Sunrise Free Radical School

Society for Free Radical Biology and Medicine

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Physiological Fluxes of Reactive Oxygen Species During Inflammation: *Reality Check*

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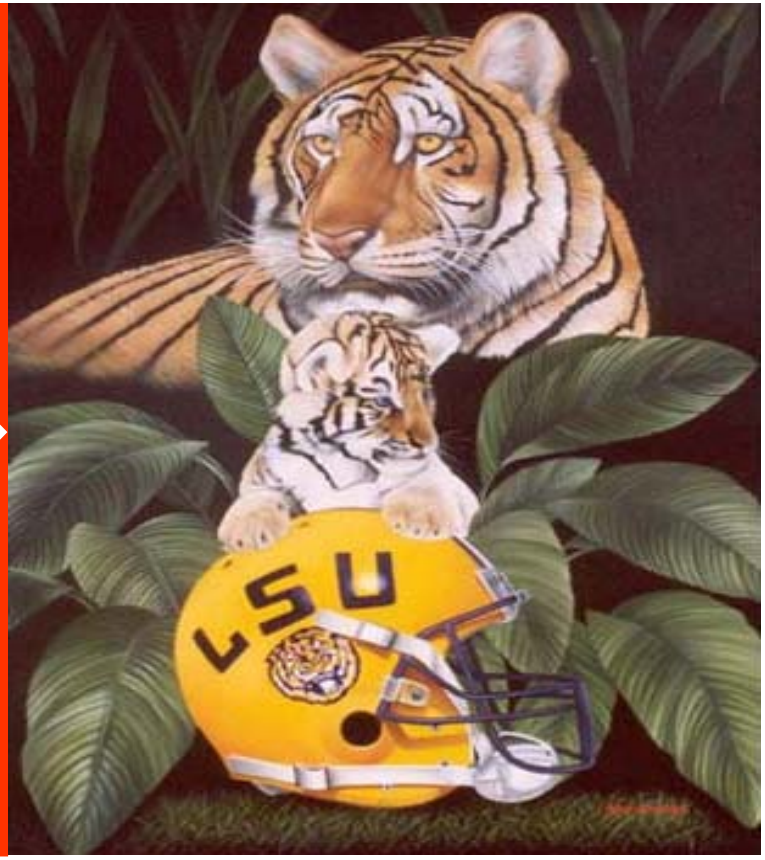
Physiological Fluxes of Reactive Oxygen Species During Inflammation: *Reality Check*



Inflammation **is a** ***protective*** **response designed to destroy invading pathogens**

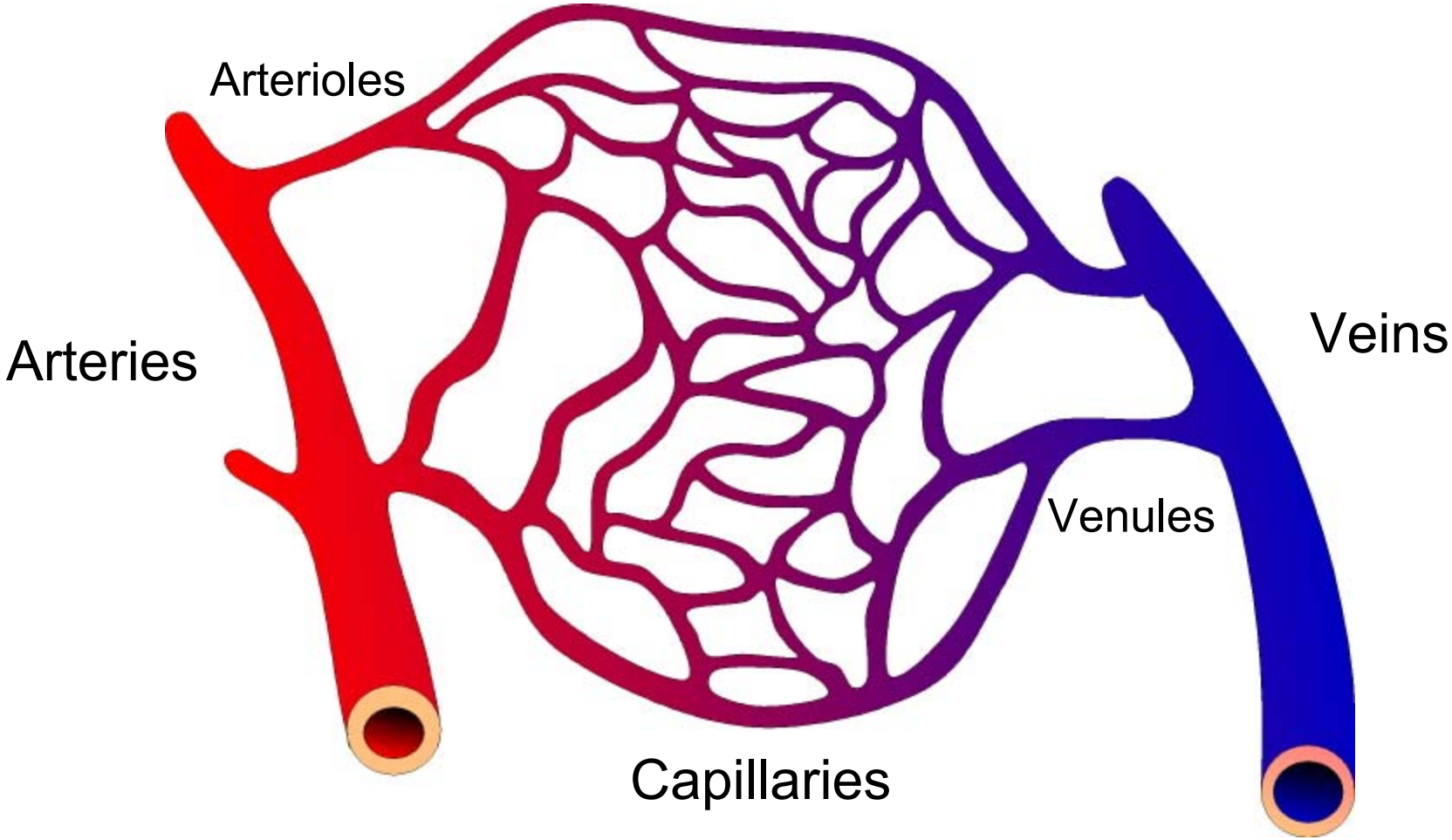
- **Redness (rubor)**
- **Heat (calor)**
- **Swelling (tumor)**
- **Pain (dolar)**

Inflammation is a Double-Edge Sword

- ***Defects*** **in mounting an inflammatory response may lead to grave illness or death.**

- ***Excessive inflammation*** **leads to tissue injury, pain and loss of function.**

Location:
Tissue Microcirculation



PMN-Endothelial Cell Interactions In Response to Infection: Post-Capillary Venules

Circulating PMNs

↓

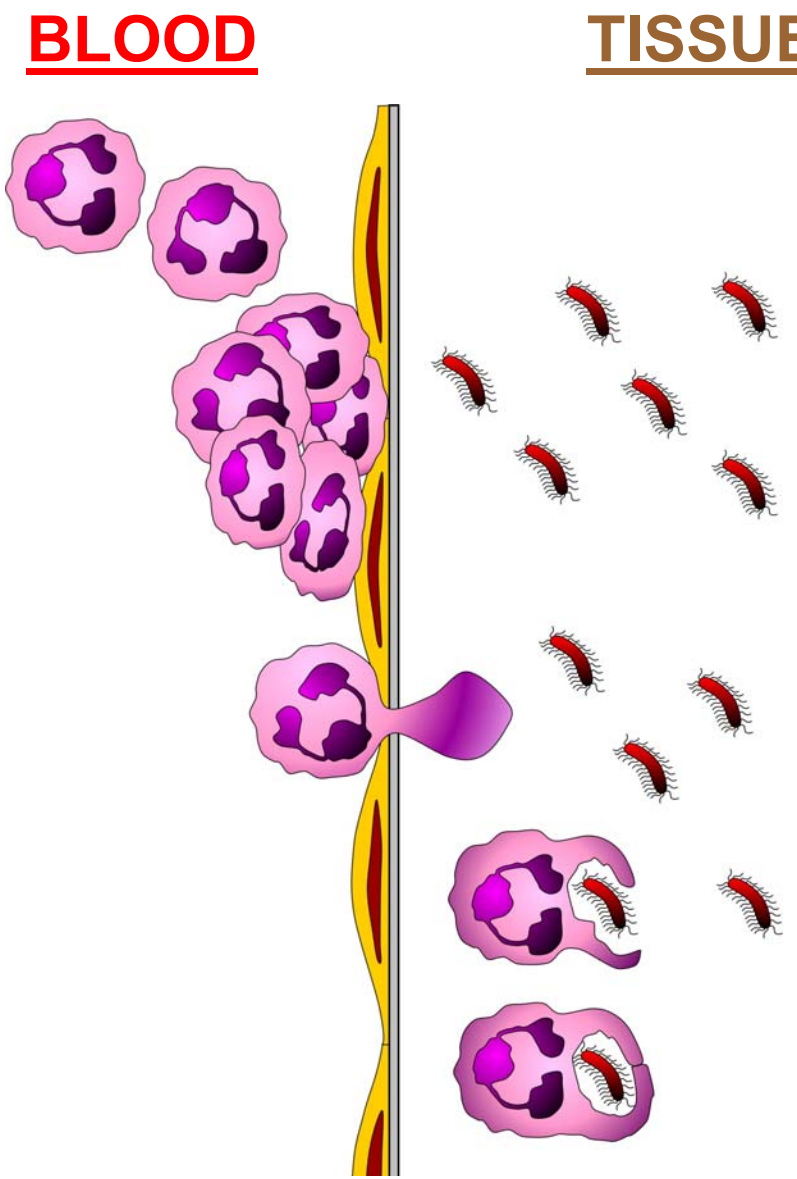
Adhesion

↓

Extravasation

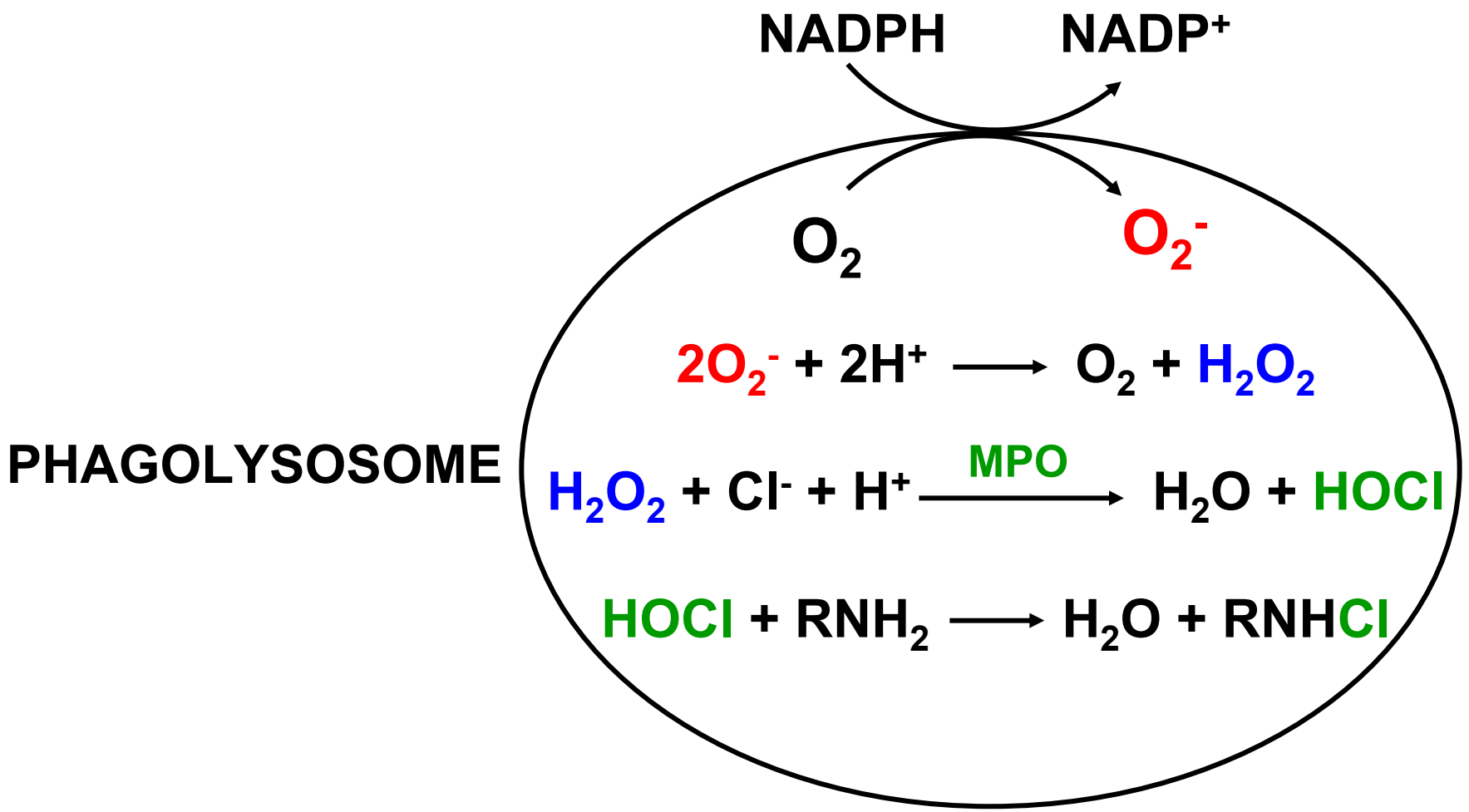
↓

Phagocytosis and Destruction of Bacteria

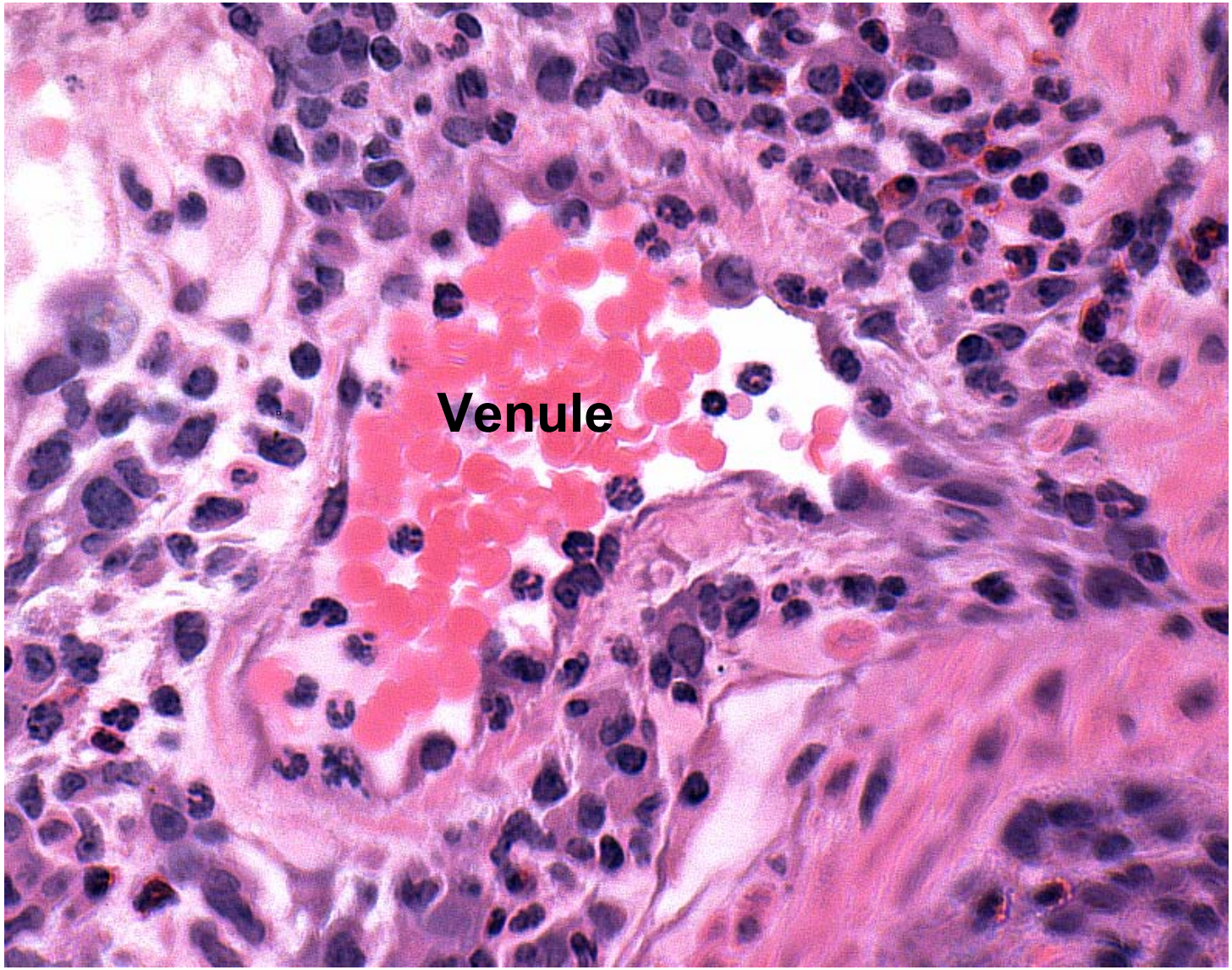



ECKERD
NDC# - 19458-9091-1
Hydrogen Peroxide
U.S.P. 3%
ACTIVE INGREDIENT: HYDROGEN PEROXIDE (H_2O_2) 3%
DIRECTIONS FOR USE: MAY BE USED FULL STRENGTH OR DILUTED WITH WATER
CAUTION: IN CASE OF DEEP OR PUNCTURE WOUNDS OR SERIOUS BURNS,
CONSULT PHYSICIAN. IF REDNESS, IRRITATION, SWELLING OR PAIN PERSISTS OR
INCREASES, OR INFECTION OCCURS, DISCONTINUE USE AND CONSULT
PHYSICIAN. IN CASE OF ACCIDENTAL INGESTION, CONSULT A PHYSICIAN OR
CONTACT A POISON CONTROL CENTER IMMEDIATELY.
STORE WELL TIGHTLY CLOSED.
STORE AT ROOM TEMPERATURE.
KEEP OUT OF REACH OF CHILDREN.
NET 16 FL OZ (1 PT) 473 ML
CLOROX
ULTRA
UNBEATABLE WHITENING
CLOROX
FRESH WILDFLOWERS
BLEACH
3 QTS (96 FL OZ)

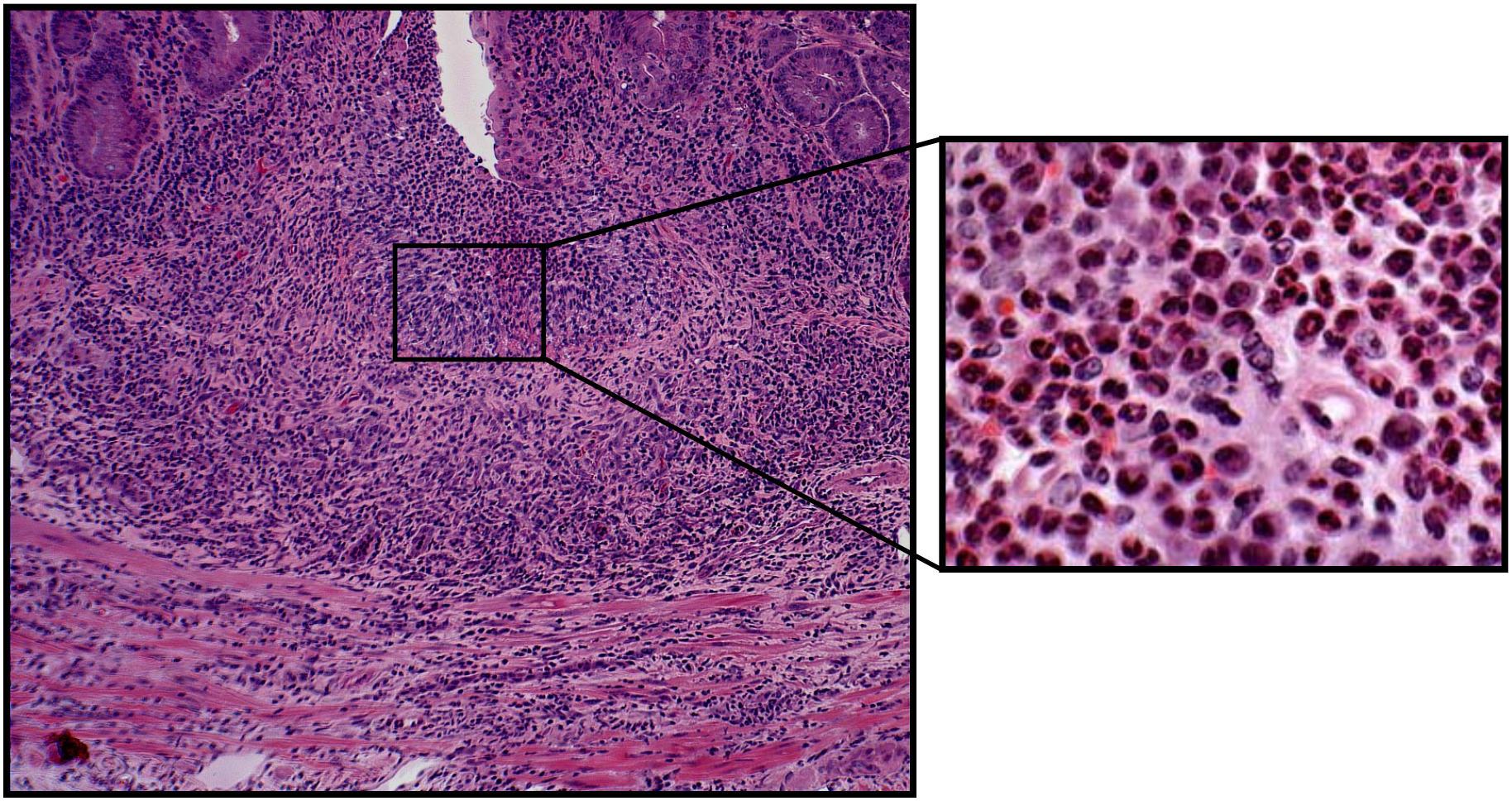
OXIDATIVE BURST



Large Numbers of PMNs Invade Tissue During an Inflammatory Response

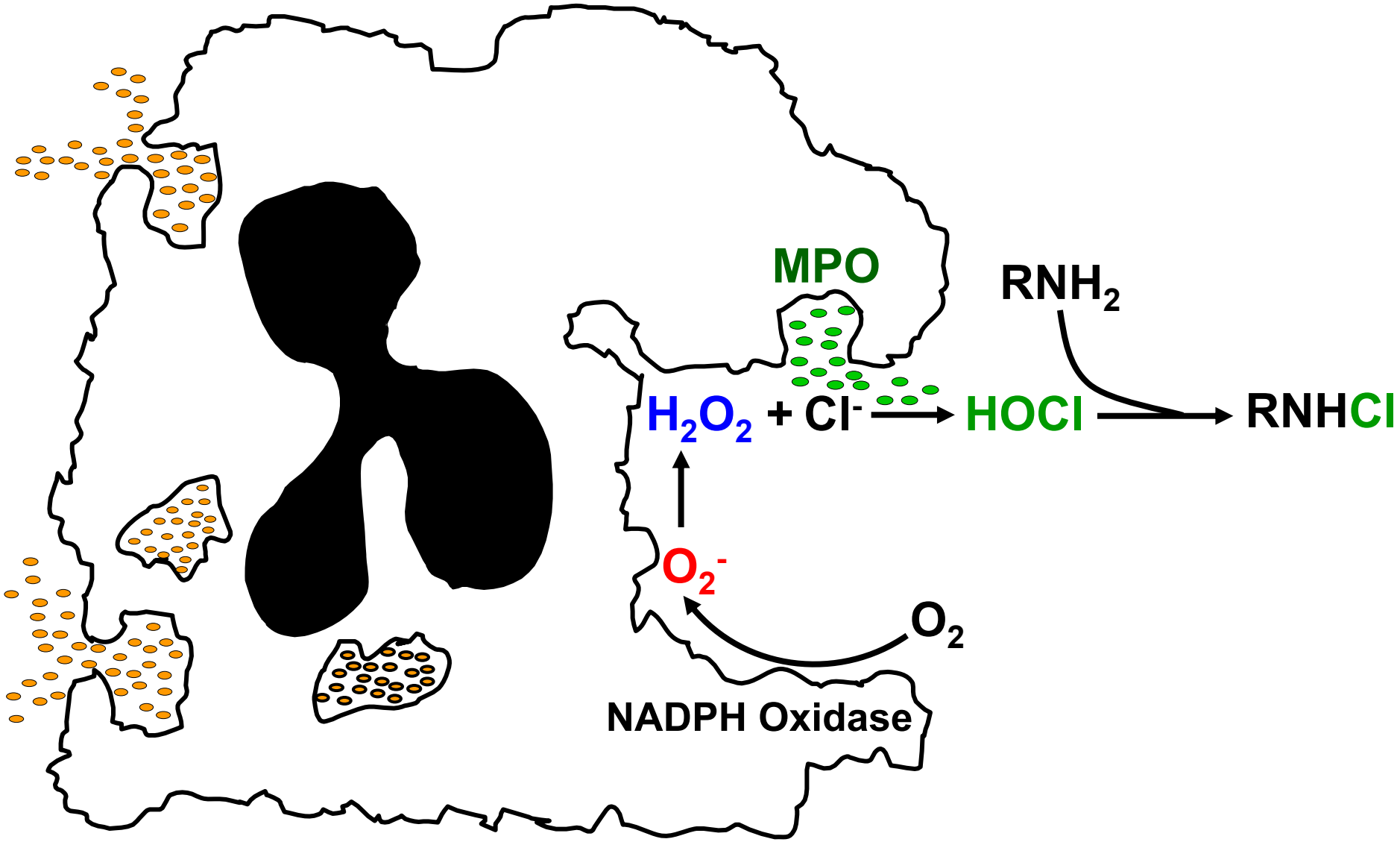


Chronic Gut Inflammation is Characterized By the Infiltration of Large Numbers of PMNs

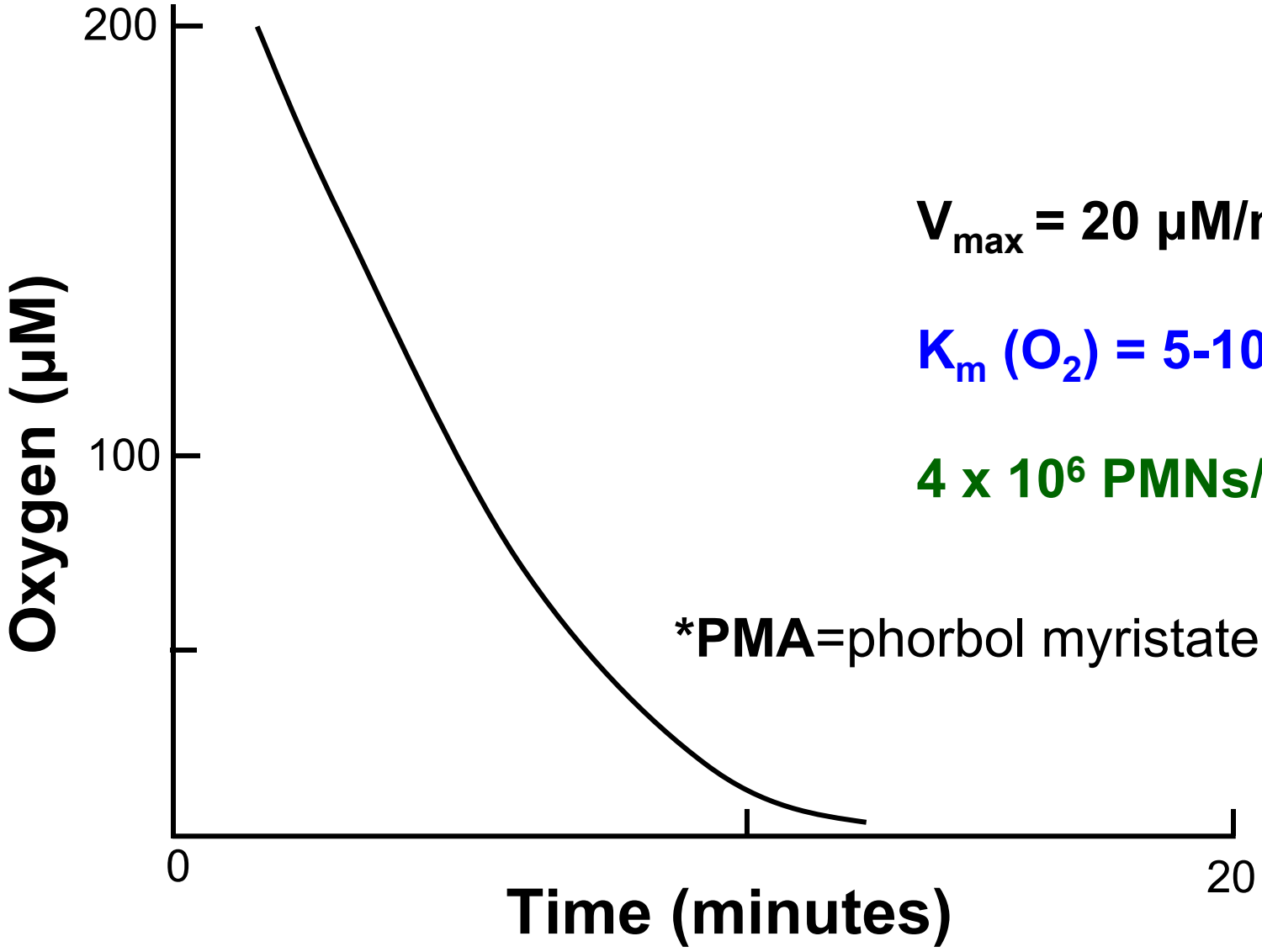


Grisham 2006

ROS Production by *Activated* PMNs



Oxygen Consumption by PMA*-Activated PMNs in Suspension



V_{max} = 20 µM/min

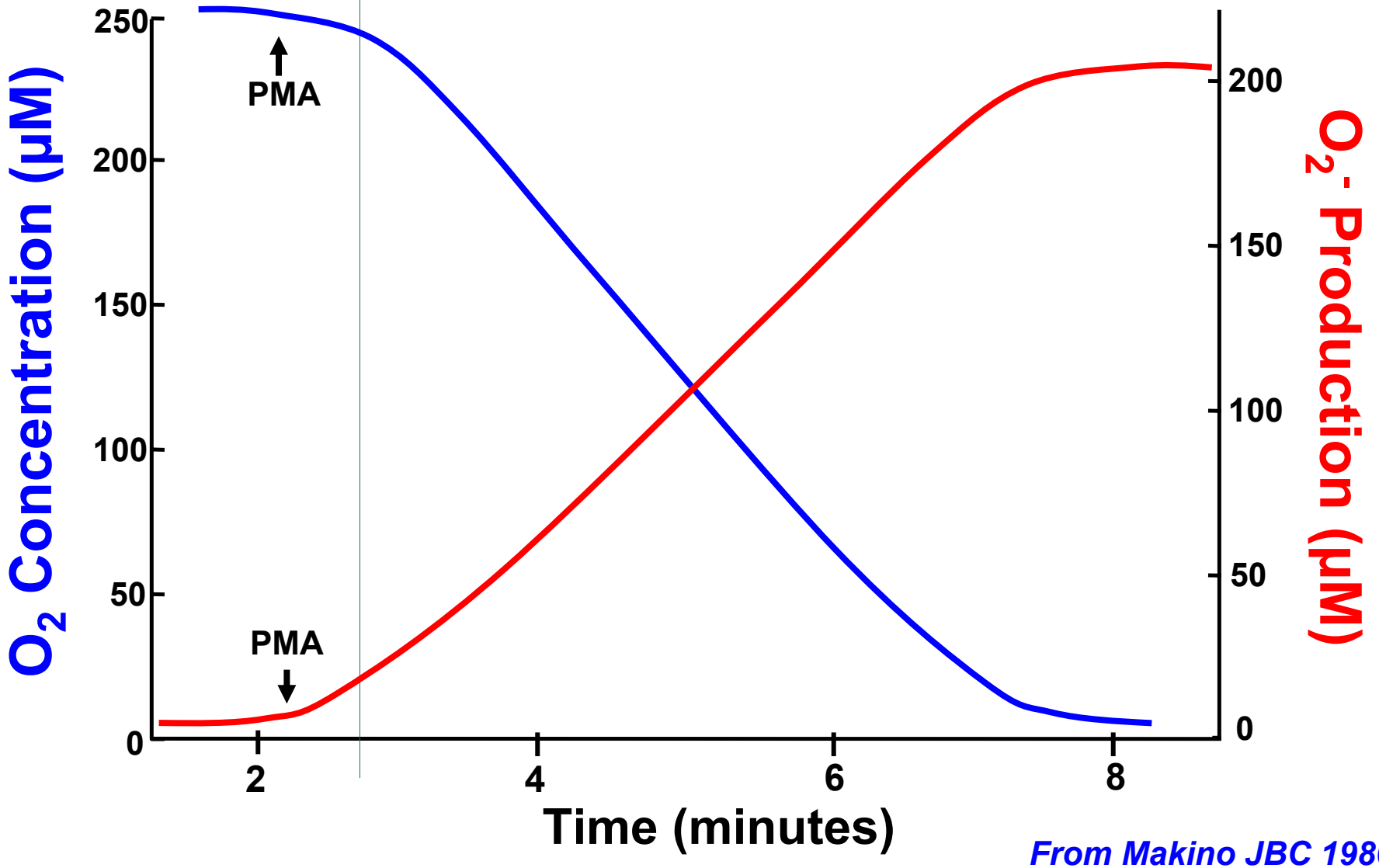
K_m (O_2) = 5-10µM

4 x 10^6 PMNs/ml

***PMA**=phorbol myristate acetate

Grisham et. al. 1983

Stoichiometric Conversion of Oxygen to Superoxide By Activated Neutrophils



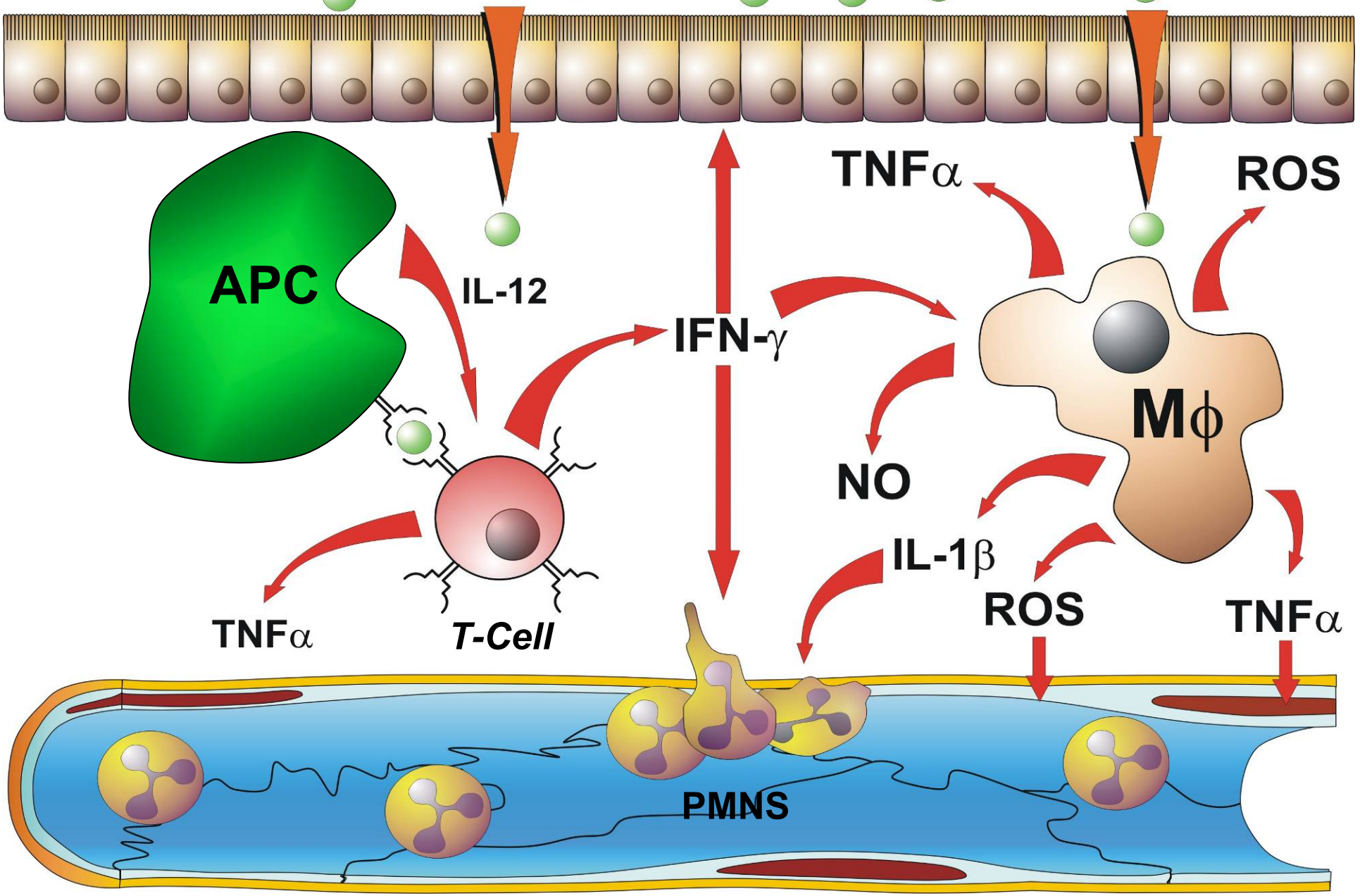
From Makino JBC 1986

Reality Check 1

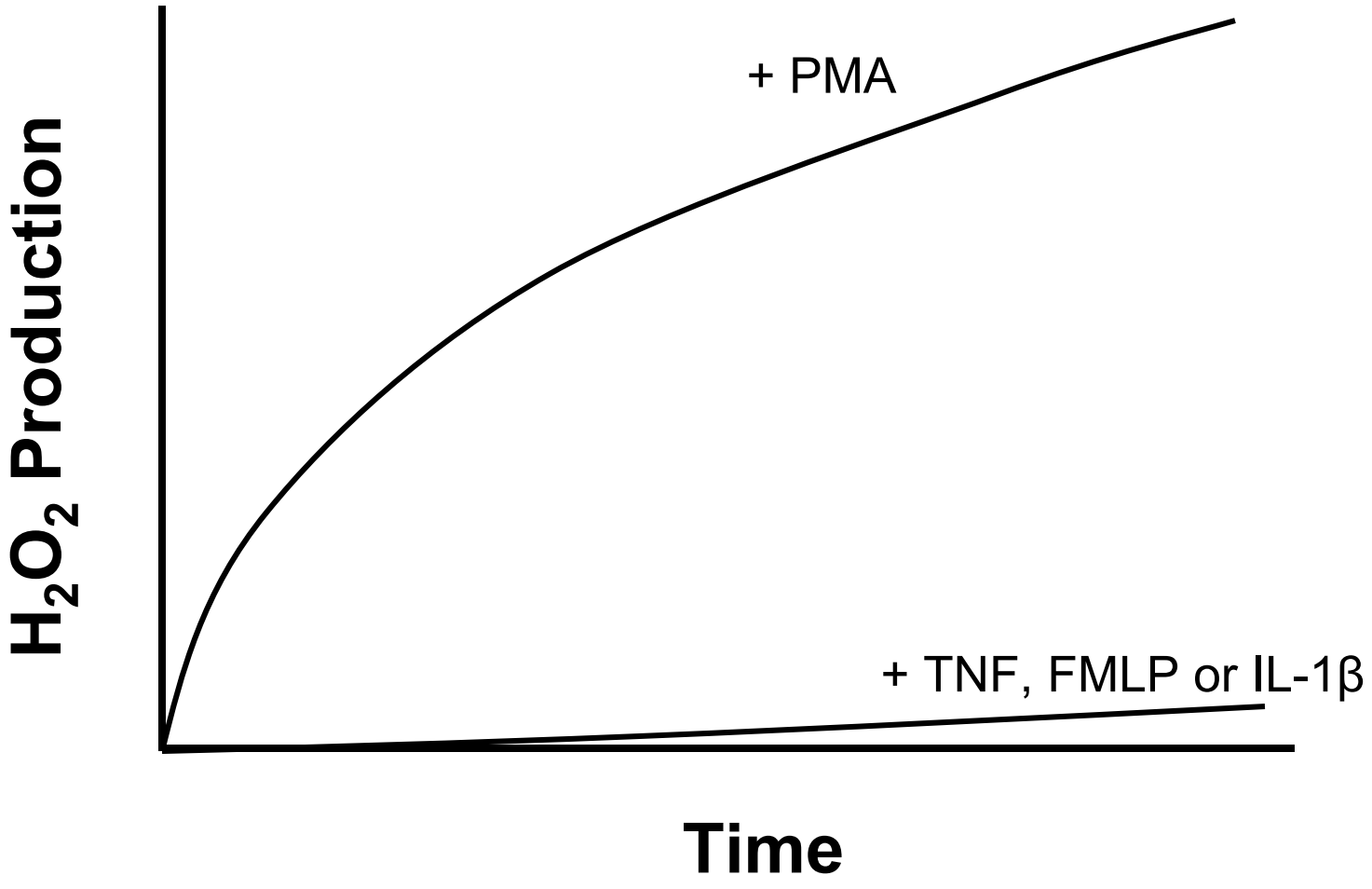
PMA-Stimulated ROS Production by PMNs in *Suspension*:

Is this Physiological?

Inflammatory Mediators (not PMA) Interact with PMNs Associated with Endothelial Cells and the ECM



Biologically-Relevant Inflammatory Mediators Do *NOT* Activate PMNs *in Suspension*



PMN-Endothelial Cell and *PMN-ECM* Interactions In Response to Infection

Circulating PMNs

↓

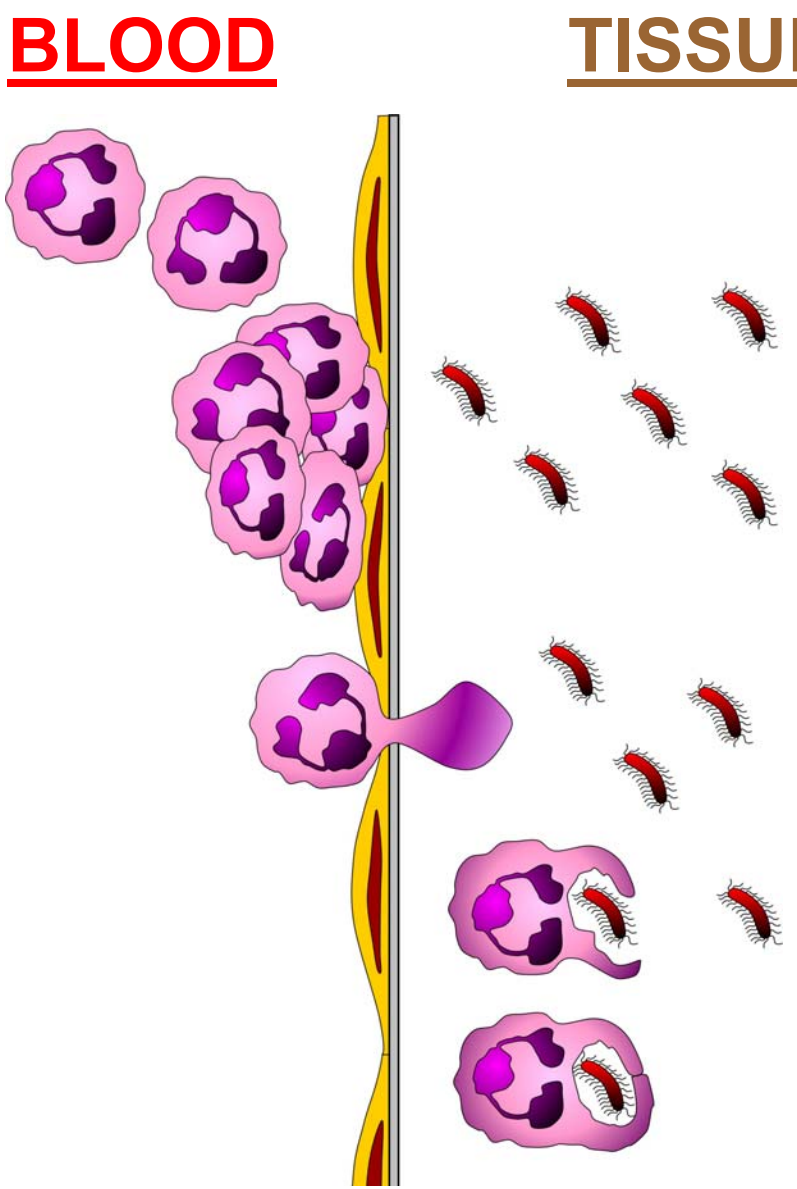
Adhesion

↓

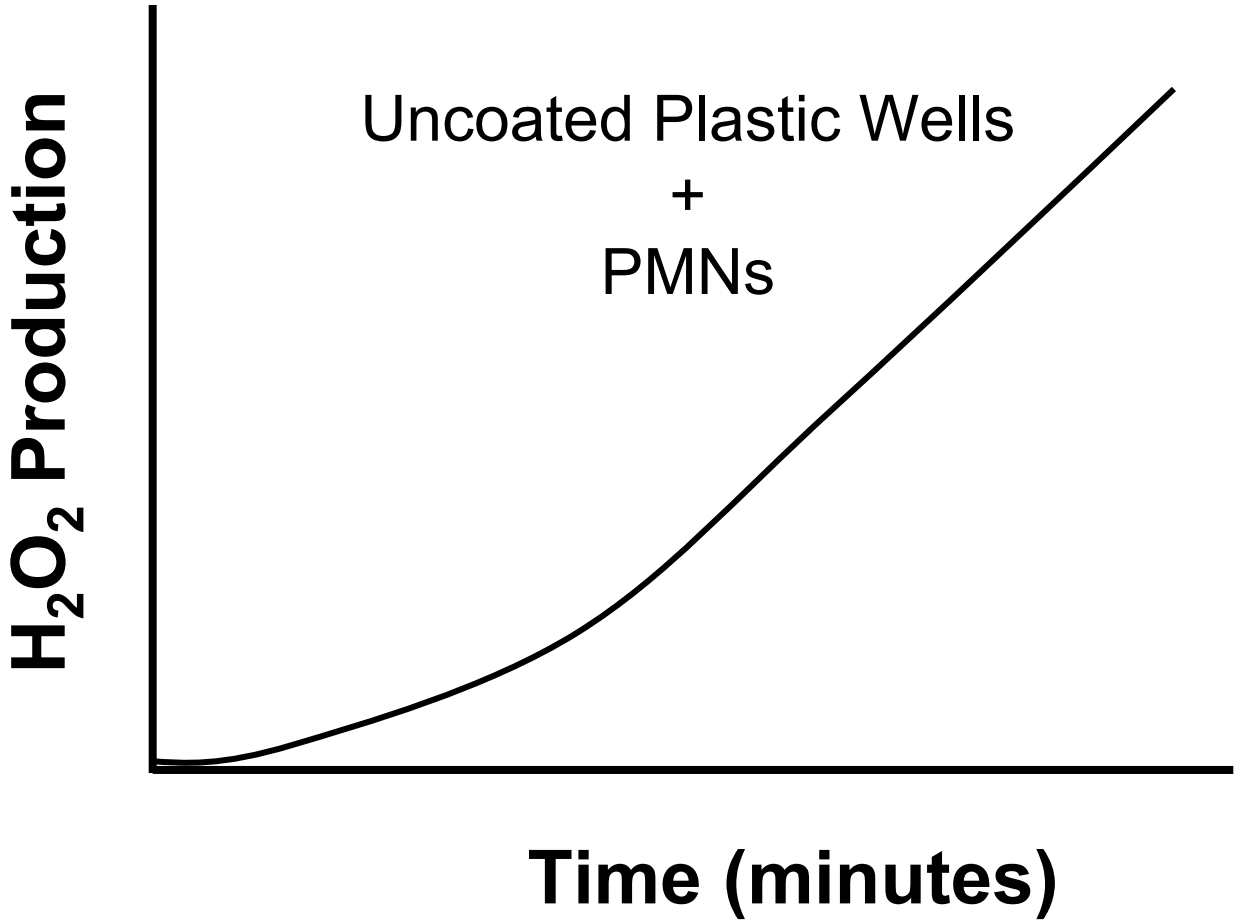
Extravasation

↓

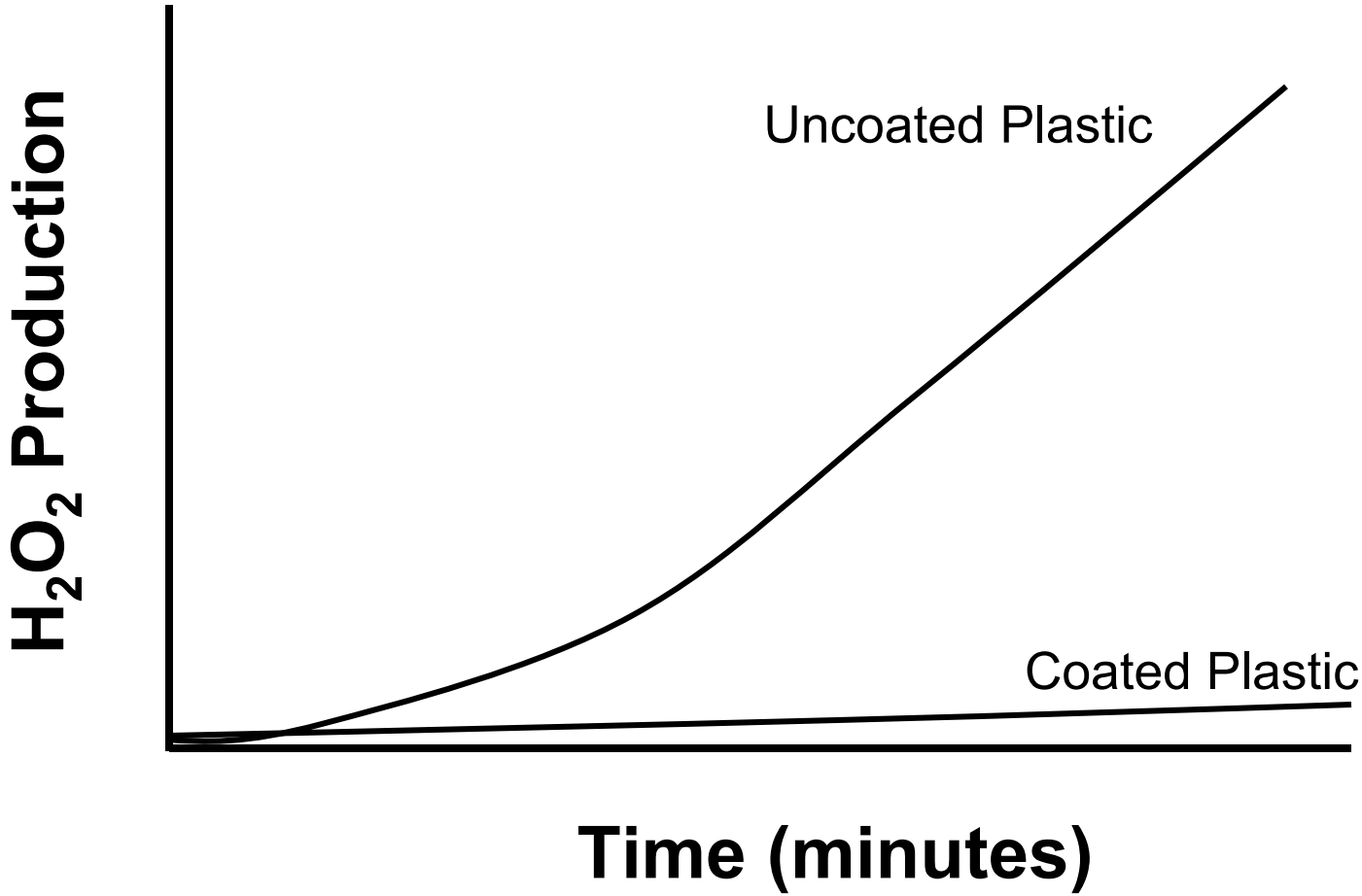
Phagocytosis and Destruction of Bacteria



Plastic Surfaces Activate PMNs

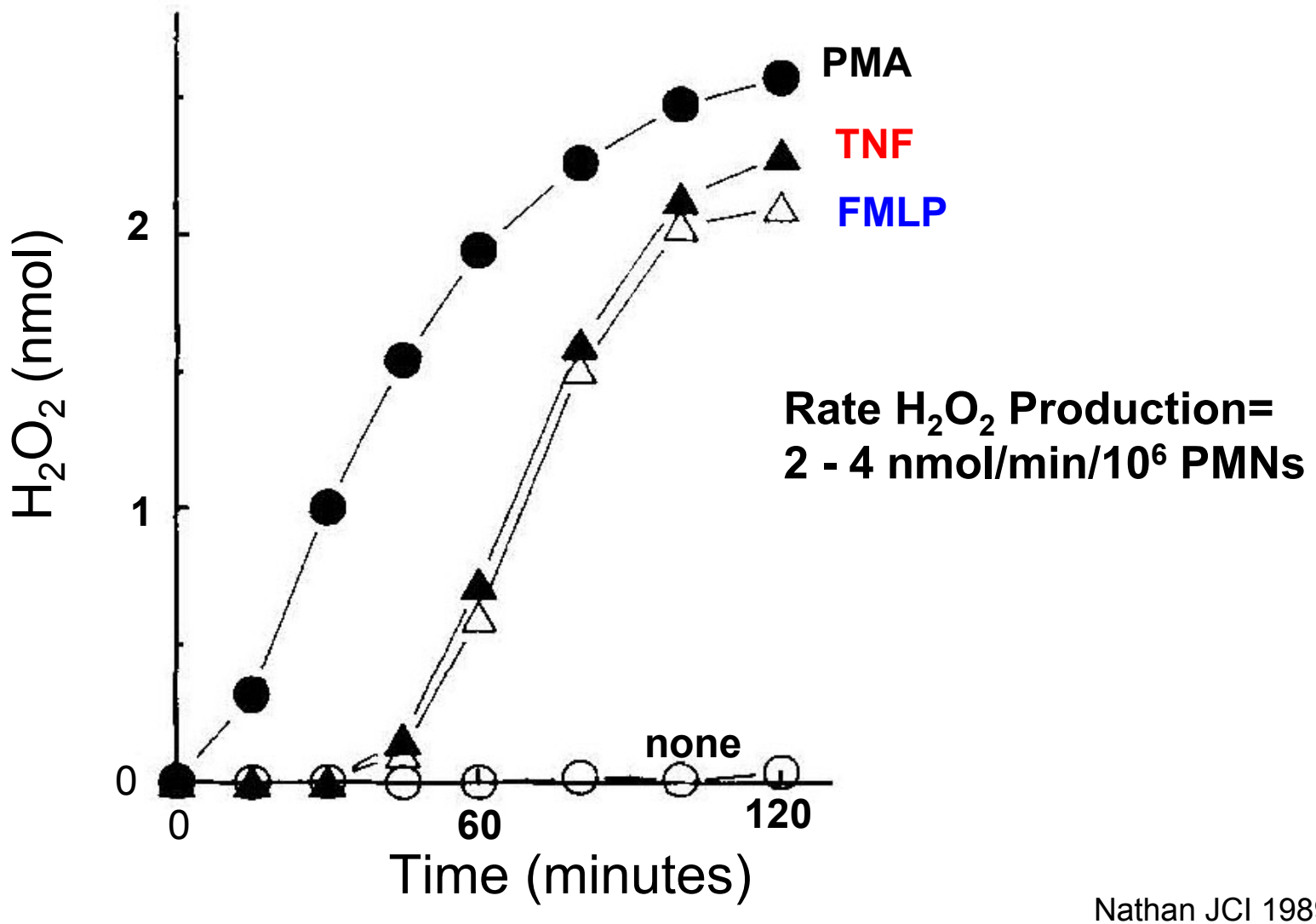


Coating Plastic Surface with Extracellular Matrix Proteins (Serum, Fibronectin or Laminin) Eliminates Spontaneous Activation of PMNs



C.F. Nathan, 1986-90

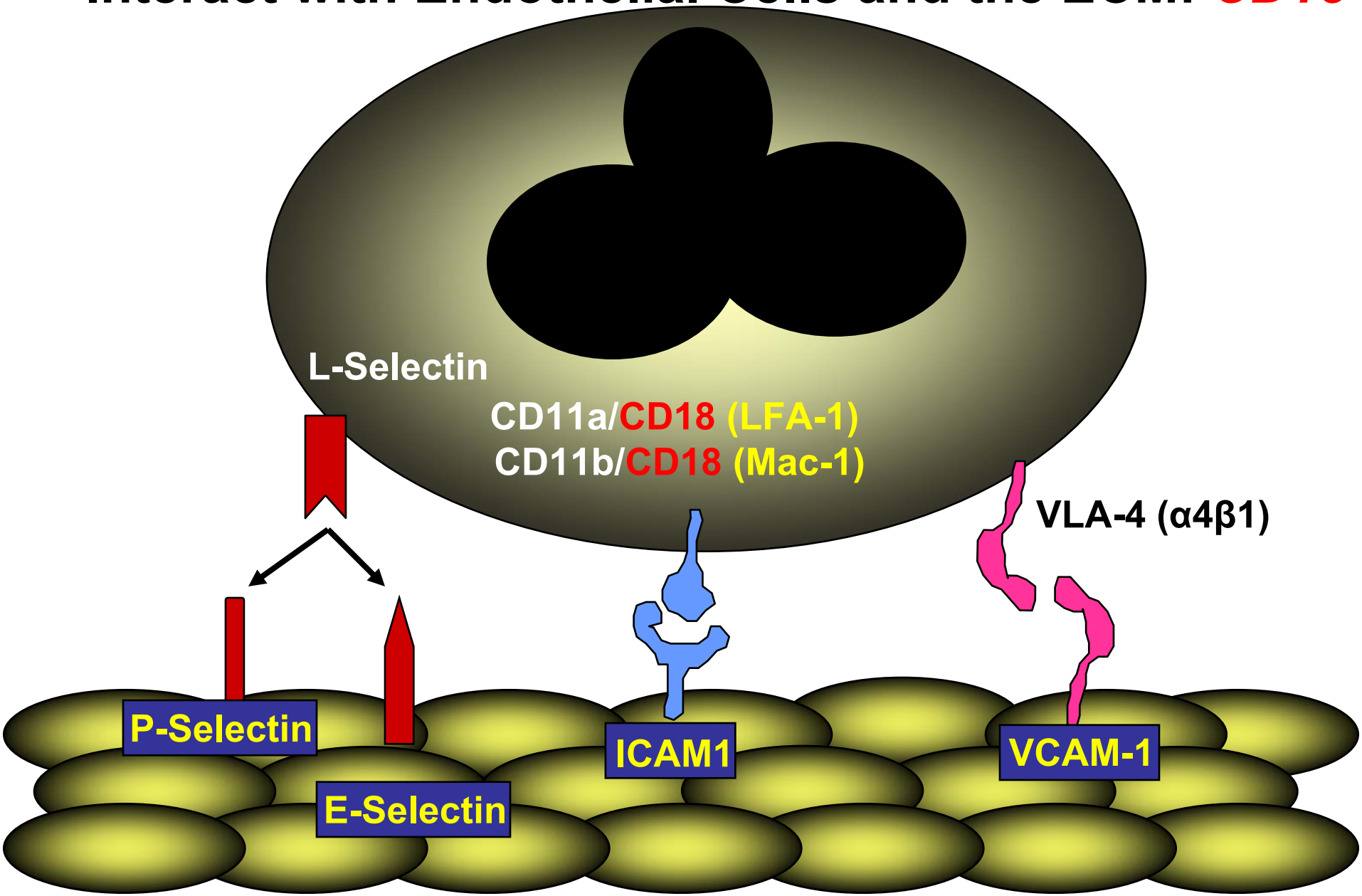
TNF-α or FMLP Induce Large and Prolonged Release of H_2O_2 When PMNs are Plated on Serum, Fibronectin or Laminin-Coated Plastic Surfaces



Rate H_2O_2 Production=
2 - 4 nmol/min/10^6 PMNs

Nathan JCI 1989

PMNs use a Variety of Adhesion Molecules to Interact with Endothelial Cells and the ECM: *CD18*

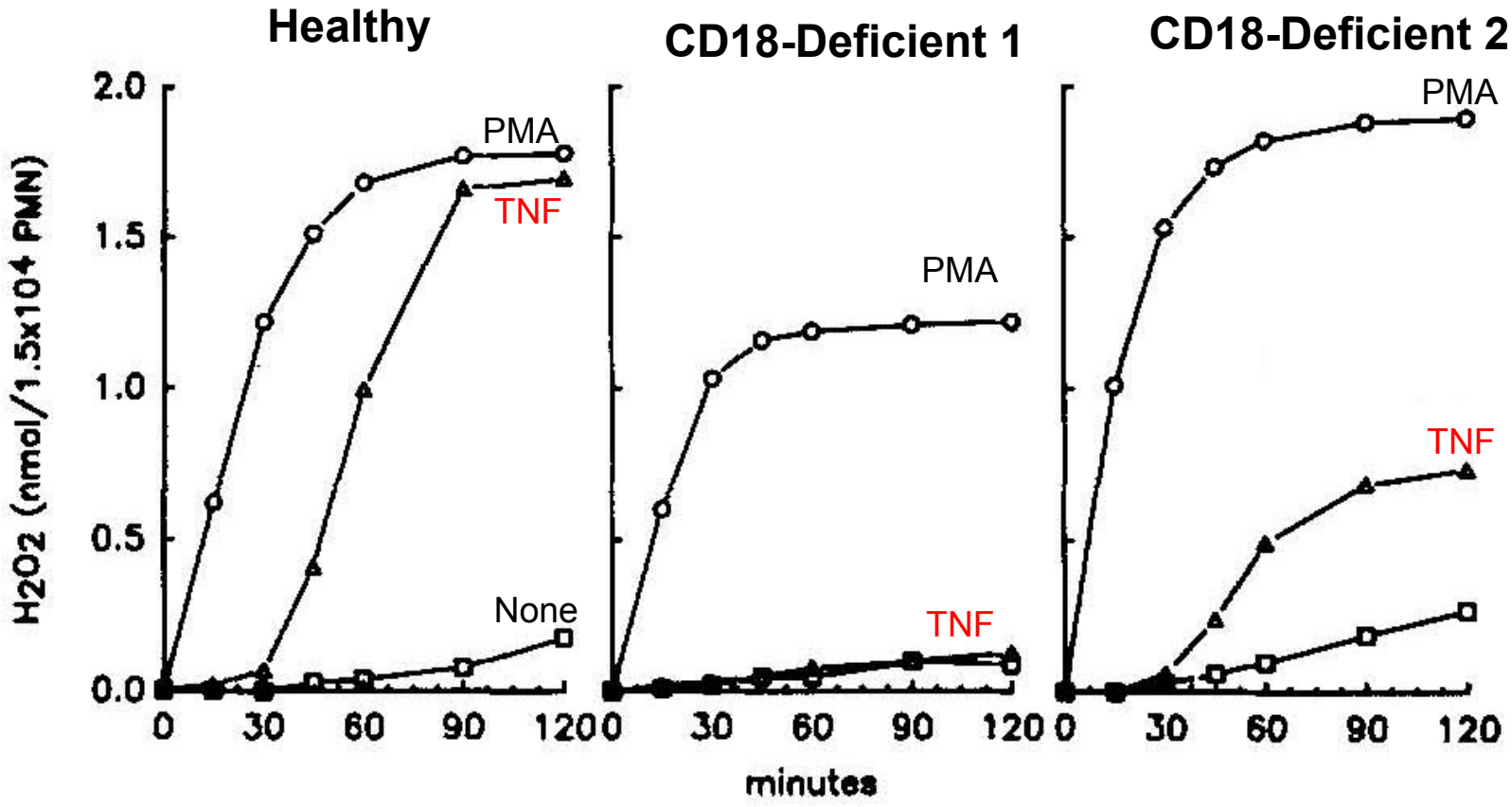


Human and Canine Leukocyte Adhesion Deficiency Disease:

CD18 Deficiency

Lymph Node Infection (Adenitis): Incision to drain abscess took *4 months to heal*

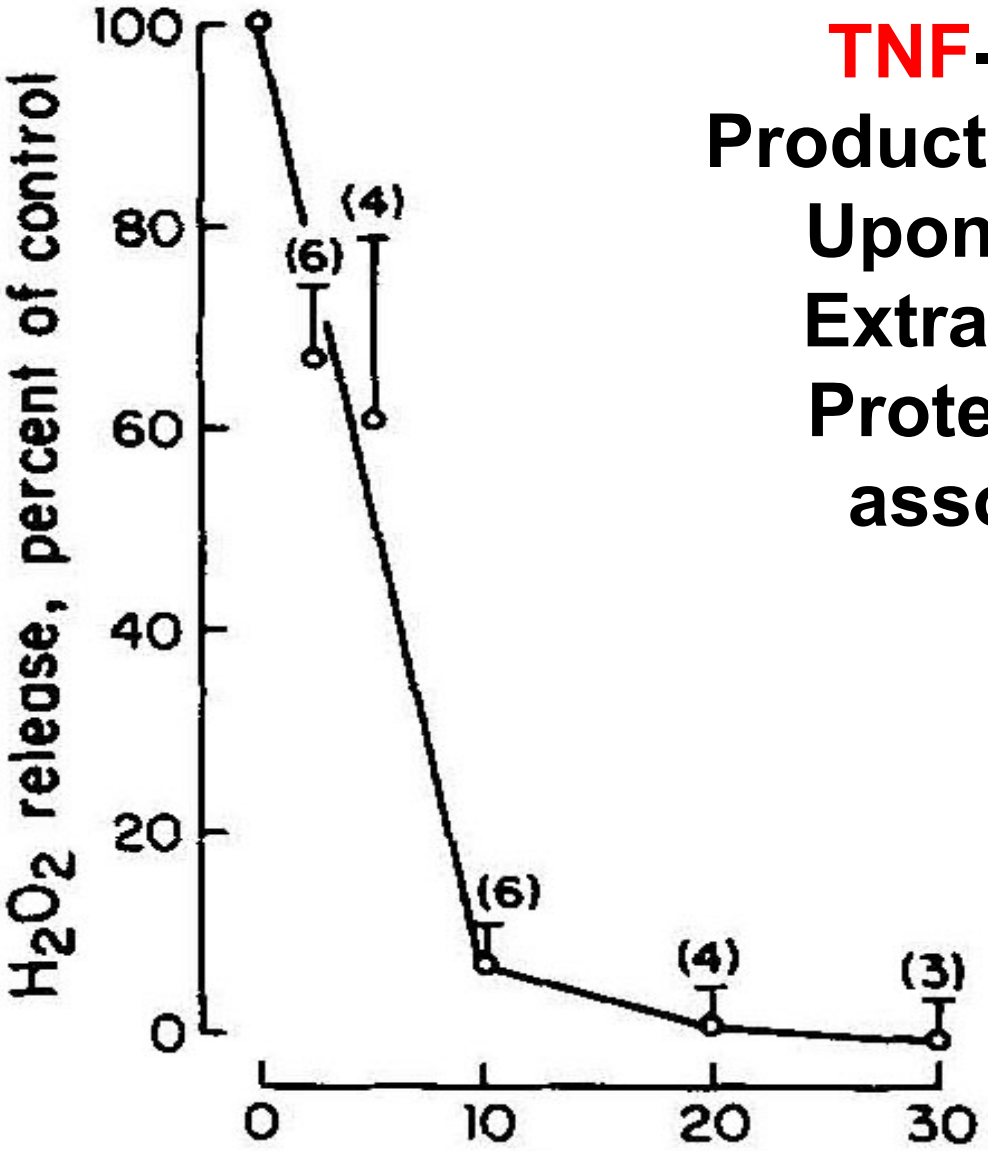
TNF-Induced ROS Production is Dependent Upon the Interaction of the β2 Integrin (CD18) With ECM Proteins



Fibronectin-Coated Wells

Nathan JCB 1989

TNF-Induced ROS Production is Dependent Upon Interaction of Extracellular Matrix Proteins with PMN-associated CD18



Fibronection-Coated Wells

Nathan JCB 1989

Endothelial Cell- PMN Interactions

BLOOD **TISSUE**

Circulating PMNs

↓

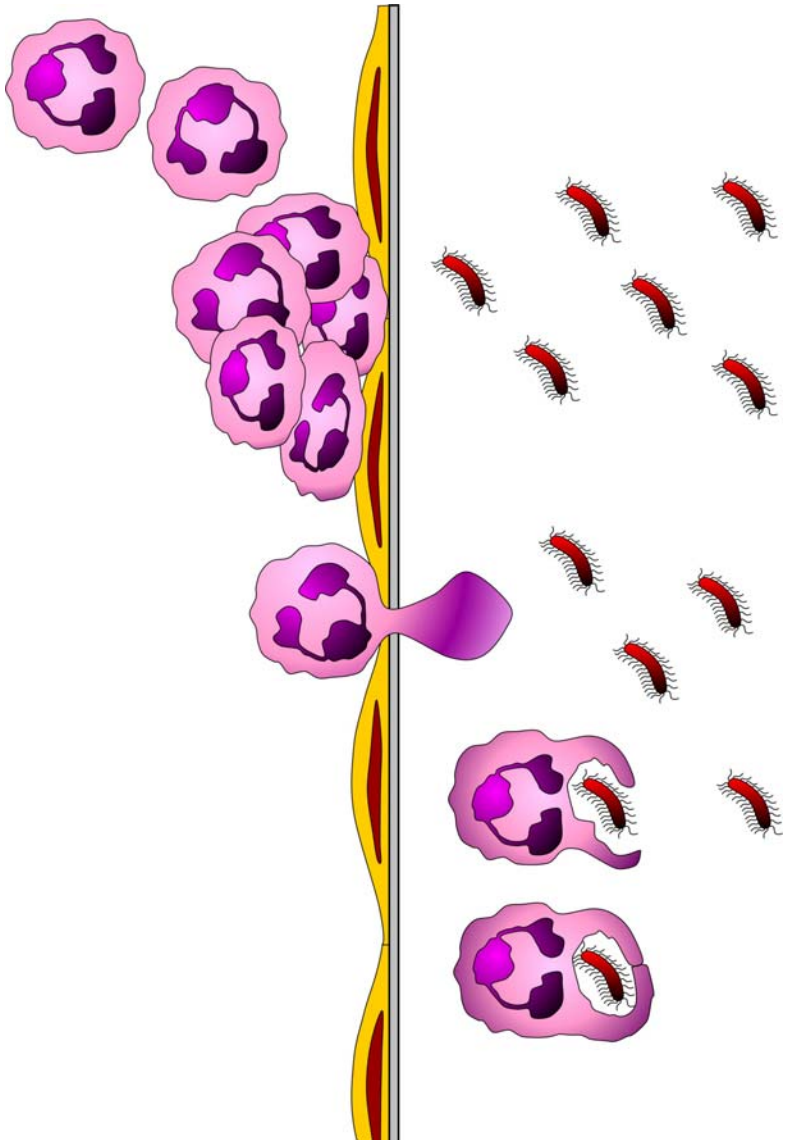
Adhesion

↓

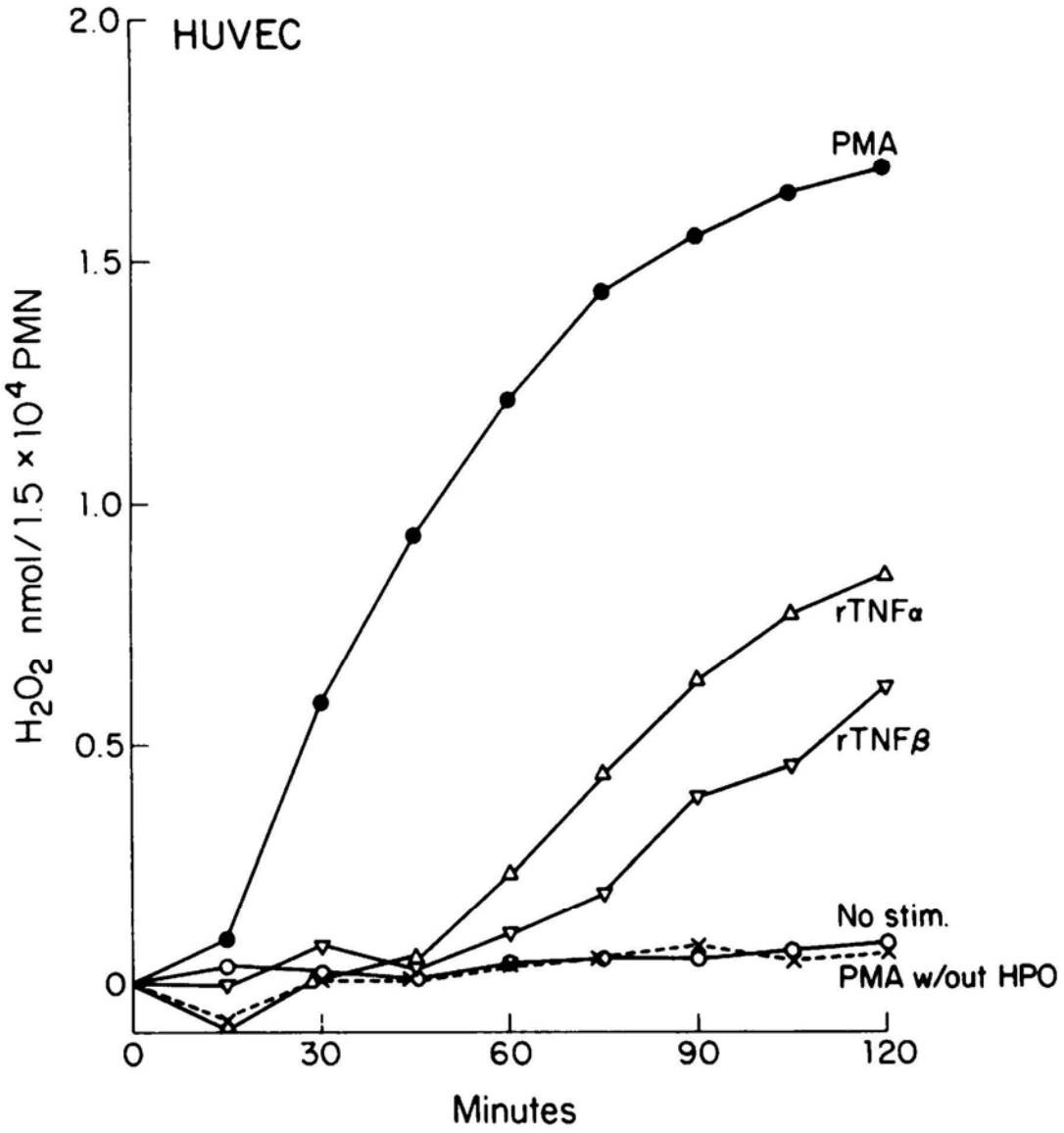
Extravasation

↓

Phagocytosis and
Destruction of Bacteria



Physiological Levels of TNF Activate PMNs Associated With Endothelial Cell Monolayers



Nathan et. al. 1990

Reality Check 2

PMN-Mediated ROS Production at Ambient O_2 Concentrations

Is this Physiological?

Not Really*: Consider the Following*

- *pO_2* is the partial pressure of O_2 in air, solution or in tissue and is expressed in *mm of Hg.* Air contains 21% O_2 which corresponds to a pO_2 of 100 mmHg or 5.2 mM.

- The pO_2 in air-equilibrated buffer *(pH 7.4; 37°C)* is also 100 mmHg (100 Torr) but because of its low solubility in water, O_2 concentration in solution is 200 µM.

Tissue pO_2 is a function of O_2 delivery, diffusion distance to nearby capillaries and O_2 consumption

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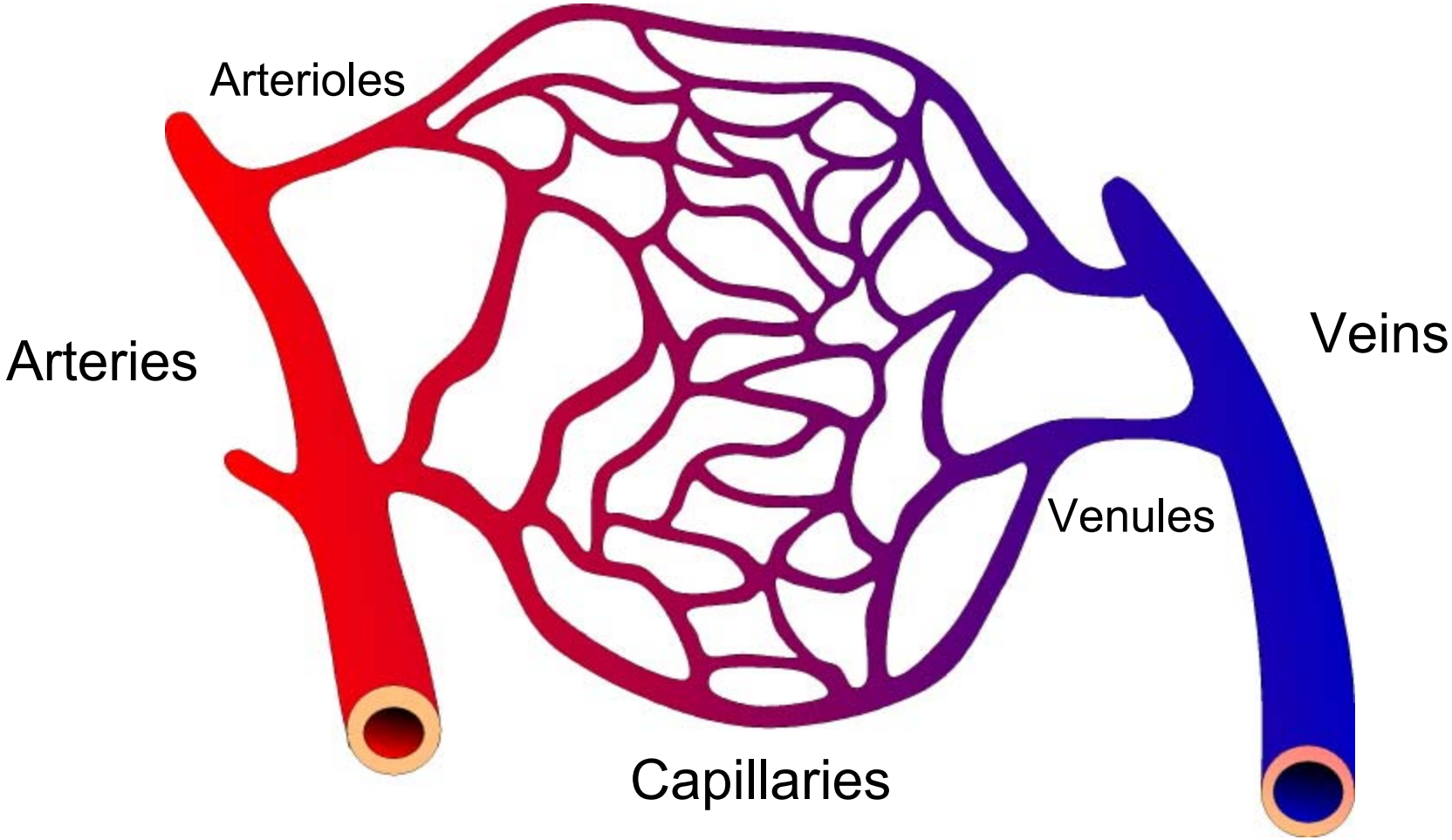
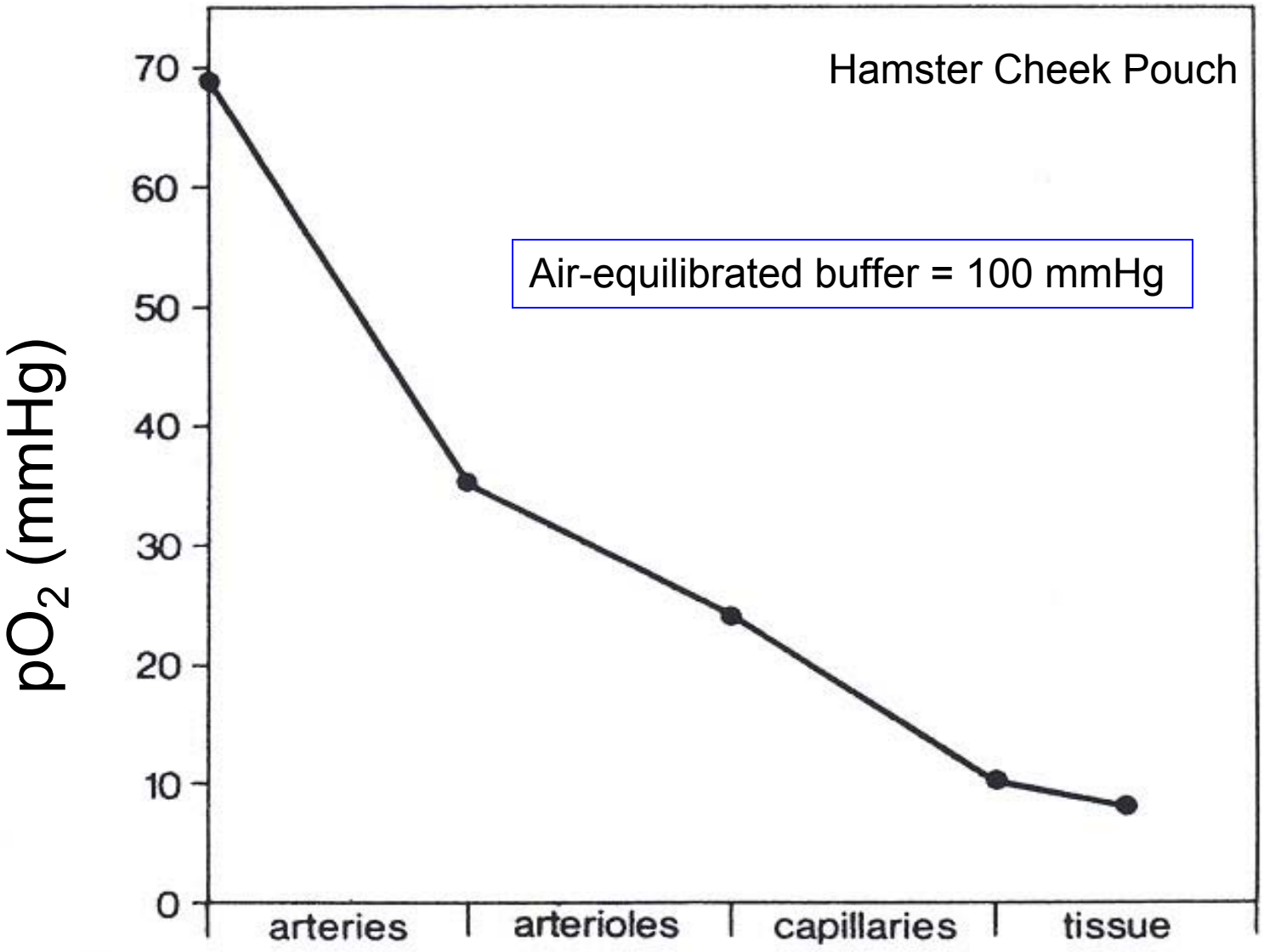
Except for the *lung, skin epidermis and cornea*, tissue ***pO_2*** ranges from ***5-40 mmHg*** or from ***10-80 µM*** (assuming an $[O_2]$ of 100 mmHg or 200 µM in solution)

Tissue pO_2 approximates venous pO_2

Inflammation, Tissue Microcirculation and pO_2



Perivascular and Tissue pO_2

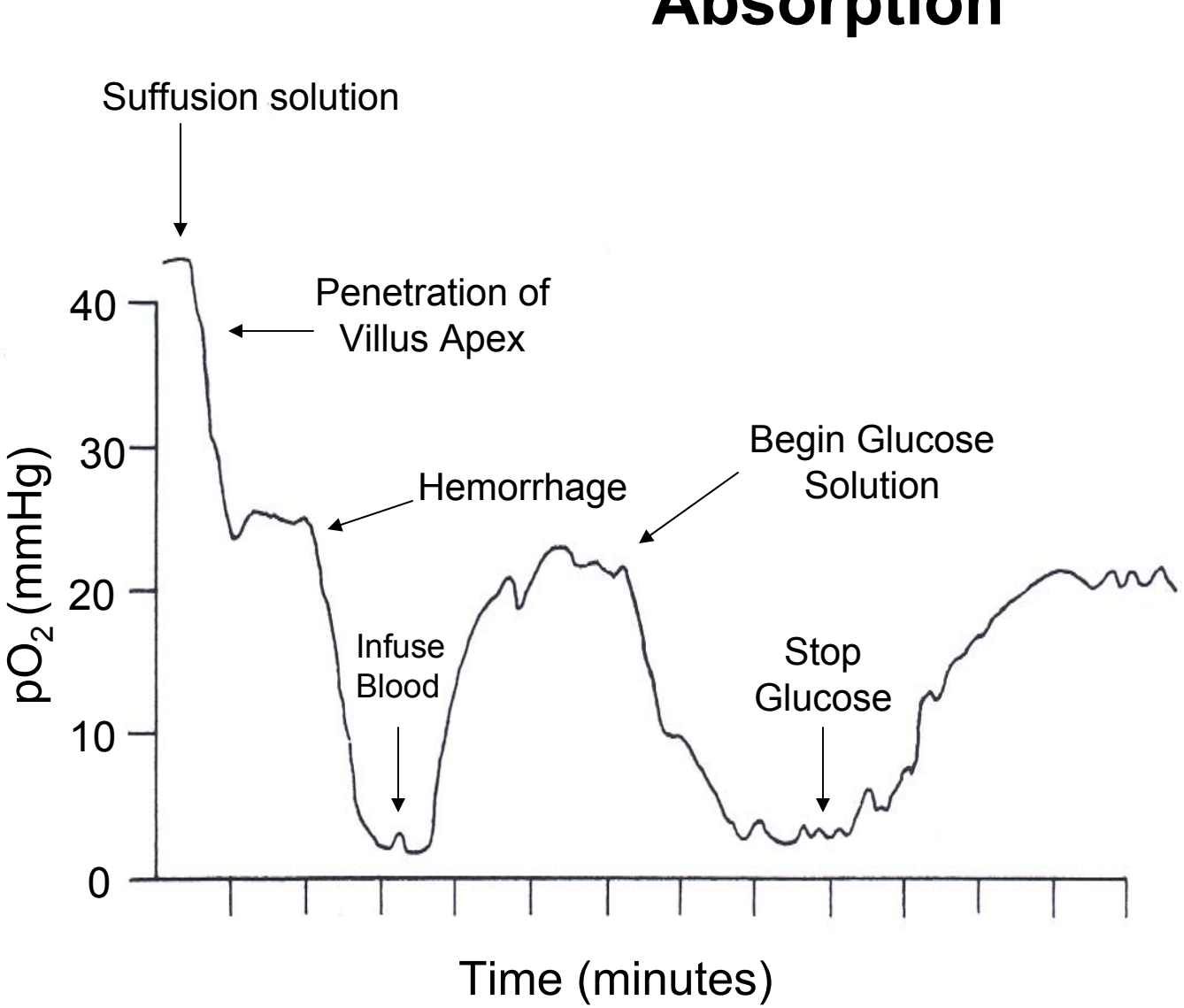


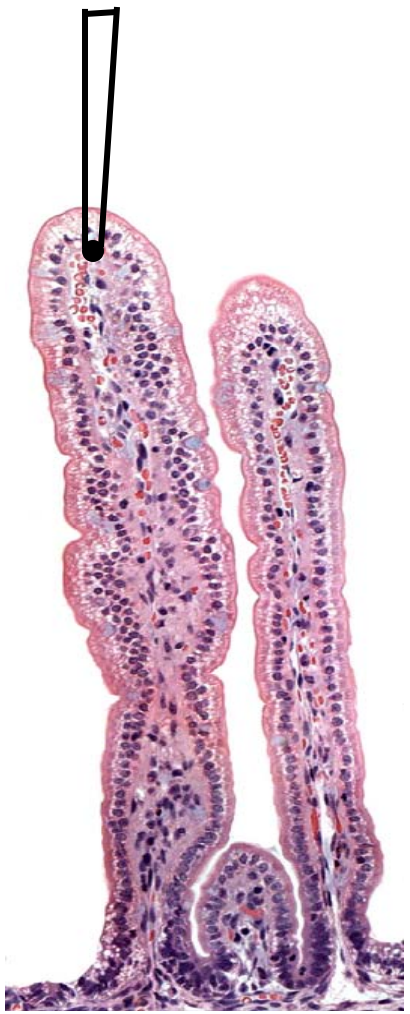
Duling and Berne

Tissue pO_2 Values

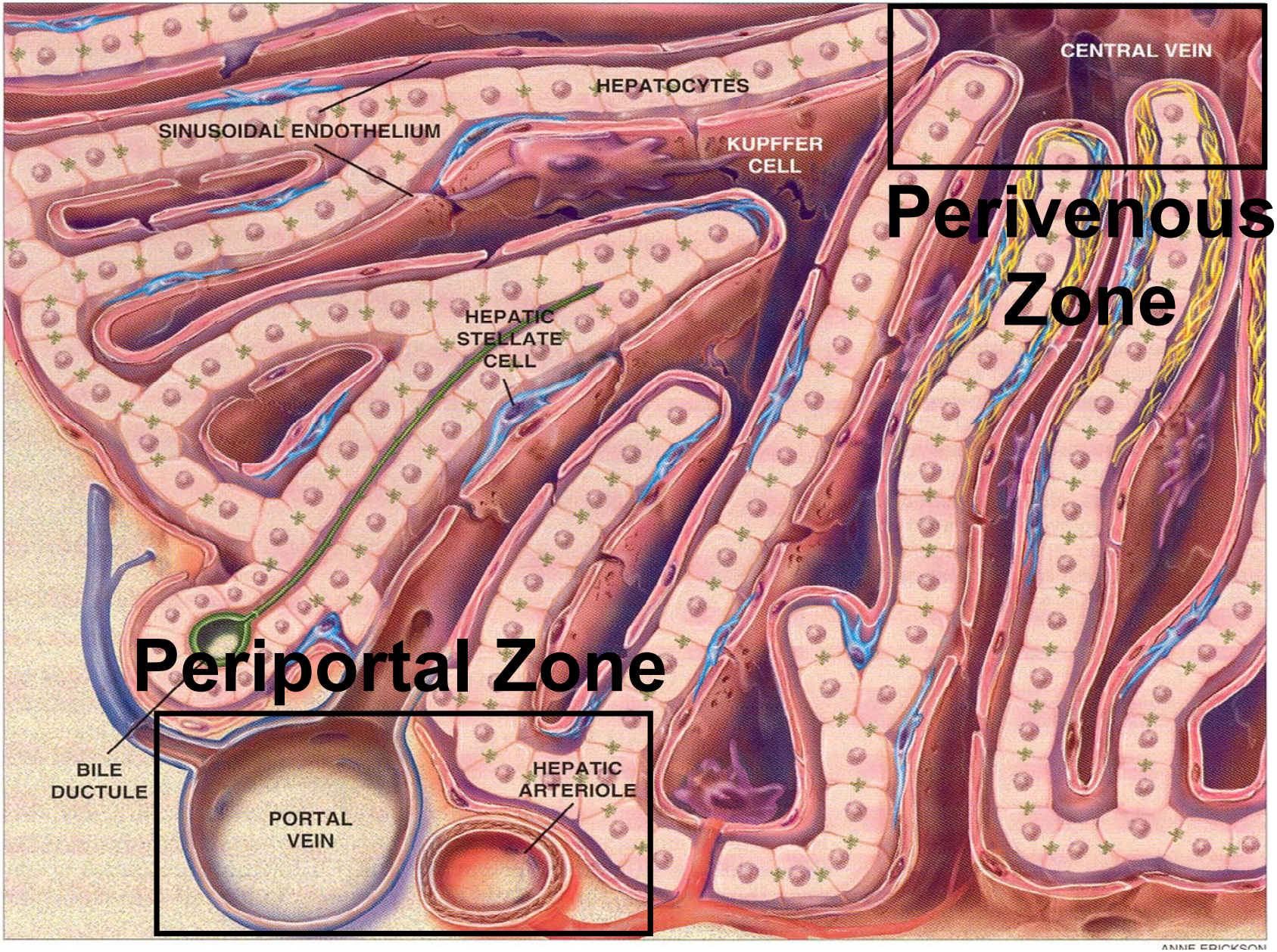
Tissue	pO_2 (mm Hg)
Kidney (cortex)	45
Kidney (medulla)	23
Skeletal muscle	25
Intestine	25
Brain	15
Liver (Periportal zone)	45
Liver (Perivenous zone)	22

Physiological pO_2 of the Intestine at Rest, Following Hemorrhage and During Nutrient Absorption



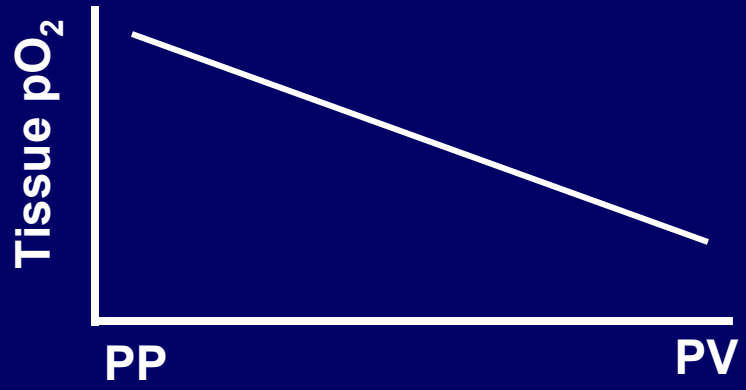


Oxygen Gradient within the Liver



Metabolic Heterogeneity of Hepatic Parenchymal Cells along the Sinusoid

Mitochondrial volume
Oxidative energy metabolism
Oxidative xenobiotic metabolism
Glucose production



pO_2= 45 mmHg (90 µM)

Periportal Zone 1

Perivenous Zone 3

pO_2= 22 mmHg (48 µM)

Glucose uptake
Glycolysis
Xenobiotic biotransformation

TY Aw

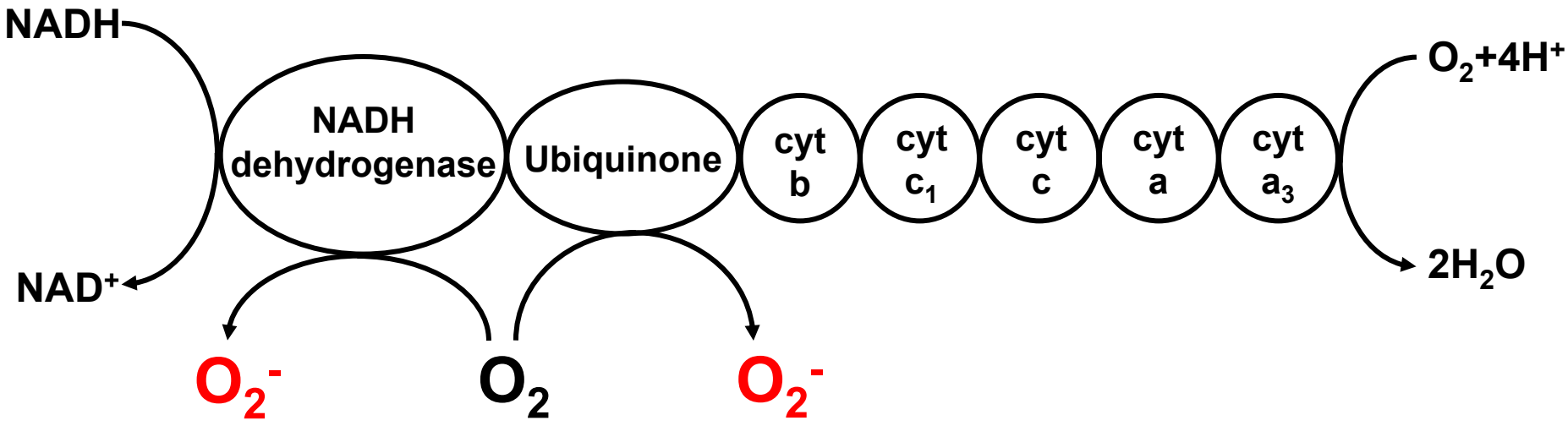
Why are these considerations important in the context of ROS generation?

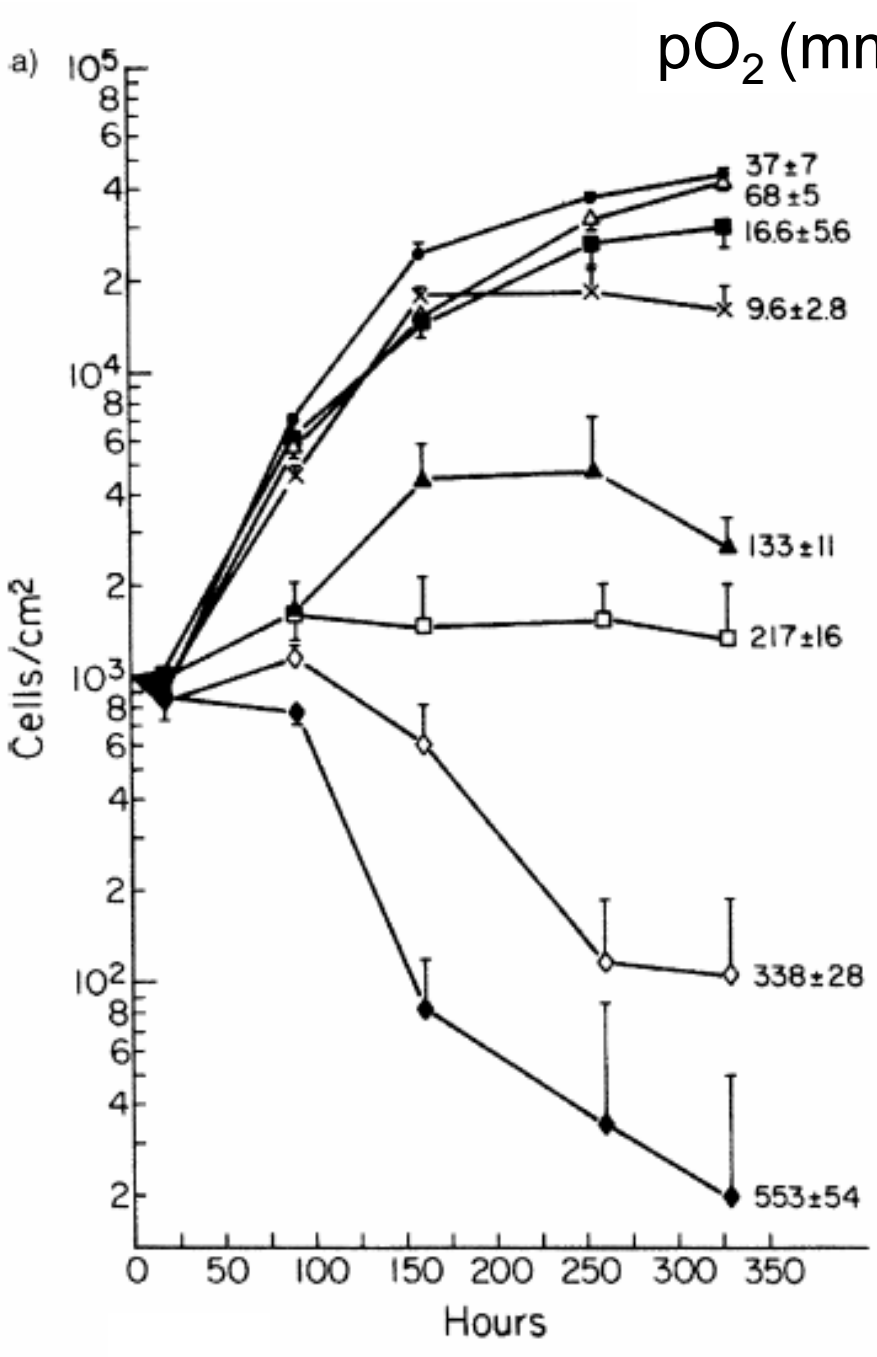
Because most cells and tissue are not exposed to ambient O_2 tension (i.e. 100 mmHg; 200μM). In reality, *physiological* pO_2 for the vast majority of cells and tissue is far below ambient oxygen tension.

Cultured Cells Grown at Ambient pO_2 are Subjected to *Hyperoxia*

$$dO_2^-/dt = k\,[O_2]\,[\text{electron donor}]$$

Doubling of the pO_2 will double O_2^- production

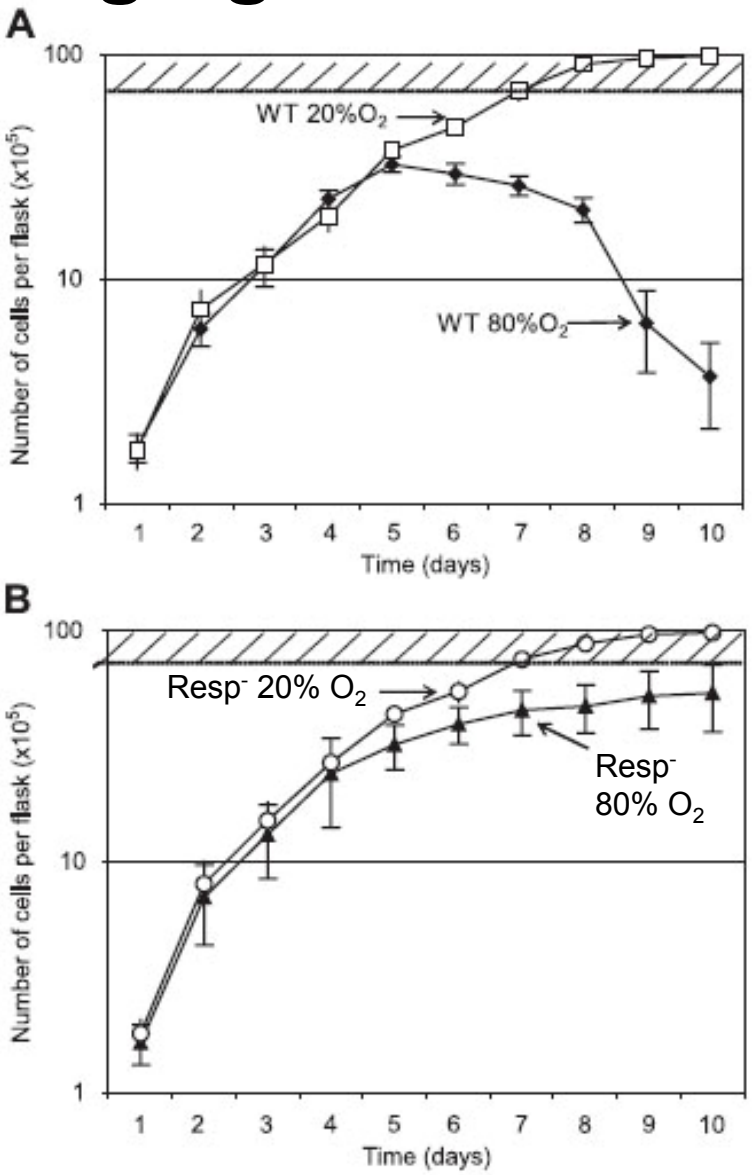




Oxygen Modulates The Growth of Fibroblasts

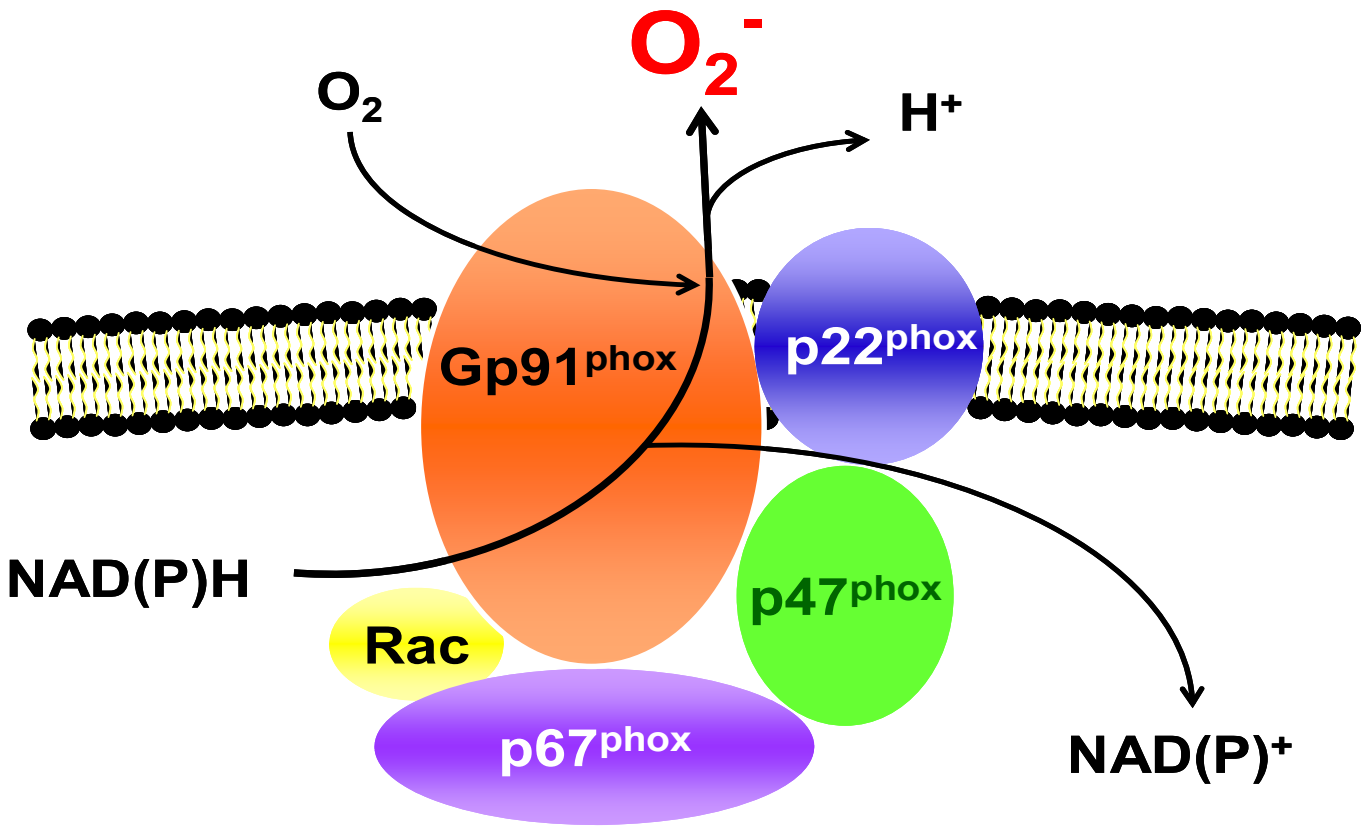
Balin and Pratt
In Vitro Cell Dev Biol
2002

Respiration-Deficient Cells are Resistant to Damaging Effects of Hyperoxia



Li et. al. FRBM 2004

Cultured Cells Grown at Ambient pO_2 are Subjected to *Hyperoxia*



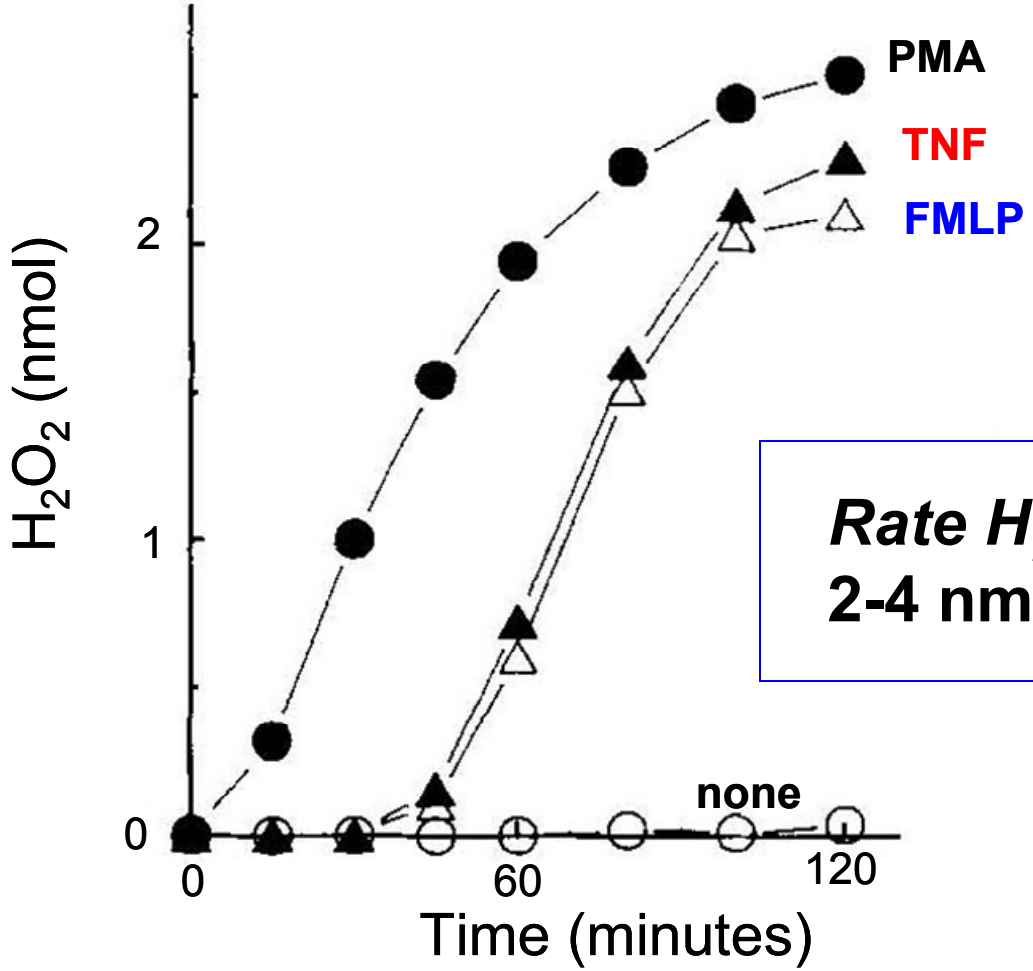
Natarajan 2003

What are the Rates of PMN-Derived ROS Generation at Physiological pO_2?

UNKNOWN. ***However one would predict that 10^6 fully-activated PMNs would be functional at pO_2 values as low as 10 mmHg (20 μM) assuming a K_mO_2 of 5-10μM for NADPH oxidase…. However:***

Reality Check 3

Rates of ROS Formation by PMNs Would Predict That all Tissue O_2 Would be Consumed Within A Matter of a Few Minutes



***Rate H_2O_2 Production* = 2-4 nmol/min/10^6 PMNs**

Nathan JCI 1989

Not Necessarily: Inflammatory Vasodilators (PGE_2, NO, adenosine) Will Promote the Delivery of O_2 to the Inflammatory Site via Enhancing Tissue Blood Flow *(O_2 Delivery = Blood Flow x [O_2])*

Poiseuille's Law: Relates pressure (P), flow (Q) and resistance (R)

$P = Q \times R$ where $R = 8L\eta/\pi r^4$

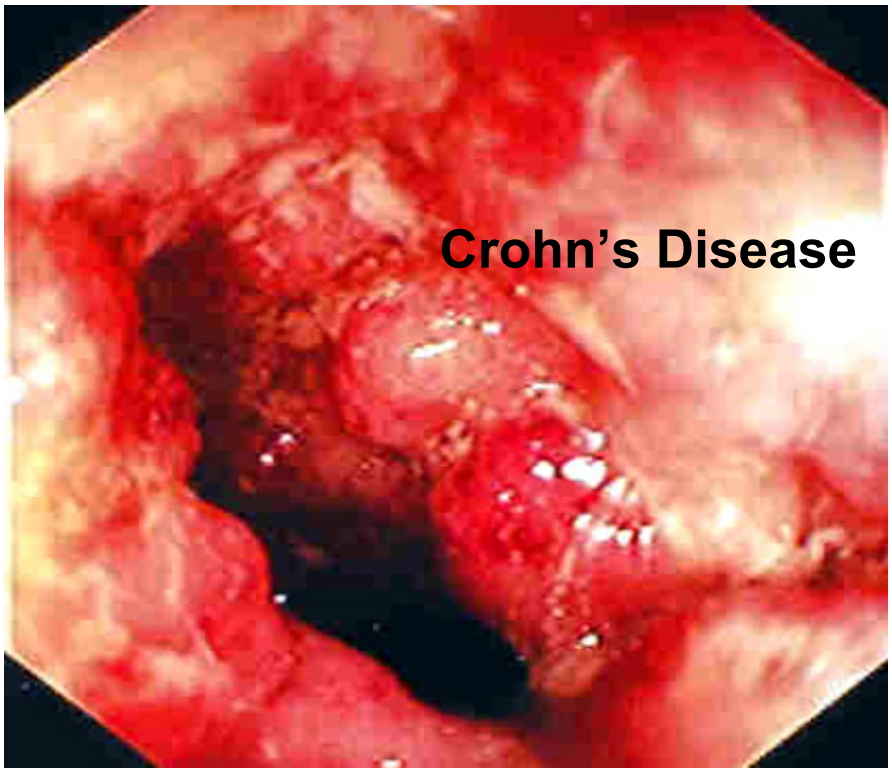
L=length, η=viscosity, ***r=radius***

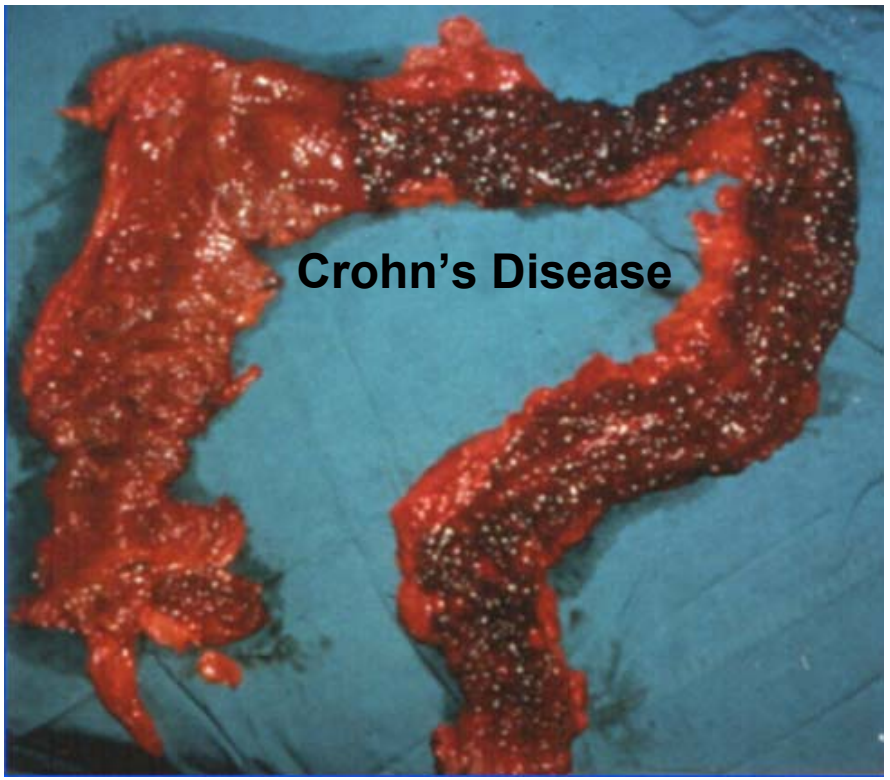
$$Q = \frac{(P)(\pi r^4)}{8L\eta}$$

Note that 2-fold increase in vessel diameter results in a 16-fold increase blood flow

Inflammation-induced hyperemia **is a good thing when responding to a bacterial infection or tissue injury.**

But what about sustained increases in blood flow associated with *chronic inflammation*?





Adding Fuel to the Fire:

Potential for Hyperoxic Insult

Summary and Conclusions

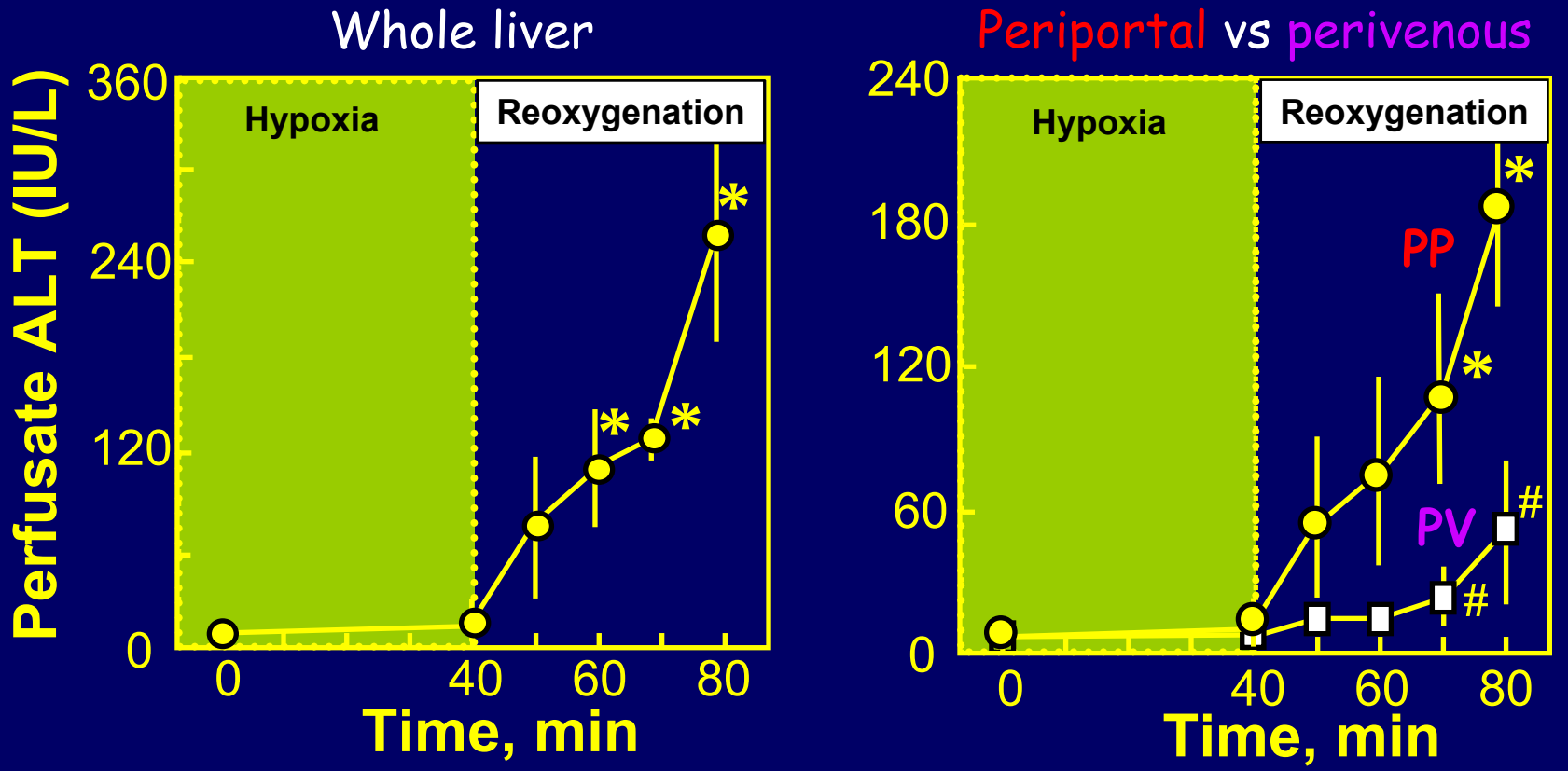
- **Inflammatory mediator (TNF-α, IL-1β)-induced ROS production by PMNs is dramatically enhanced by the interaction of PMN-associated CD18 with extracellular matrix (ECM) proteins.**

- **Except for the lung, tissue pO_2 is far below ambient pO_2 and approximates venous oxygen tension (≈ 40 mmHg).**

- **Blood flow affects tissue pO_2 and thus may regulate the magnitude and duration of PMN-derived ROS generation during times of infection and inflammation.**

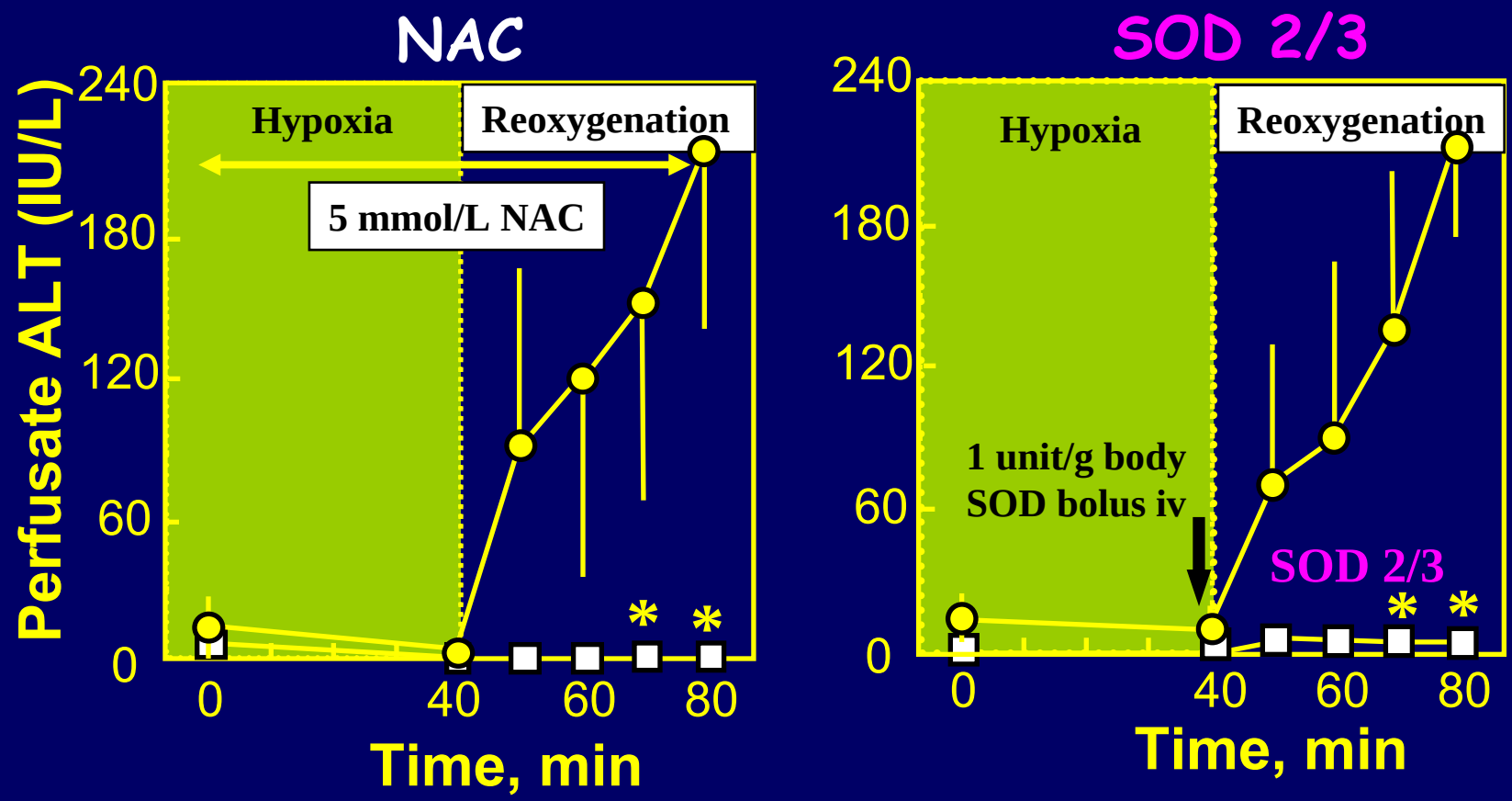
Physiological fluxes of PMN-derived ROS are dependent upon the physical association of these phagocytes with endothelial cells and ultimately the ECM as well as tissue pO_2

Susceptibility of Periportal vs Perivenous Zones to Hypoxia/Reoxygenation



AW et. al.

ROS involvement in HR-induced Periportal injury



Aw et. al.