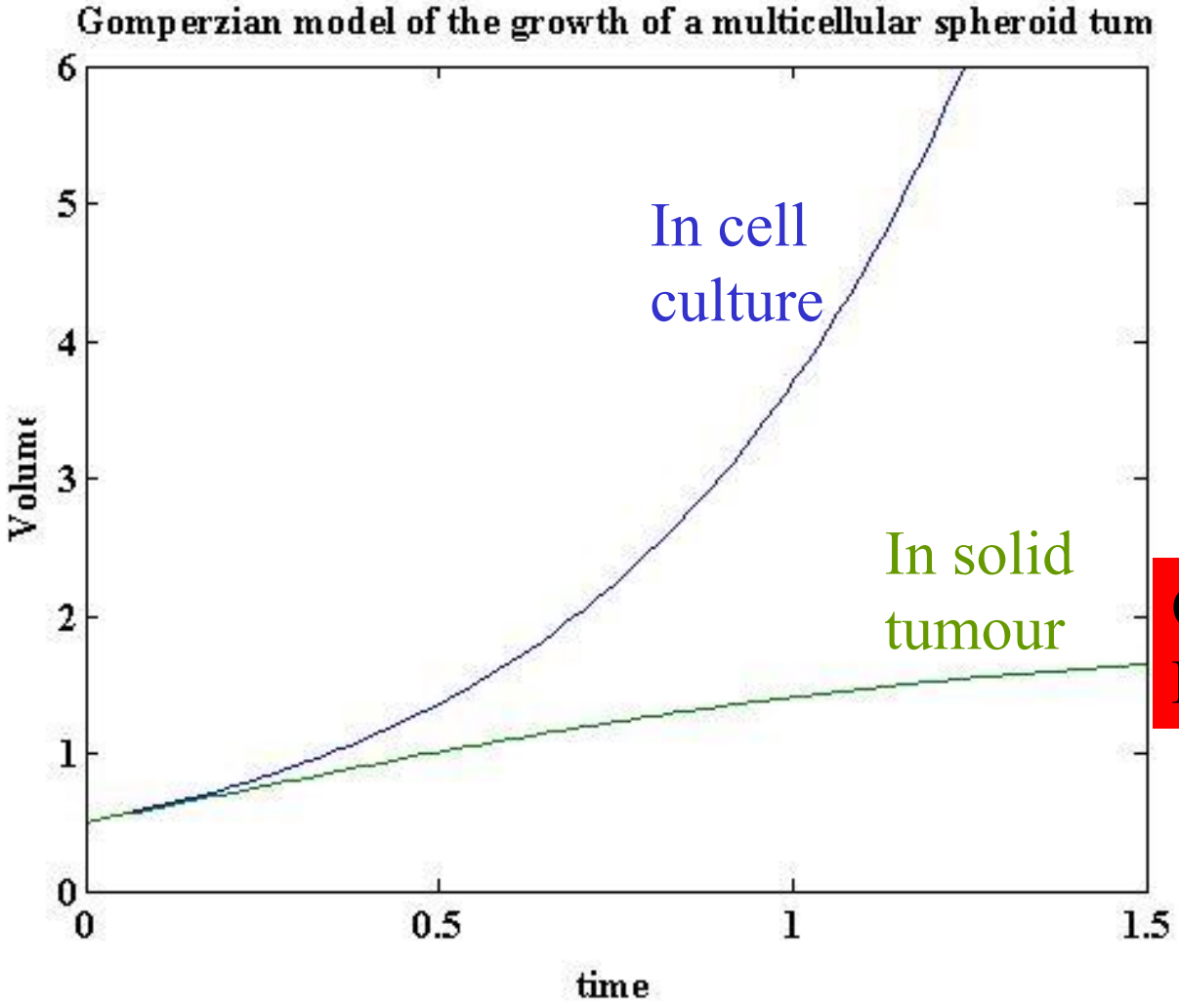


# Gomperzian Model

*predicts zero growth for large tumors*




Oxygen Deprivation ?

# Oxygen Diffusion Limit
## (Rmax)

$$P(r) = Pa - \frac{VO_2}{6 \cdot D \cdot k} \cdot (R^2 - r^2)$$

$VO_2$ -consumption rate per unit volume

R – Tumour radius

r – radial distance from cente

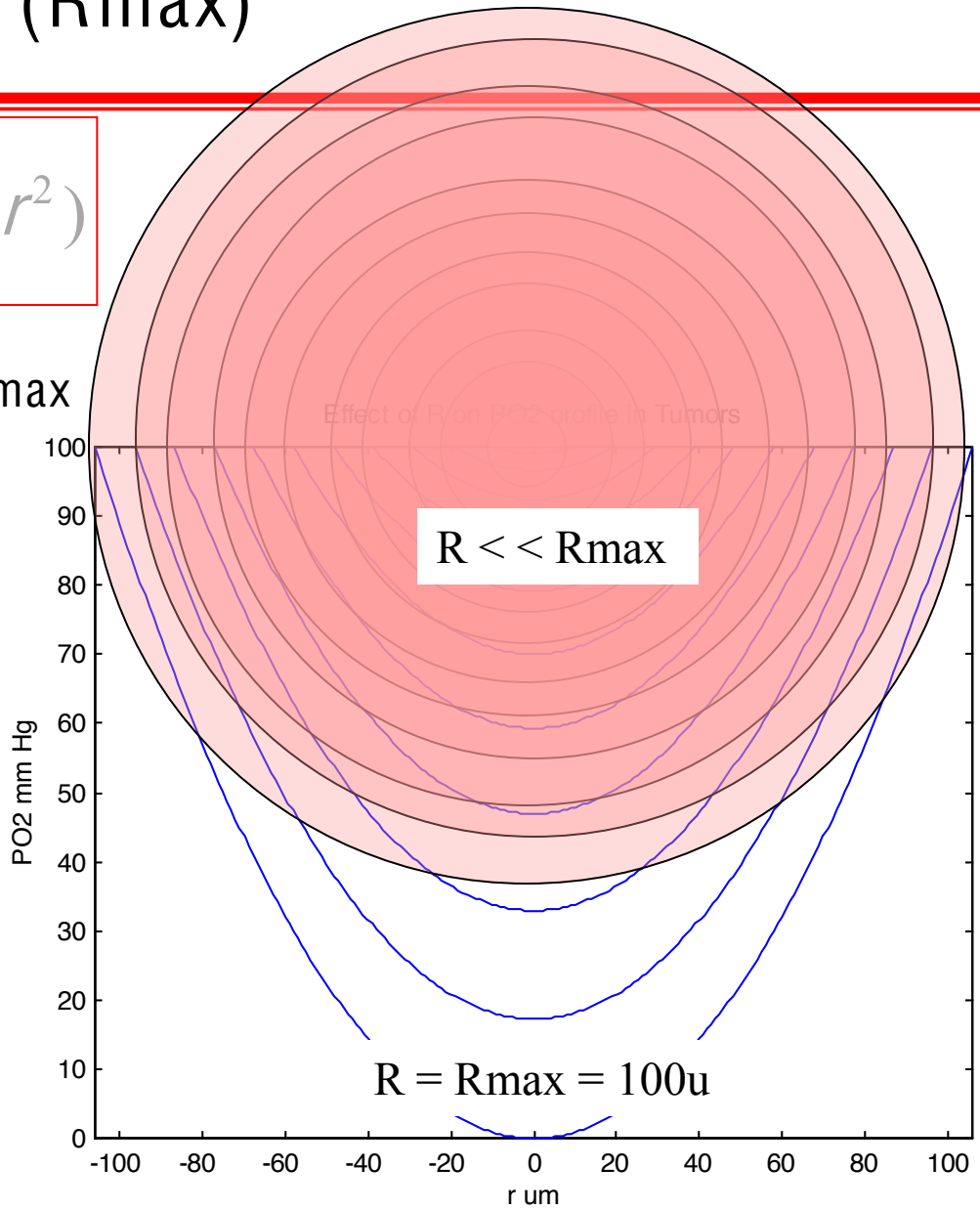
D – $O_2$ Diffusion constant

k - Solubility

As tumour volume grows,

R = Rmax, then

$pO_2$ falls to zero at centre



# Tumour Size ($R > R_{max}$)

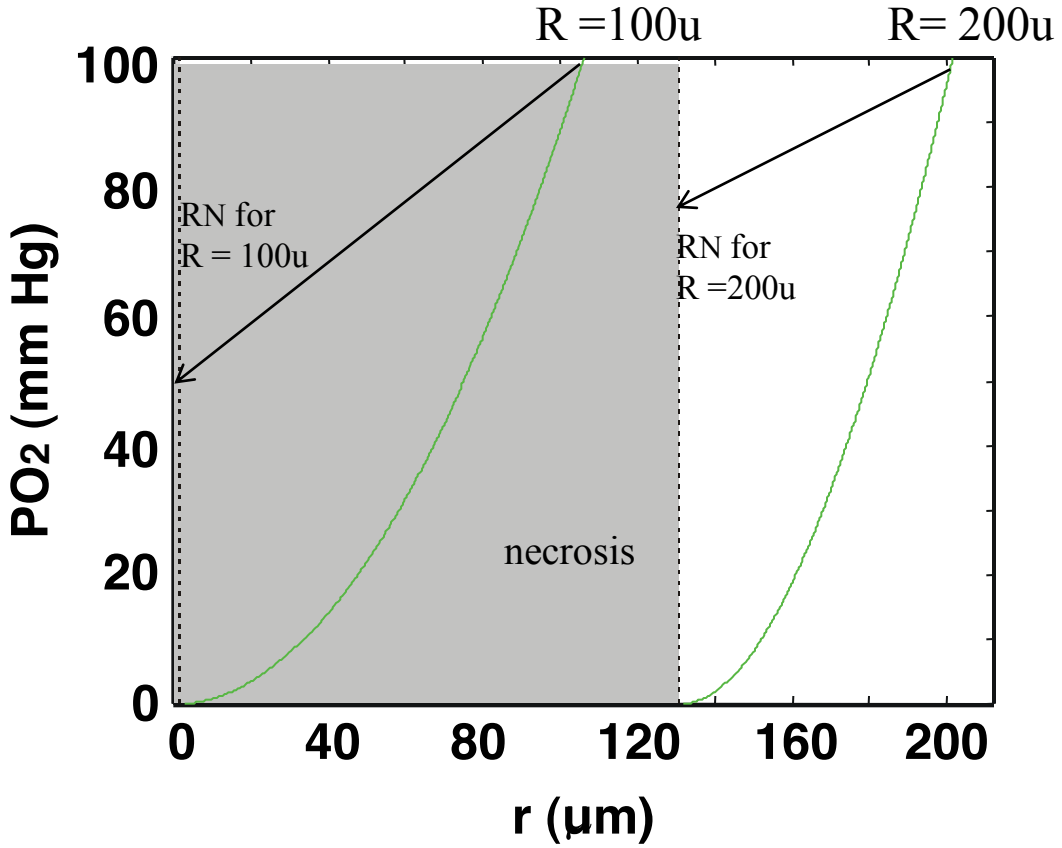
$R_N$ – necrotic radius

Doubling the tumor radius from ~100 to 200-um increases $R_N$ from um's to 130-um

*Necrotic core radius growing faster than tumor radius !!*

## Spherical Tumor with Necrosis

*Radius of necrotic core for two tumors*



# Observation of Tumour **Viable "Rim"**



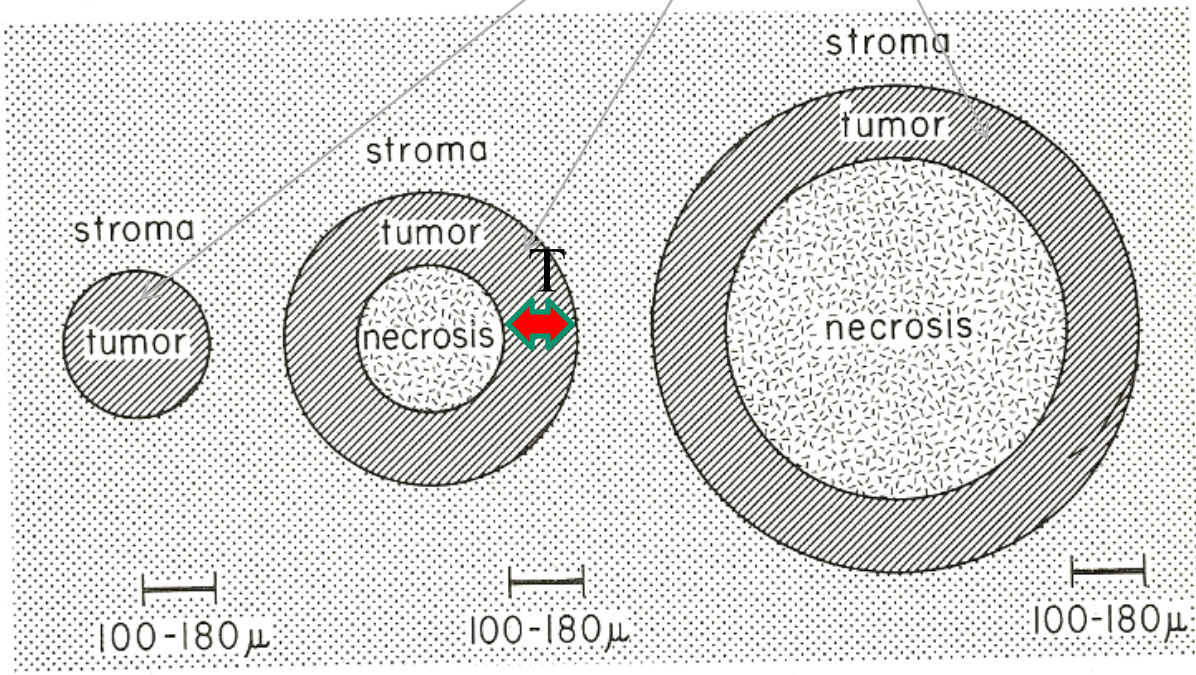



***FIGURE 6.7*** ● The conclusions reached by Thomlinson and Gray from a study of histologic sections of human bronchial carcinoma. No necrosis was seen in small tumor cords with a radius of less than about 160 $\mu$m. No tumor cord with a radius exceeding 200 $\mu$m was without a necrotic center. As the diameter of the necrotic area increased, the thickness of the sheath of viable tumor cells remained essentially constant at 100 to 180 $\mu$m.

T – thickness of viable rim

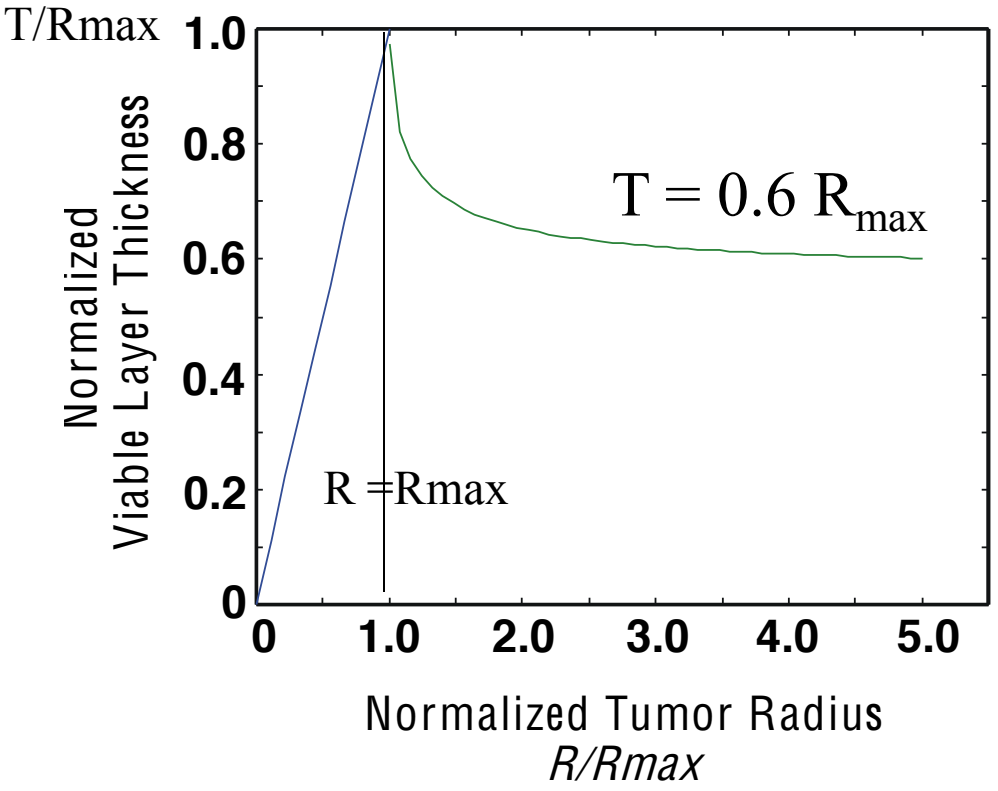
# Normalized Viable Rim ($T/R_{max}$)

$R_{max}$ is a characteristic of oxygen diffusion for all tumor sizes

Divide all dimensions by $R_{max}$ to normalize solution for all spheroid tumors…

*… one curve fits all!*

Normalized Viable Layer Thickness versus Normalized Tumor Radius



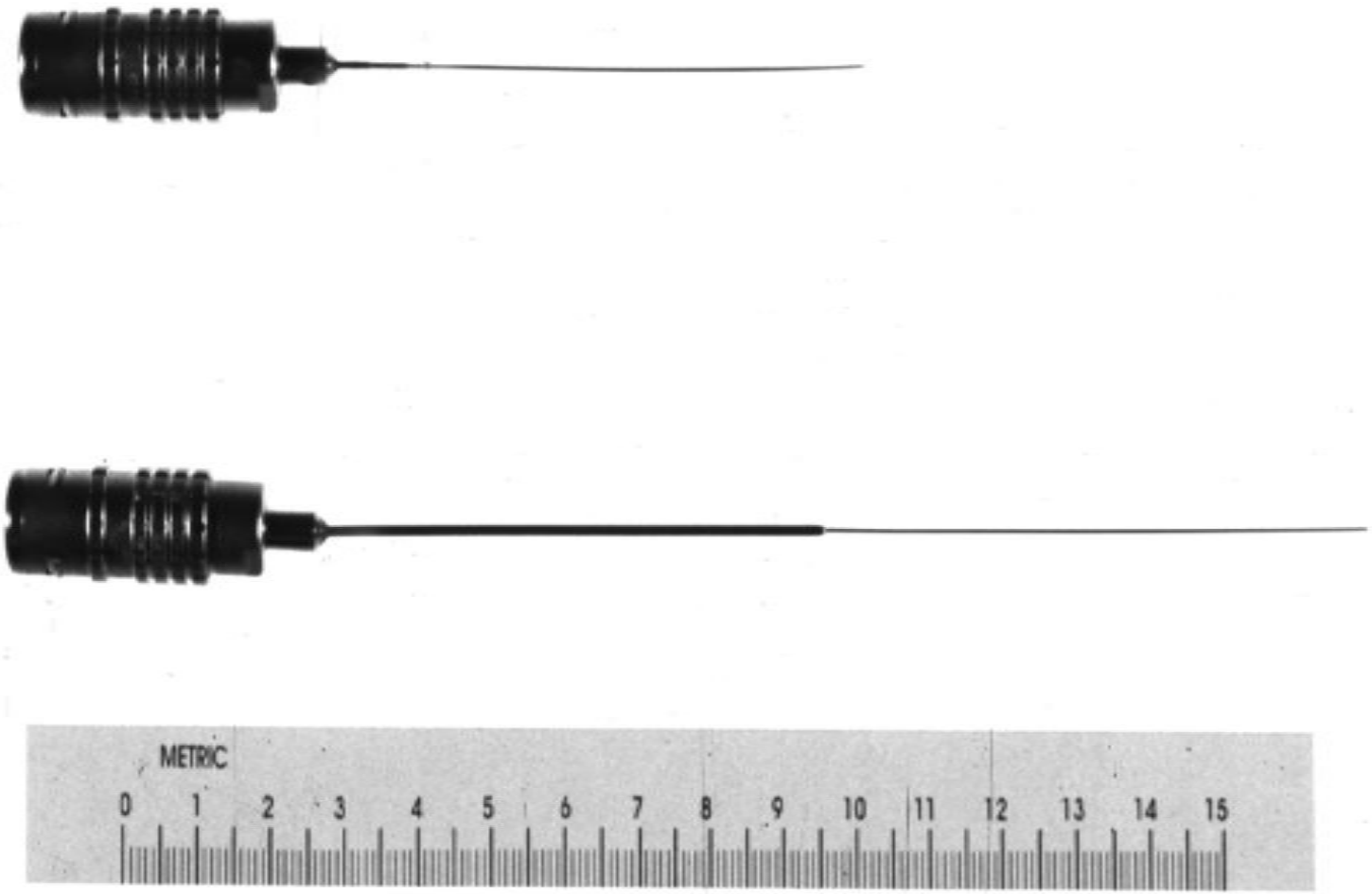
# Hypoxia Measurements

- **Eppendorf $pO_2$microelectrodes/Oxylite®probes**

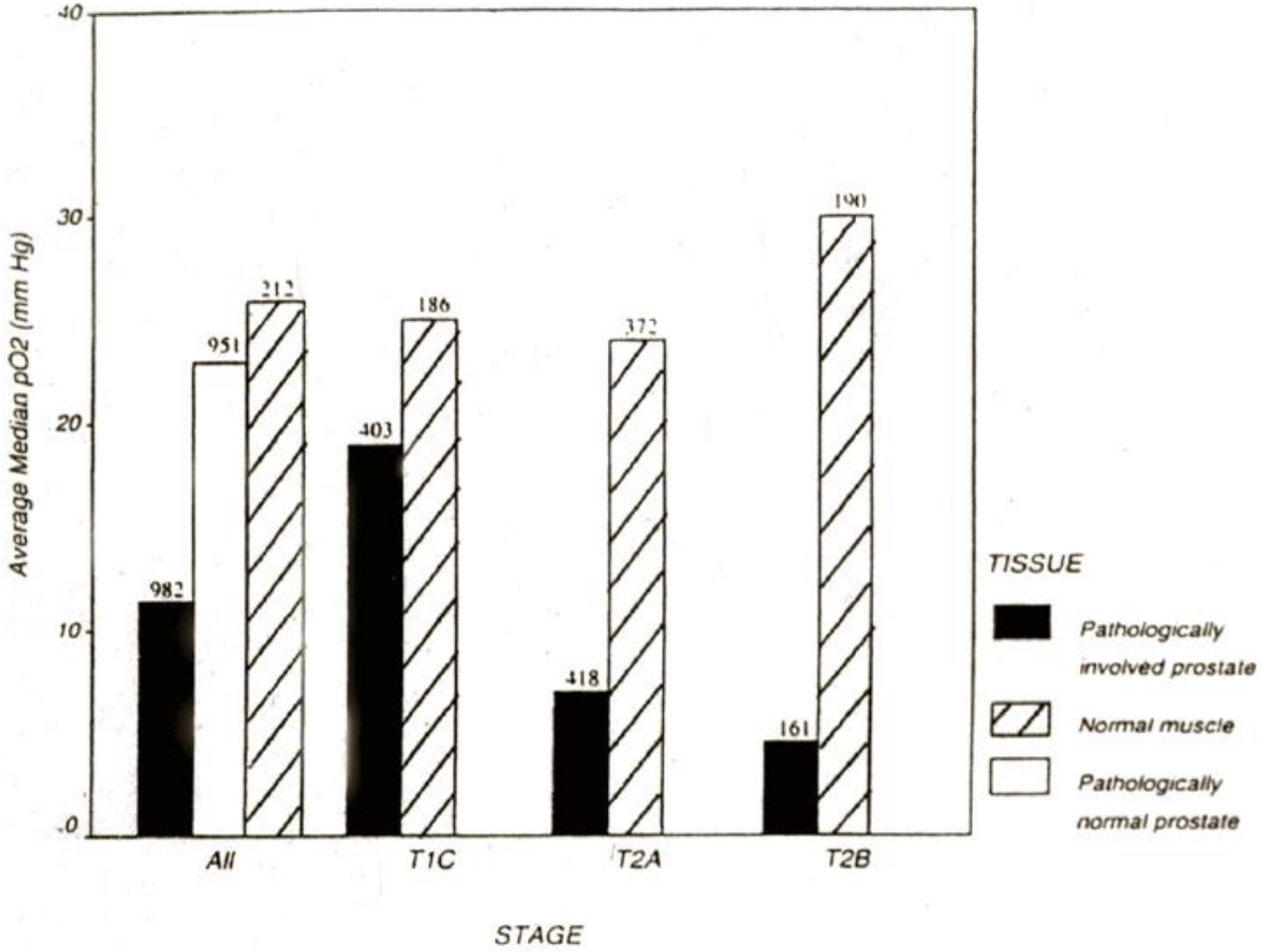
- **Histochemical staining of hypoxia specific factors (HIF-1α, carbonic anhydrase9, etc.) including hypoxic markers (EF-5, pimonidazole, etc.)**
- **EPR of oxygen-dependent reporter probes**
- **Fluorescence/IR detection of haemoglobin oxygen**

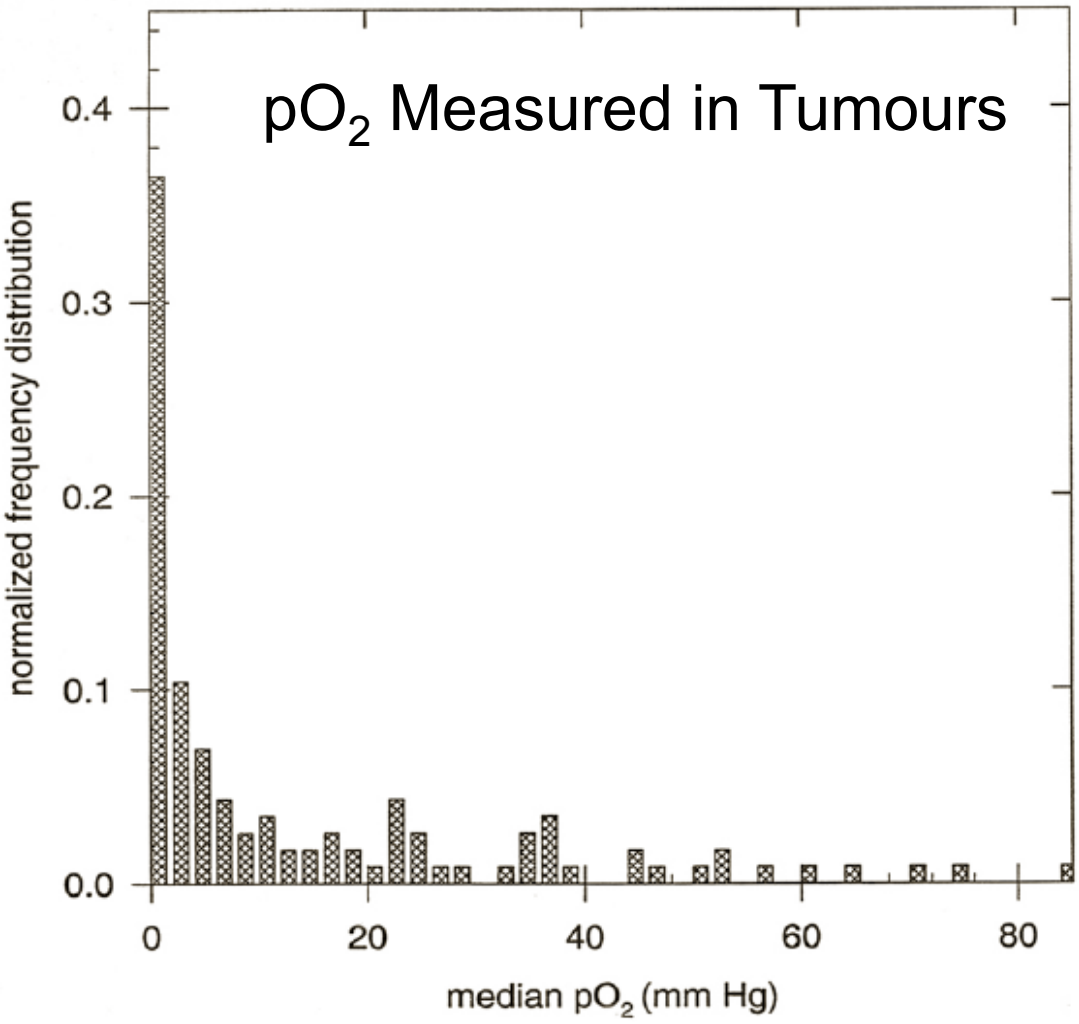
- **MRI/MRS measurement of energetic phosphorous states or oxygen-dependent reporter probes (hexafluorobenzene)**
- **Nuclear medicine imaging of radiolabeled hypoxic markers**

# Eppendorf Needles



# Prostate Tumour Samples





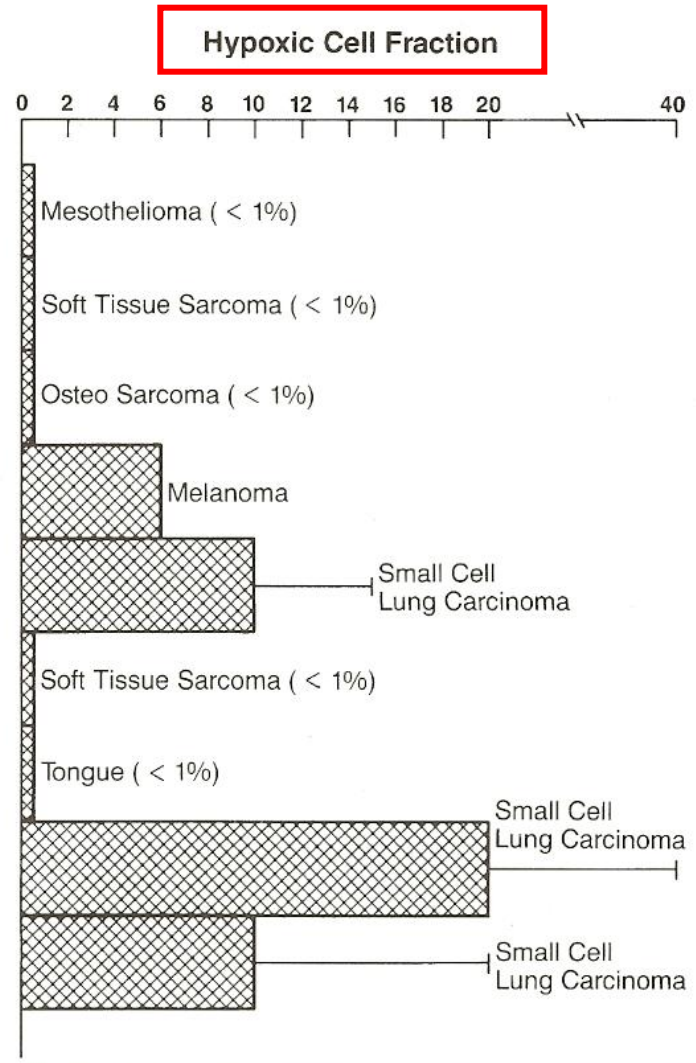




**Figure 25.12.** Summary of the labeling levels found in the first eight patients to be administered radioactive-labeled misonidazole. Only four of the eight patients show levels of labeling commensurate with the presence of hypoxic cells. (Courtesy of Drs. J. D. Chapman and R. Urtasun.)

# Hypoxia Measurements

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- **Histochemical staining of hypoxia specific factors (HIF-1α, carbonic anhydrase9, etc.) including hypoxic markers (EF-5, pimonidazole, etc.)**
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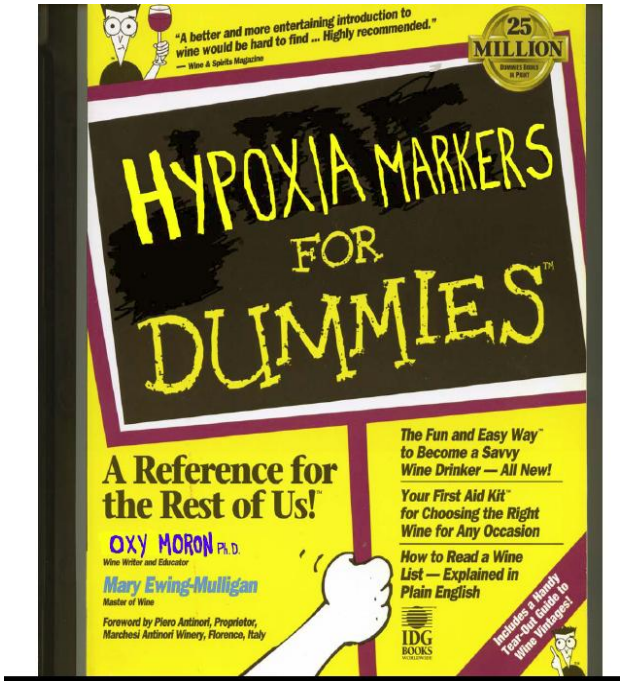
# Hypoxic Cell Sensitizers and Markers (Thanks go to Dr. Don Chapman)

**VANILLA**–Azomycin-containing chemicals that are bio-reduced within cells to intermediates that covalently bind to molecules at rates inversely proportional to oxygen concentration (MISO, F-MISO, β-D-IAZGP, EF-5).

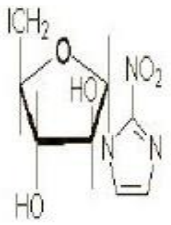
**CHOCOLATE**–Metal-containing ligands that deposit their activity in tissue via some reduction mechanism ([Tc-99m]HL-91, [Cu-64]ATSM).

**CHOCOLATE/SWIRL**–Ligands that contain azomycin and are labeled with various radioactive-metals.

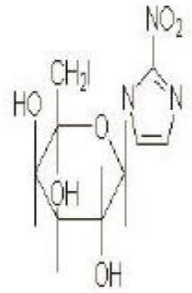



See Chapter 23

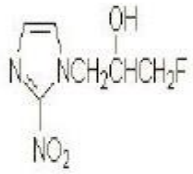
# Hypoxia Imaging Markers



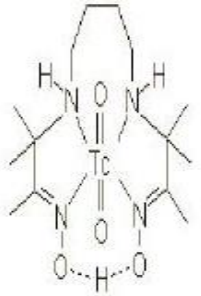
α-D-IAZA



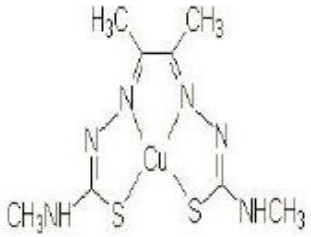
β-DIAZGP



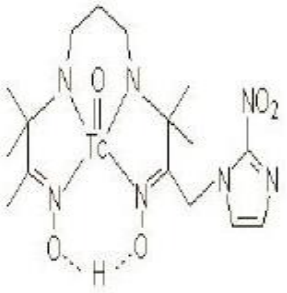
FLUOROMISONIDAZOLE



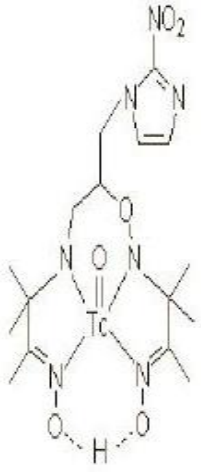
Tc99m-HL-91



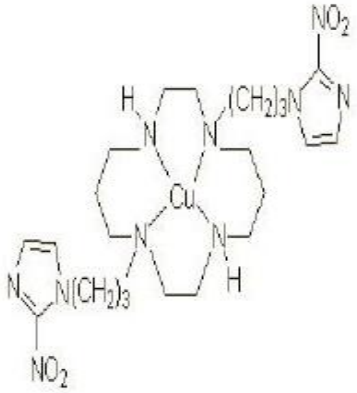
Cu-ATSM



Tc99m-BMS-181321



Tc99m-BMS-194796



Cu-EC-327

D. Chapman

# Autoradiography of Hypoxia

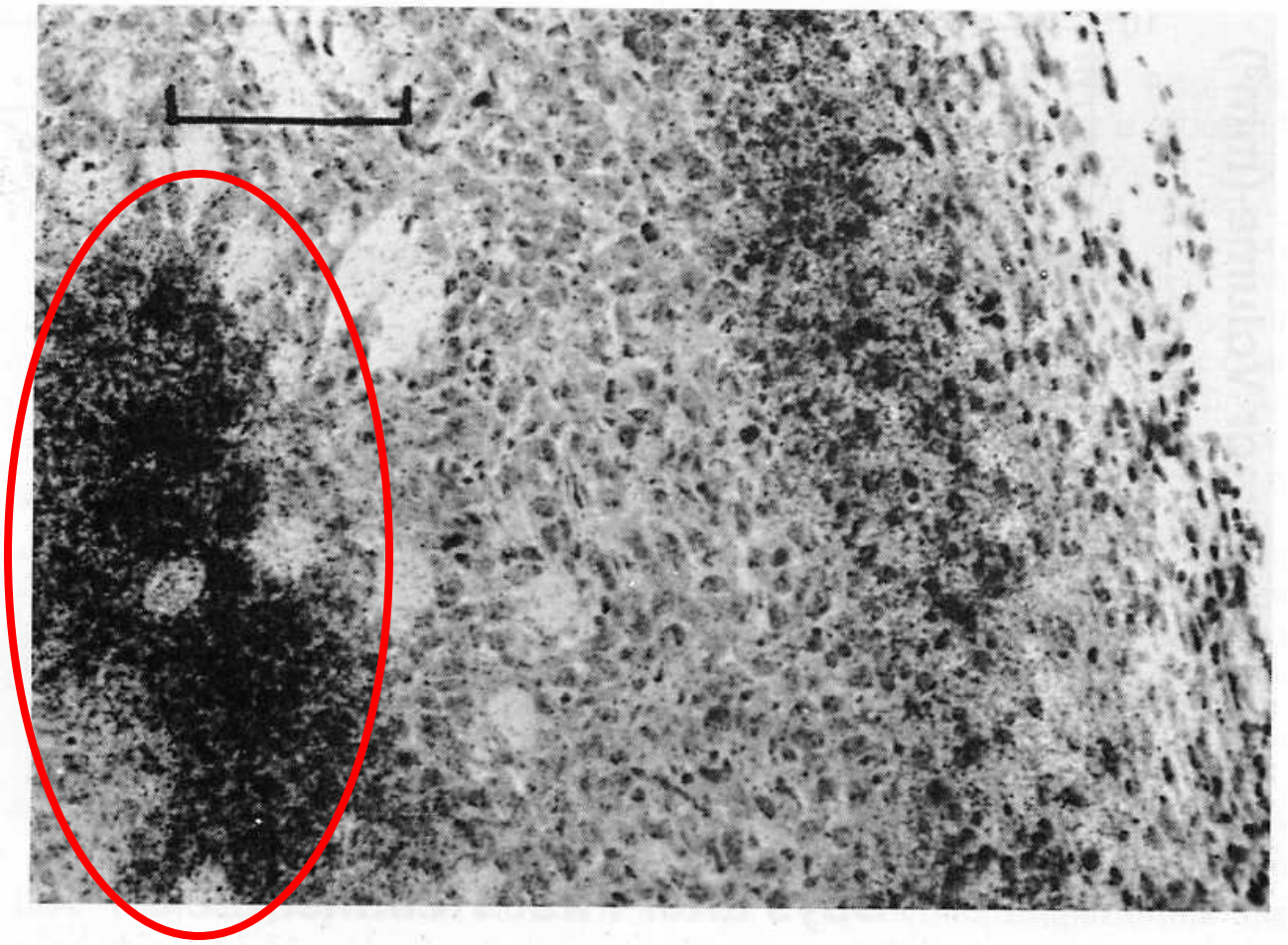
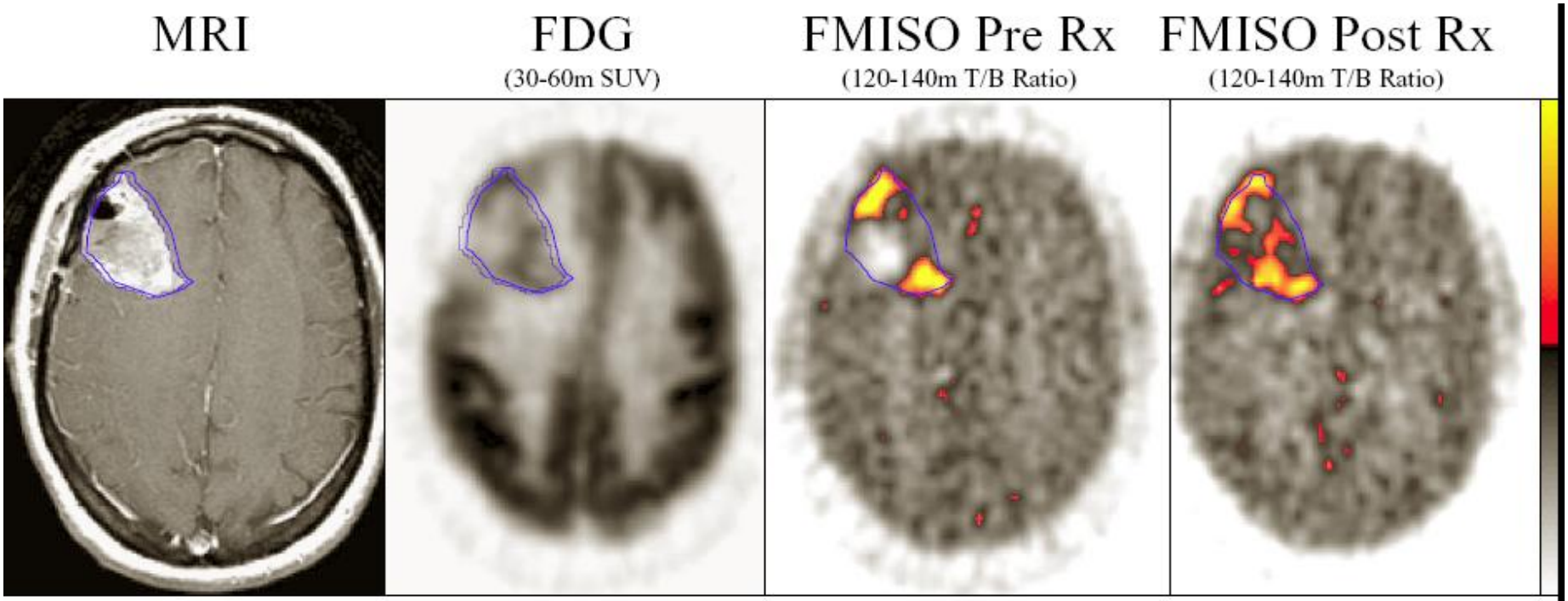


***FIGURE 25.11*** ● Autoradiograph of a section of a human small-cell lung carcinoma from a patient who received radioactive-labeled misonidazole the previous day. There are areas of intense labeling (many grains in the emulsion), suggesting the presence of hypoxic regions in the tumor. In areas deficient in oxygen, the misonidazole undergoes anaerobic metabolism and is broken down, and the radioactive label is deposited. (Courtesy of Drs. J.D. Chapman and R. Urtasun.)




49 yo F with rt frontal GBM resected 9/19/01

MRI, FDG, FMISO pre therapy

Treated with 15 Gy neutrons, 3 / wk

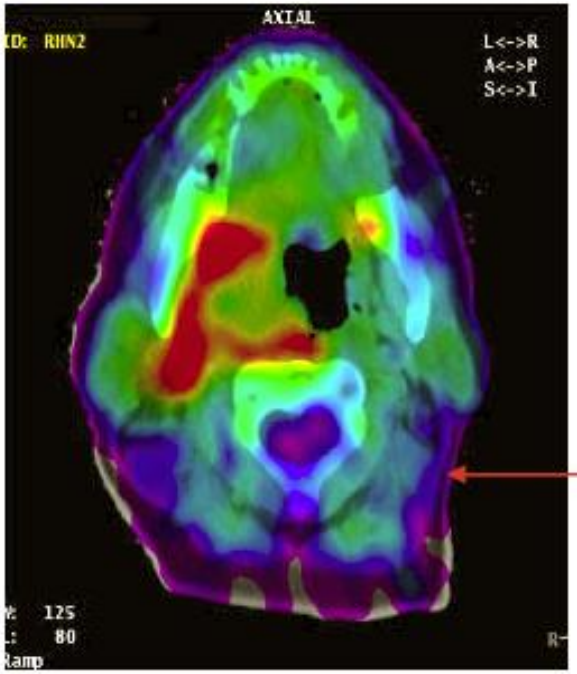
Post neutron image shows no reoxygenation

Patient was continued with photon therapy

The thermometer is B/W in the normal range (<1.2) and changes to color above the hypoxia threshold of 1.2.

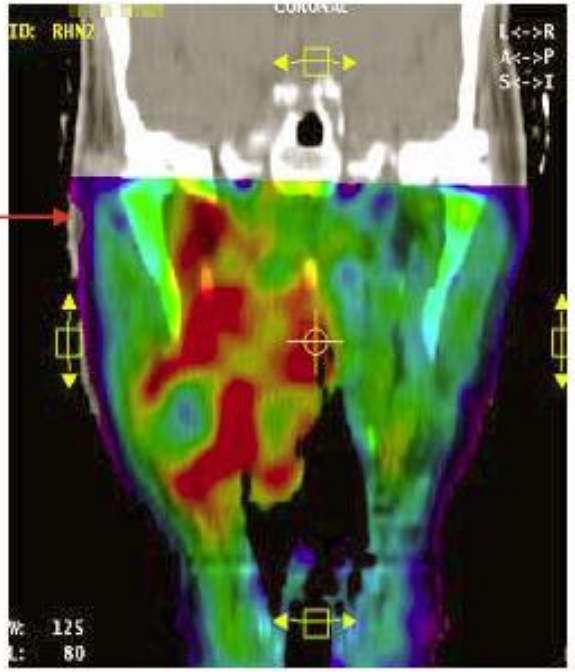
**K Krohn, University of Washington, Seattle**

# Delineation of Hypoxic GTV by $^{60}$Cu-ATSM PET



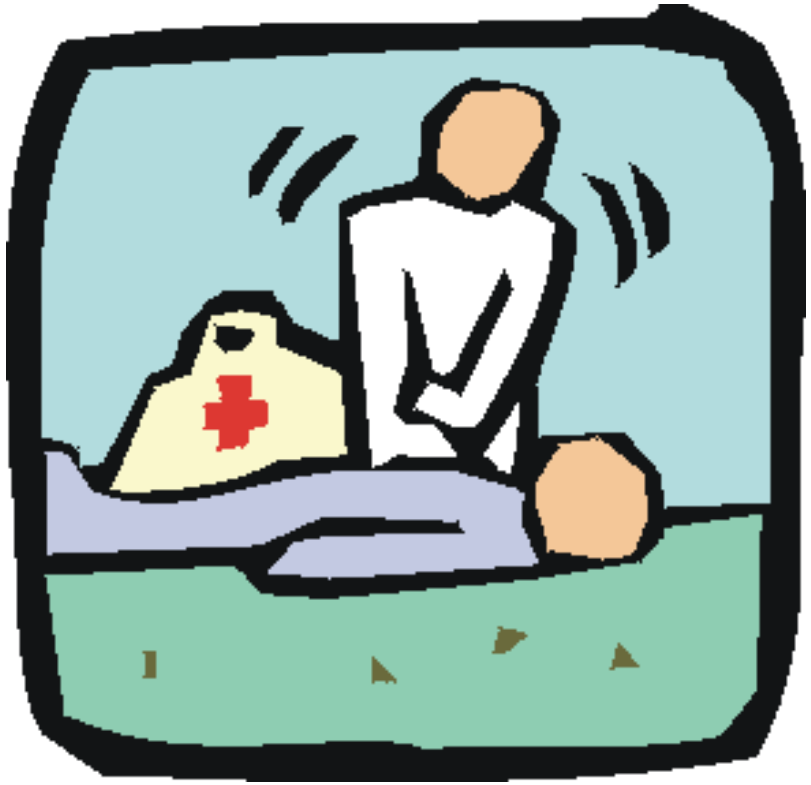

Coronal view

Axial view




*Chao, IJROBP 2001; 49(4): 1171-1182*

# Radiotherapy Strategy: Re-Oxygenate Tumour !



# Cell Survival Curves – Mixed Cell Distribution

Hypoxic Fraction

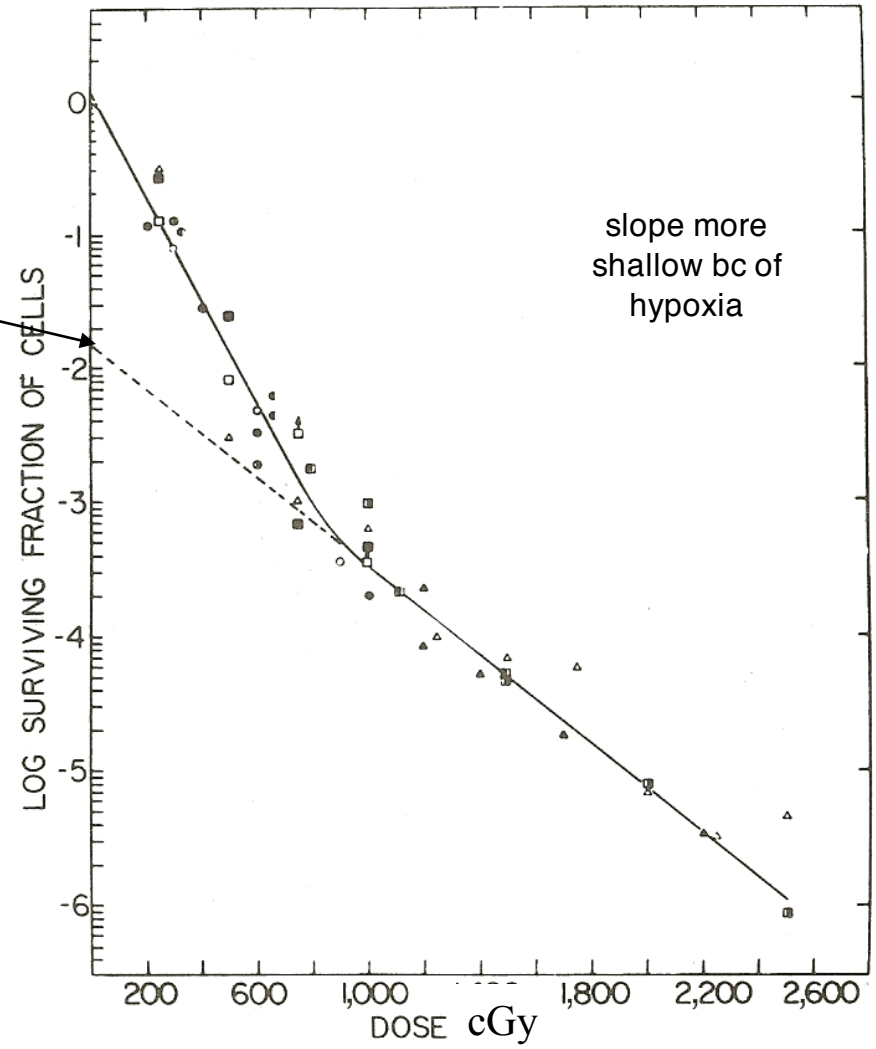



***FIGURE 6.10*** Fraction of surviving cells as a function of dose for a solid subcutaneous lymphosarcoma in the mouse irradiated *in vivo*. The first part of the curve has a slope $D_0$ of 1.1 Gy (110 rad); the second component of the curve has a shallower slope $D_0$ of 2.6 Gy (260 rad), indicating that these cells are hypoxic. (From Powers WE, Tolmach LJ: A multicomponent x-ray survival curve for mouse lymphosarcoma cells irradiated *in vivo*. *Nature* 197:710–711, 1963, with permission.)

# Fractionate to Re-Oxygenate !

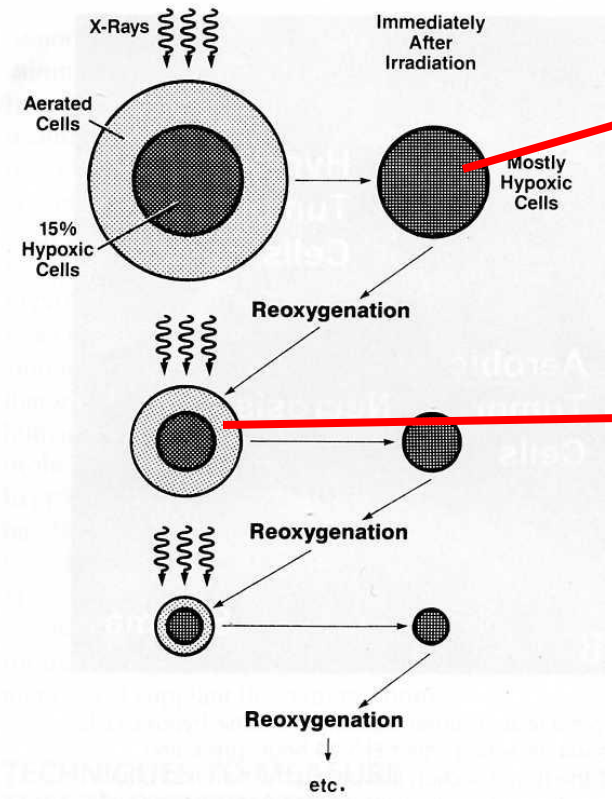



***FIGURE 6.13*** ● The process of reoxygenation. Tumors contain a mixture of aerated and hypoxic cells. A dose of x-rays kills a greater proportion of aerated cells than hypoxic cells because aerated cells are more radiosensitive. Therefore, immediately after irradiation, most cells in the tumor are hypoxic. However, the preirradiation pattern tends to return because of reoxygenation. If the radiation is given in a series of fractions separated in time sufficient for reoxygenation to occur, the presence of hypoxic cells does not greatly influence the response of the tumor.

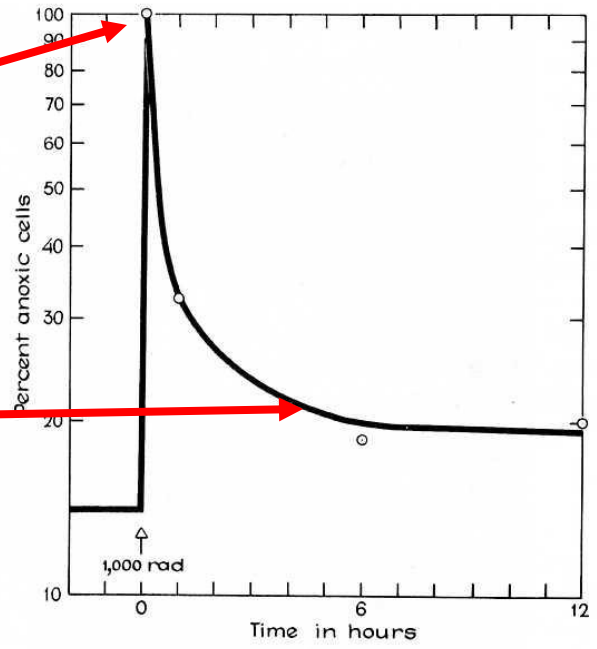



***FIGURE 6.14*** ● Percentage of hypoxic cells in a transplantable mouse sarcoma as a function of time after a dose of 10 Gy (1,000 rad) of x-rays. Immediately after irradiation, essentially 100% of the viable cells are hypoxic, because such a dose kills a large proportion of the aerated cells. In this tumor, the process of reoxygenation is very rapid. By 6 hours after irradiation, the percentage of hypoxic cells has fallen to a value close to the preirradiation level. (From Kallman RF, Bleehen NM: Post-irradiation cyclic radiosensitivity changes in tumors and normal tissues. In Brown DG, Cragle RG, Noonan JR [eds]: *Proceedings of the Symposium on Dose Rate in Mammalian Radiobiology, Oak Ridge, TN, 1968*, pp 20.1–20.23. USAEC Report CONF-680410. Springfield, VA, Technical Information Service 1968, with permission.)

# Another Reason to reduce Hypoxia

Oxygenated Pathway

Hypoxic Pathway

Induced Tumour Growth

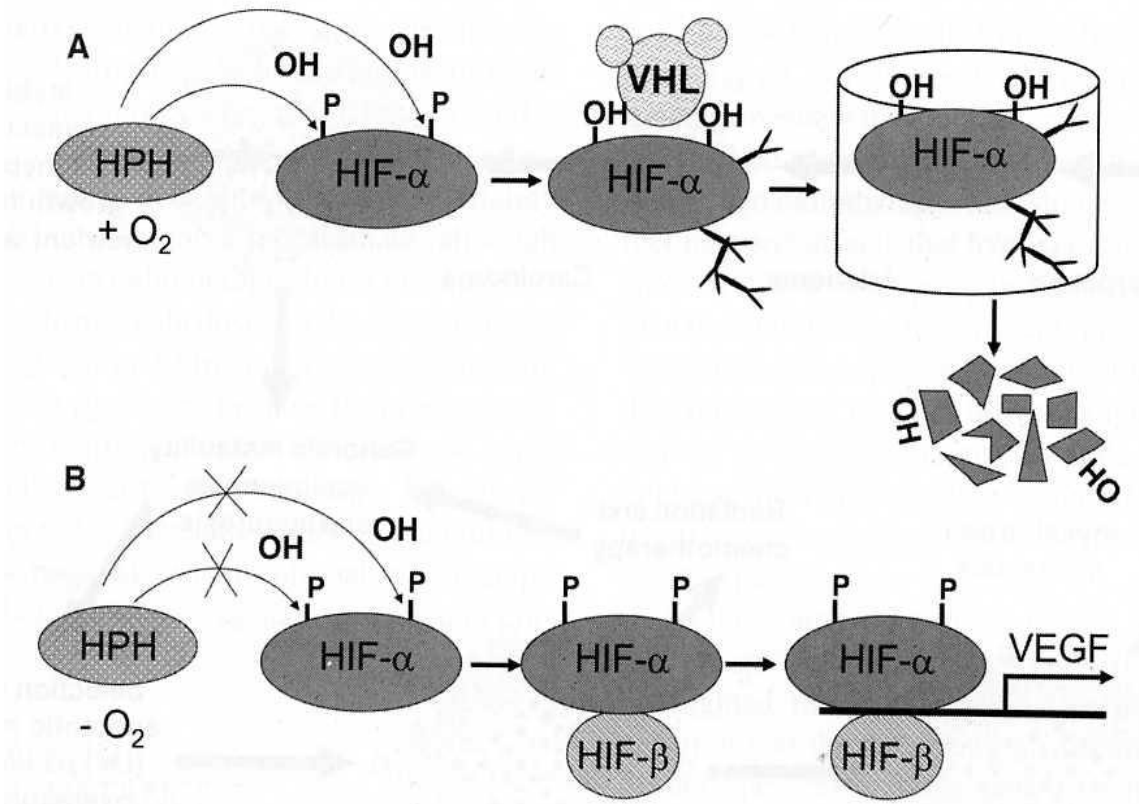



***FIGURE 6.17*** ● Critical steps in the regulation of the hypoxia-inducible transcription factor HIF-1α. **A:** Under normoxic conditions, a group of enzymes called prolyl hydroxylases (HPHs) add hydroxyl groups (OH) to two proline residues of HIF-1α. The hydroxylation of HIF-1α by HPH allows the VHL tumor-suppressor gene to bind and promotes the addition of ubiquitin groups. The addition of ubiquitin groups targets HIF-1α for degradation in the proteasome. **B:** Under hypoxic conditions, the HPHs cannot hydroxylate HIF-1α on proline residues owing to their requirement for molecular oxygen, and HIF-1α becomes stabilized. It then binds with the HIF-1β subunit in the nucleus and promotes transcription of at least 50 target genes that regulate angiogenesis, erythropoiesis, tissue remodeling, and glycolysis. Most of the studies to date have shown that HIF is essential for tumor growth.


Angiogenesis
Angiogenesis Inhibitors
Angiogenic Factors
Adhesion Molecules and Related Proteins
Cathepsins
Extracellular Matrix
Gene Regulation and Expression
Growth Factors and Cytokines
Hormones
Lipid Signaling
Kinases
Nitric Oxide
Plasma Factors
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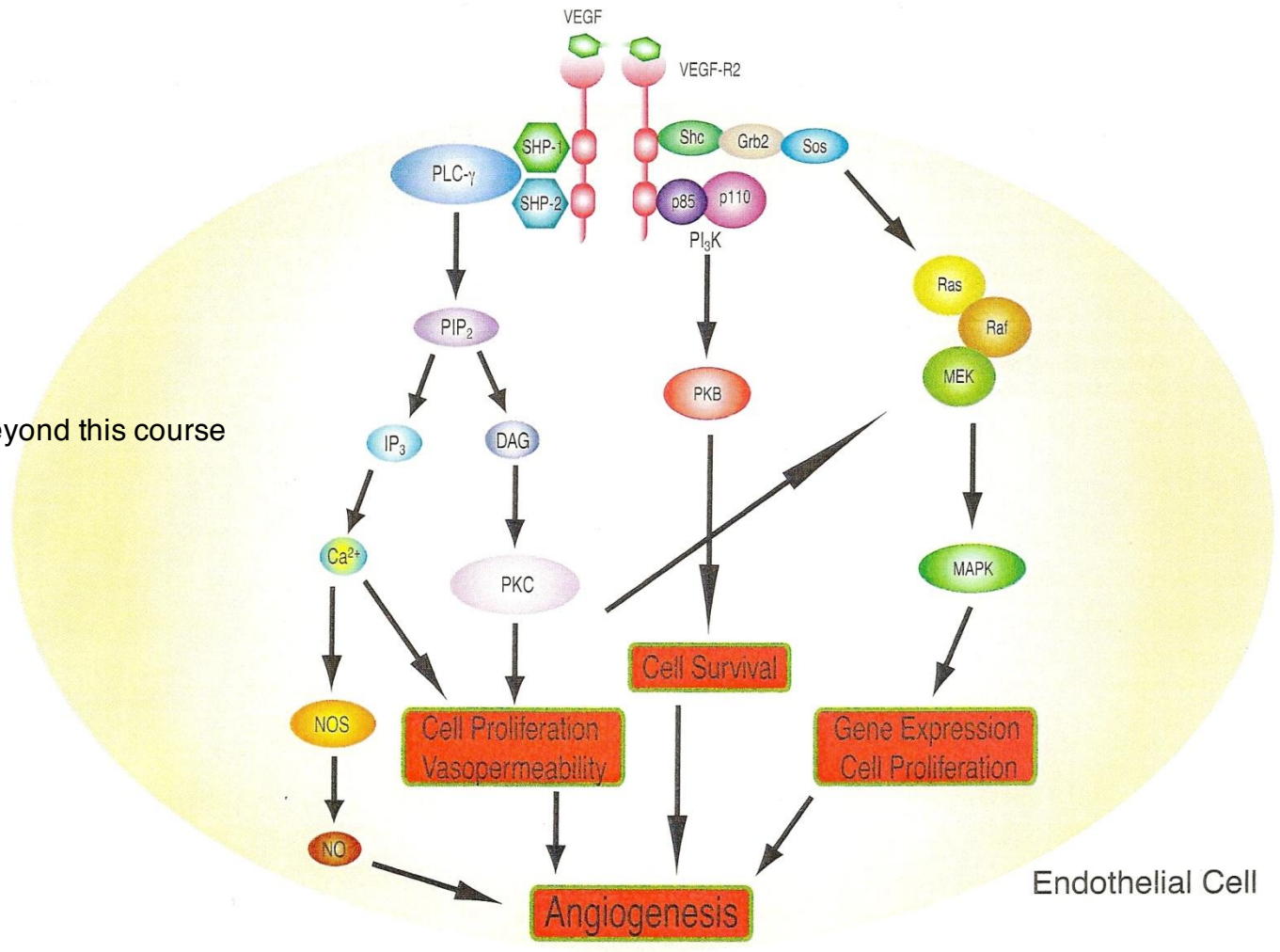
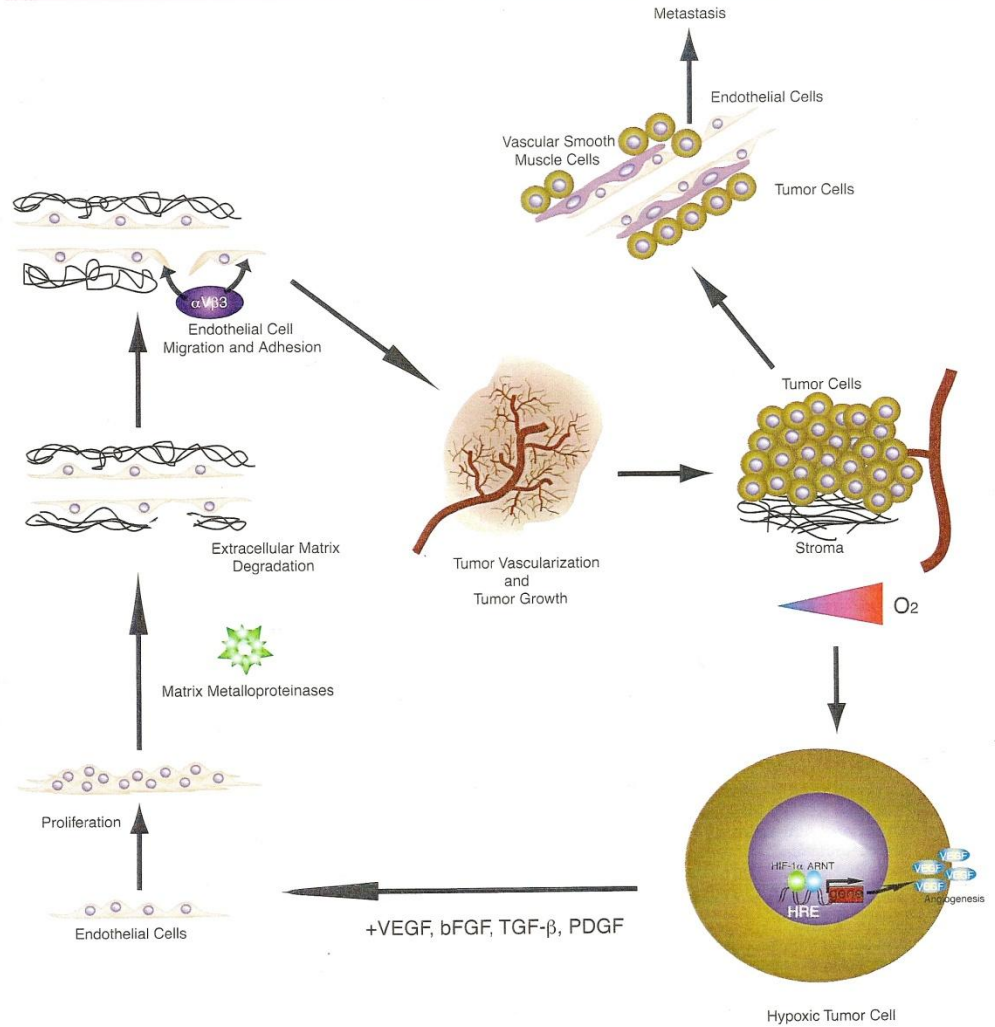
well beyond this course

## Signaling Pathways Activated by VEGF




**Signaling pathways activated by VEGF.** VEGF regulates several endothelial cell functions, including proliferation, differentiation, permeability, vascular tone, and the production of vasoactive molecules. Upon ligand binding, the receptor tyrosines are phosphorylated, allowing the receptor to associate with and activate a range of signaling molecules, including phosphatidylinositol 3-kinase ($PI_3K$), Shc, Grb2, and the phosphatases SHP-1 and SHP-2. VEGF receptor activation can induce activation of the MAPK cascade via Raf stimulation leading to gene expression and cell proliferation, activation of $PI_3K$ leading to PKB activation and cell survival, activation of PLC-γ leading to cell proliferation, vasopermeability, and angiogenesis.

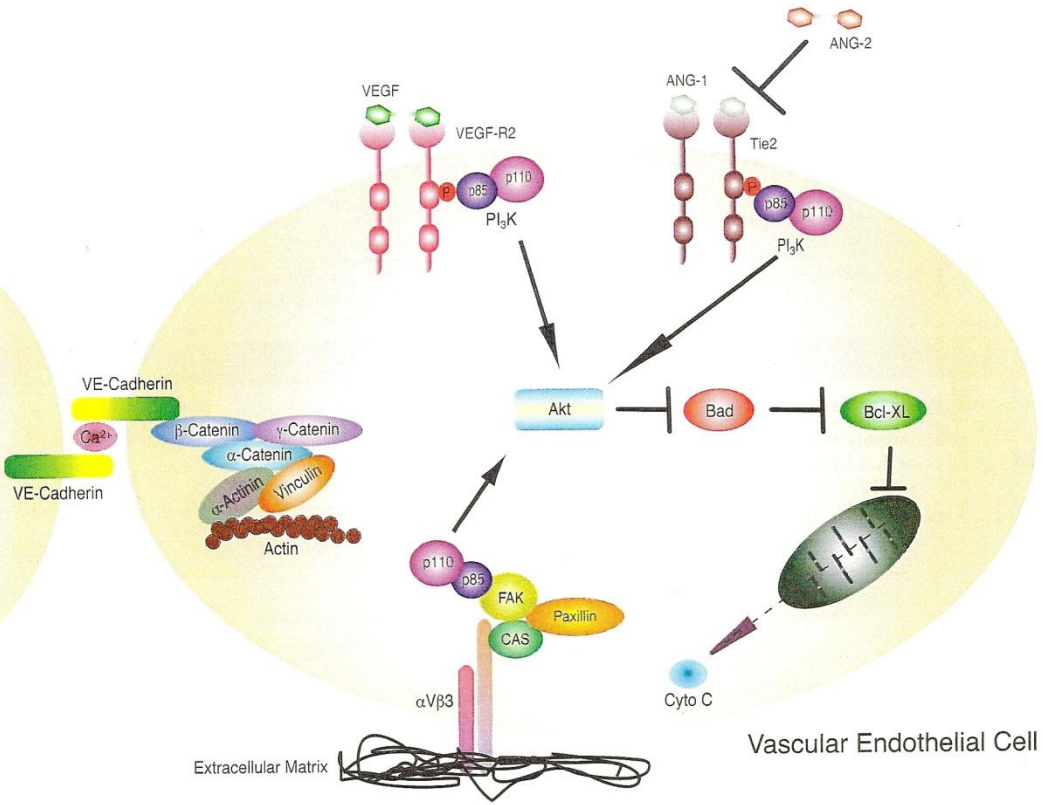
## Events Leading to Angiogenesis and Metastasis




**Events leading to angiogenesis and metastasis.** Early tumors begin as small masses no larger than about 1 $mm^3$ in diameter. In the absence of angiogenesis, tumors are unable to grow further, although active cell proliferation, counter-balanced by apoptosis, occurs continually in these tumors. The tumor cells farthest removed from the blood supply become hypoxic. In response to the hypoxic environment, hypoxia-inducible factor-1α (HIF-1α) accumulates and translocates to the nucleus where it dimerizes with its partner, aryl hydrocarbon receptor nuclear translocator (ARNT) and promotes transcription of many downstream target genes, including the gene for vascular endothelial growth factor (VEGF). In effect, an angiogenic switch is flipped, that allows for the formation of a neovasculature that is necessary for tumor growth.

Interactions between tumor cells, stromal cells, and endothelial cells trigger the secretion and activation of matrix metalloproteinases that degrade the extracellular matrix and permit the budding of new blood vessels from existing vessels. The proliferation and migration of vascular endothelial cells are triggered by angiogenic growth factors secreted by tumor cells, such as VEGF, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF). Blood vessels that are established in the tumor tissue permit the invasion of tumor cells into the bloodstream, which carries these cells to additional sites in the body, where they may establish new tumors or metastases.

## Vascular Endothelial Cell Interactions and Signaling




**Vascular endothelial cell interactions and signaling.** Endothelial cells use cell adhesion molecules, such as integrins and cadherins to attach themselves to each other and to the vascular extracellular matrix. Vascular endothelial (VE)-cadherin mediates the calcium-dependent interactions between neighboring endothelial cells. These adherens junctions are believed to provide a mechanical barrier to interendothelial leakage. The short cytoplasmic tail of VE-cadherin is able to interact with the plakoglobin complex (α-, β-, and γ-catenin) and this complex interacts with α-actinin and vinculin to link the VE-cadherin-catenin complex to the actin cytoskeleton. VE-cadherin coordinates with VEGFR-2 (Flk-1) to mediate $PI_3K$/Akt-dependent endothelial cell survival. Additionally, endothelial cells adhere to the extracellular matrix (ECM) through interactions with cell surface heterodimeric integrins (e.g., αVβ3). The ECM is a dense network of collagen and elastin contained in a complex of proteoglycans and glycoproteins. The engagement of integrins with the ECM causes the activation of focal adhesion molecules, such as focal adhesion kinase (FAK). Activated FAK recruits Src, which phosphorylates FAK on additional sites allowing for the subsequent recruitment of signaling molecules such as phosphatidylinositol 3-kinase ($PI_3K$), CAS, and paxillin to focal adhesions. In addition to adhesion-dependent cell survival, endothelial cells can also respond to receptor tyrosine kinase-mediated survival signals via VEGF through its receptor VEGFR-2 and via angiopoietin-1 (ANG-1) through its receptor Tie-2. Both VEGF and ANG-1 allow endothelial cells to escape apoptosis (anoikis) upon detachment from the ECM.

# Does Hypoxia Matter Clinically ?

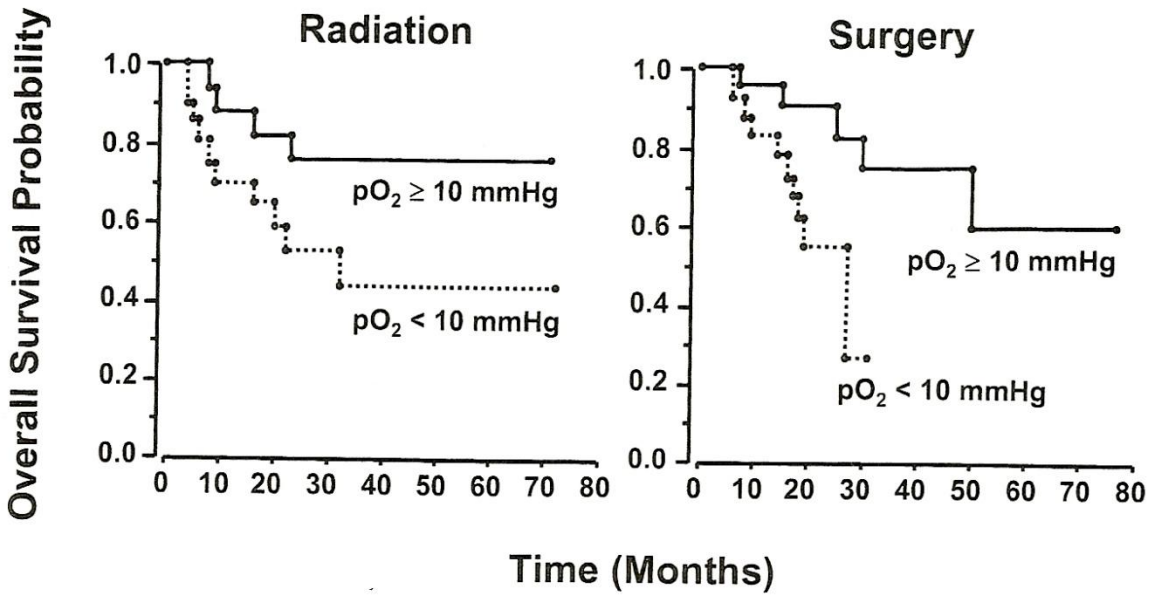



***FIGURE 23.5*** Recurrence-free survival in patients with advanced carcinoma of the cervix treated either by a combination of external-beam radiotherapy and high-dose-rate brachytherapy or by surgery. The patients were divided into two groups on the basis of pretreatment oxygen-probe measurements that indicated mean $pO_2$ values of less than, or of greater than or equal to 10 mm Hg. The natural interpretation of the radiotherapy trial is that hypoxia compromises the efficacy of radiation. The fact that poorer survival is seen after surgery when tumors are hypoxic suggests that hypoxia results in more aggressive tumors. (Left panel adapted from Höckel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P: Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res* 56:4509–4515, 1996; right panel adapted from Höckel M, Knoop C, Schlenger K, et al.: Intratumoral $pO_2$ predicts survival in advanced cancer of the uterine cervix. *Radiother Oncol* 26:45–50, 1993, with permission.)

# Recap of 4 R’s

# Summary of Differential Effects on Tumour and Normal Tissues

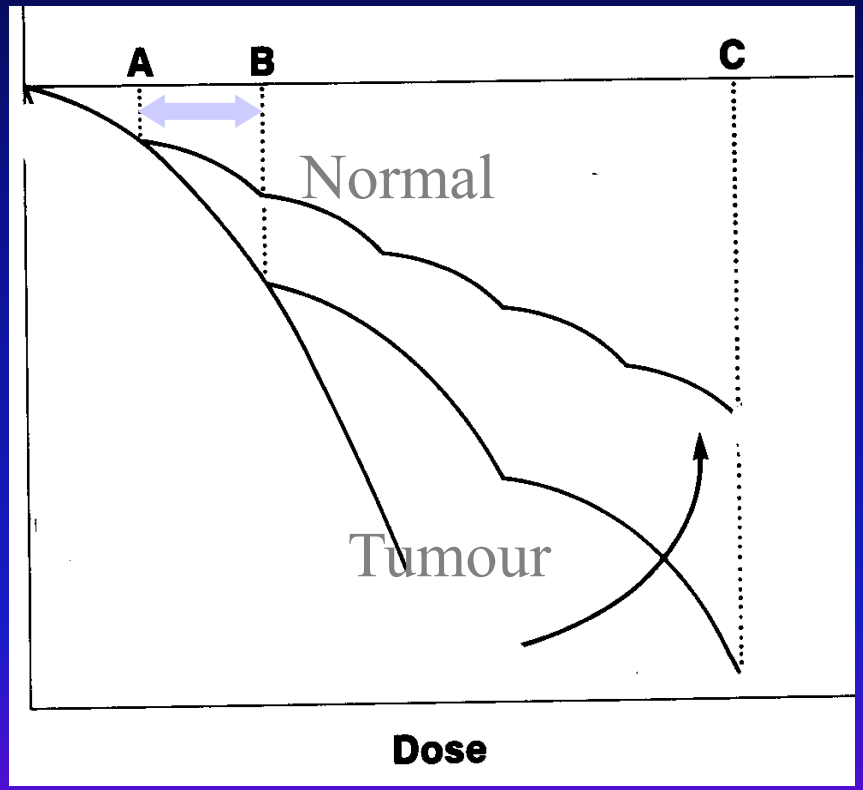
| 4Rs | Tumour Cells | Normal Cells |
|---|---|---|
| Repair |  |  |
| Re-Assortment |  |  |
| Re-Population |  |  |
| Re-Oxygenation |  |  |

Balance is affected by Dose and Dose Rate/Fractionation

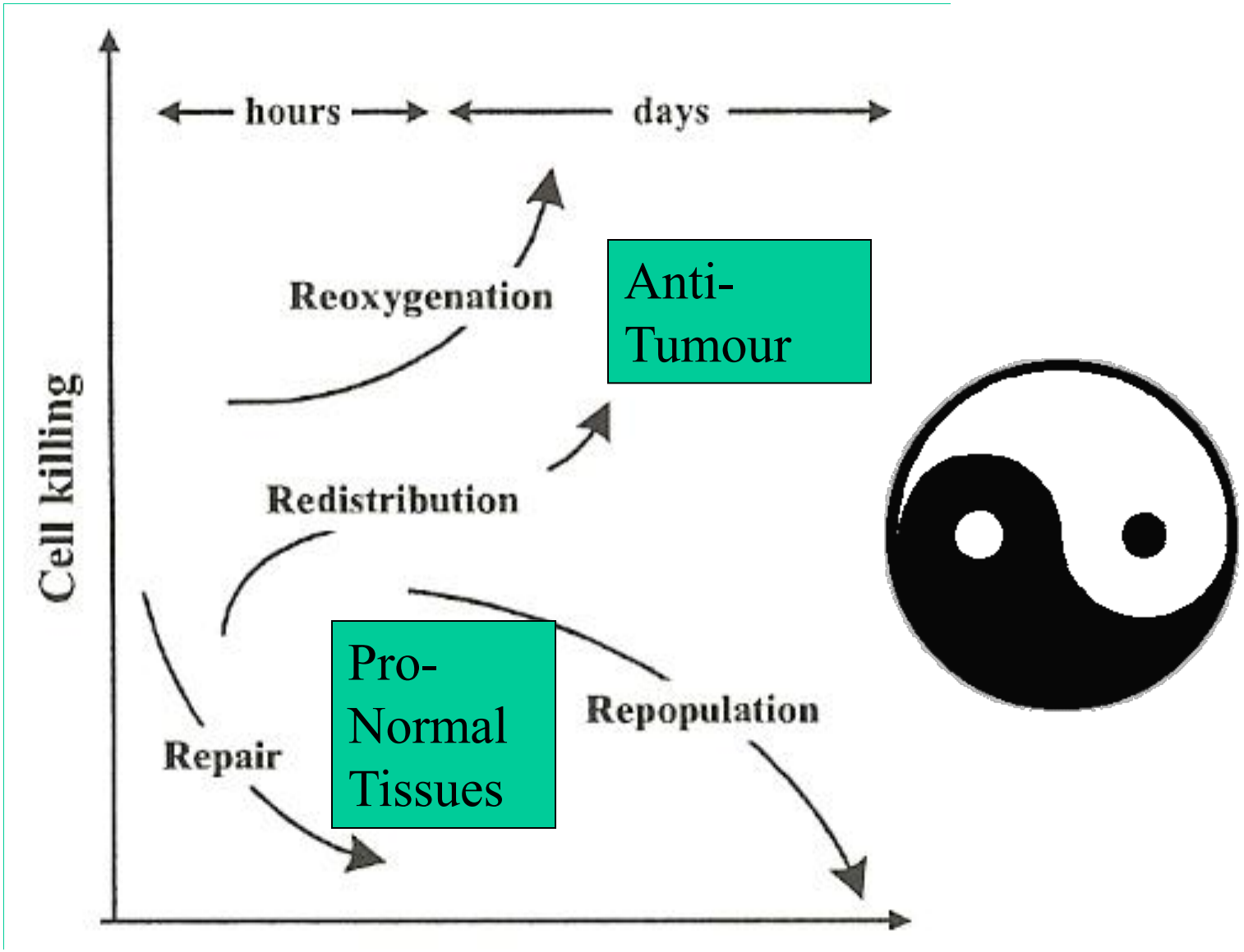
# Time: Dose Fractionation

- Radiation does not "recognize" tumour cells *versus* normal cells
- BUT… but cells express radiation damage differently over time
- PLUS…a lower dose (A) can be delivered to the normal cells *versus* tumour cells (B)

Cell Survival Curves




J²B

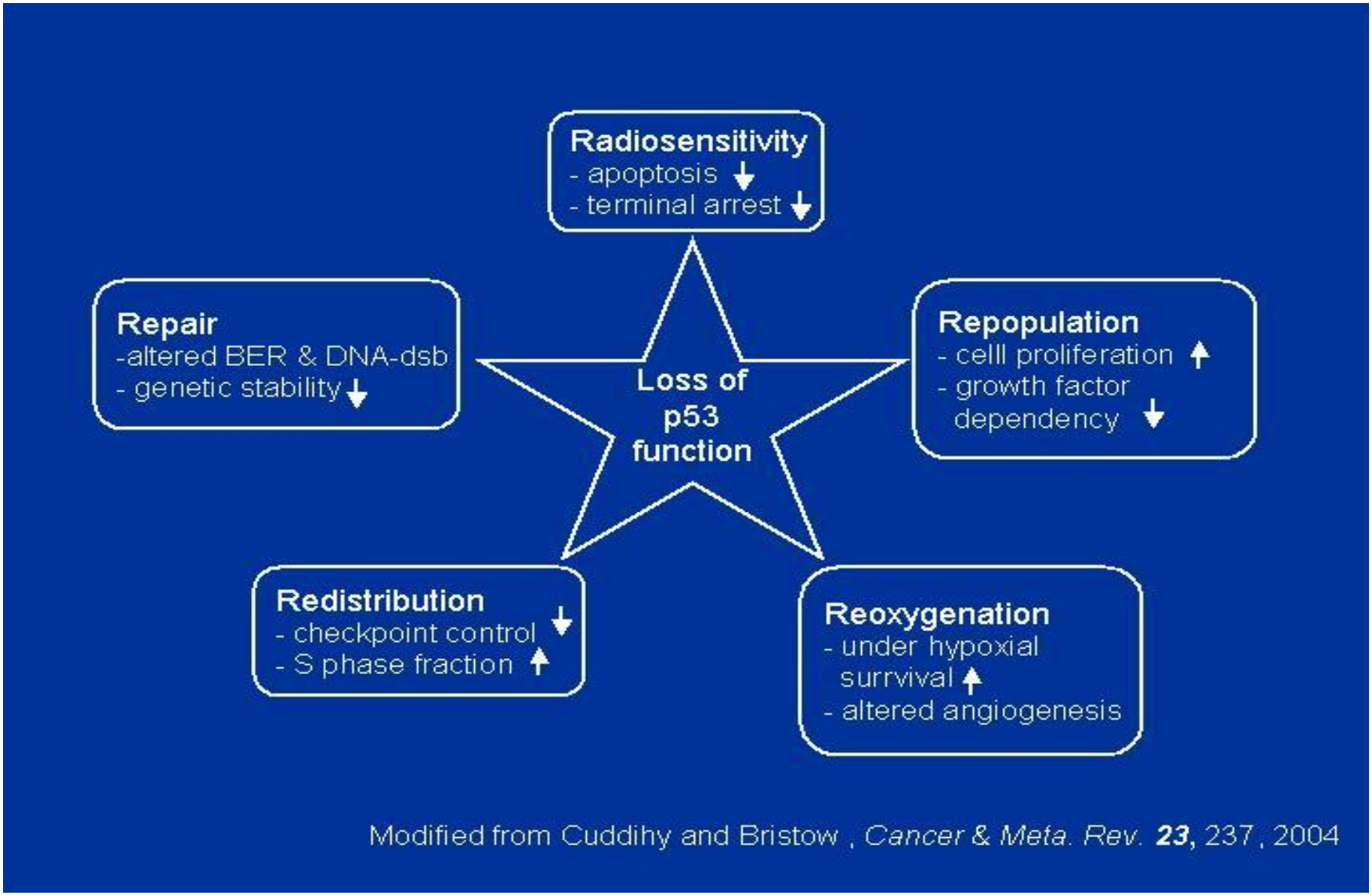



Duration of Chronic Exposure or

Time Gap between Acute Radiation Exposures

RD Stewart et al.

# p53 at the Heart of 5 R's




Modified from Cuddihy and Bristow , *Cancer & Meta. Rev.* ***23***, 237 , 2004

*"It's all a Complex Matter of Space and Time"*

$J^2B$