The Cellular Basis of Disease
Cell Injury 2

Mechanisms of Cell Injury

APPROVED

Christine Hulette MD

"Details from today are continually revised and updated with research. Lots of it changes on a daily basis so today will be a broad overview"

Objectives

- **Describe and understand mechanisms of cell injury including depletion of ATP; mitochondrial damage; entry of calcium into the cell ; increased reactive oxygen species (ROS); membrane damage**
- **Describe and understand the pathogenesis and give examples of ischemic and hypoxic injury; ischemia-reperfusion Injury; chemical Injury and radiation injury**

Minor injuries with a long duration can have a more profound effect than a major injury with short duration

Mechanisms of Cell Injury

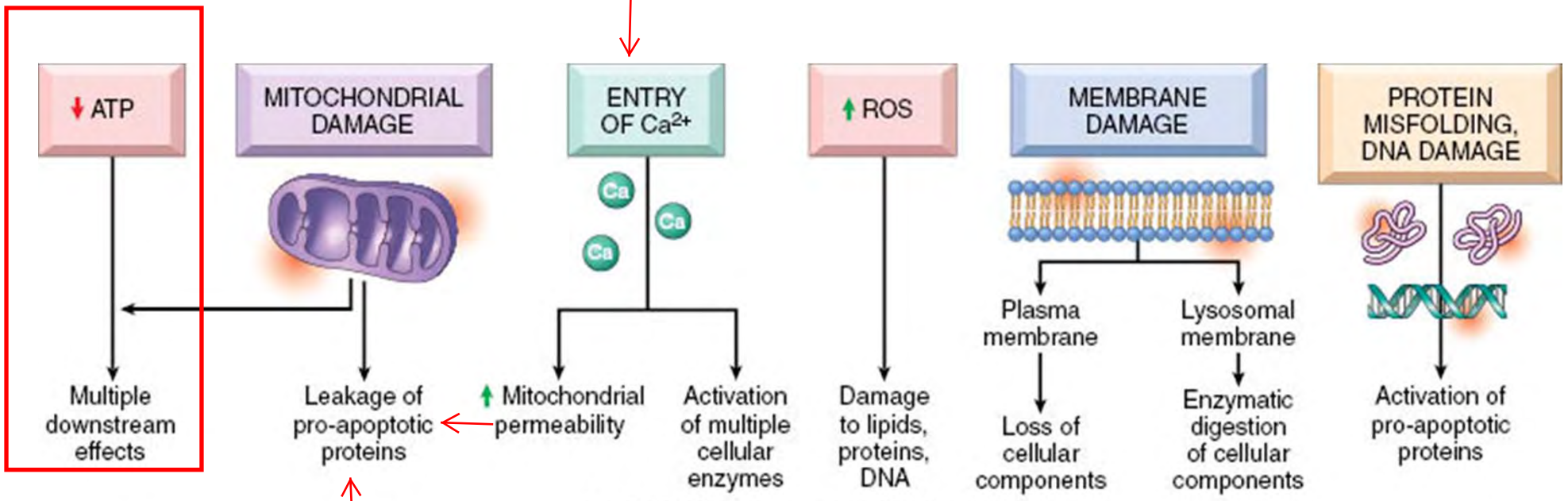
- Cellular response to injury depends on nature, duration and severity of injury.
- Consequences of injury depend on type, state and adaptability of the injured cell.
- Cell injury results from different biochemical mechanisms acting on essential cellular components.

Young people can respond better to injury than old people. The reason for this resides at cellular level

We'll talk about each of these in turn

This is critically important, and often underlies or coincides with other causes of injury

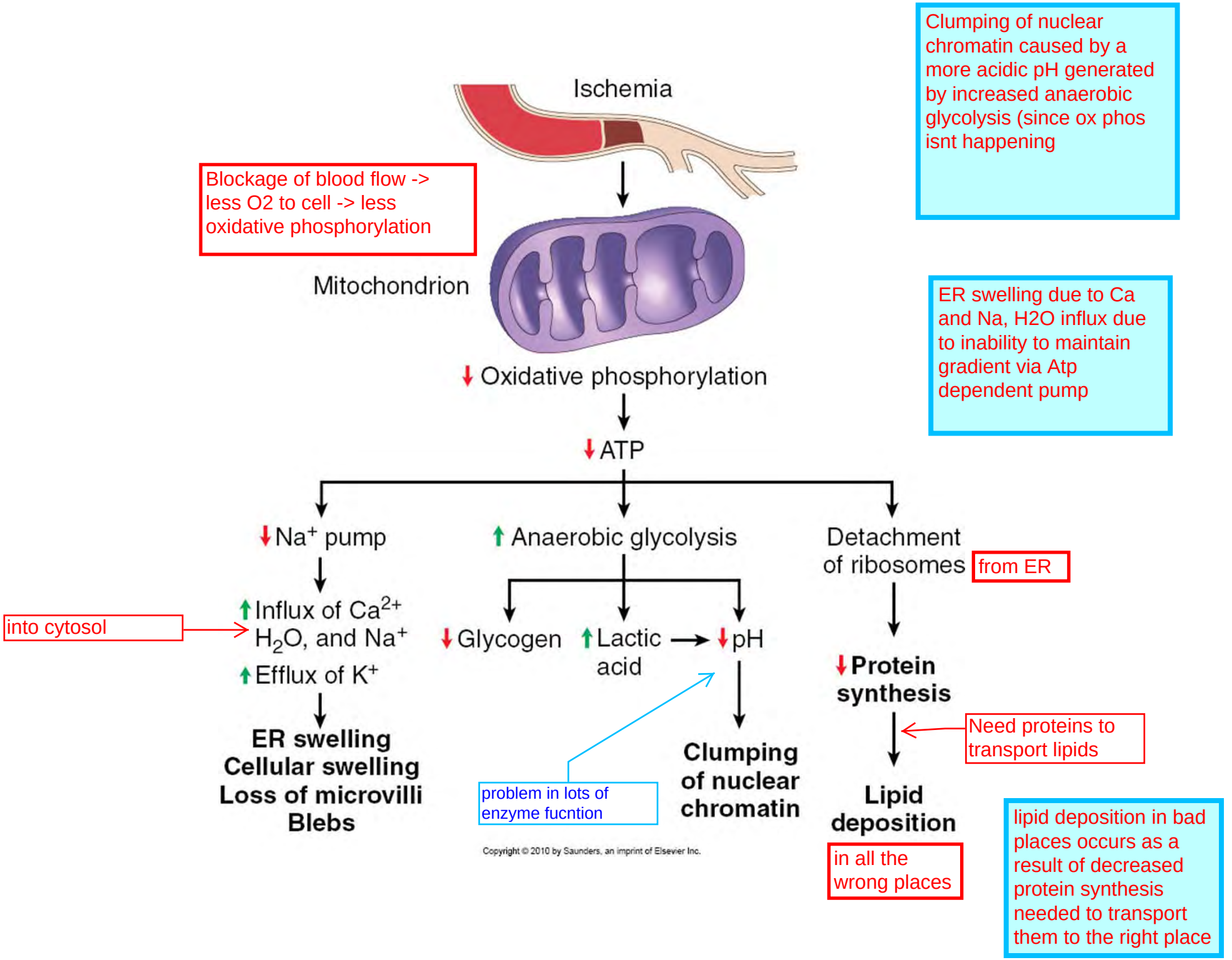
Most Ca++ is kept outside cell or inside mitochondria



May initiate apoptosis cascade

Mechanisms of Cell Injury

- **Depletion of ATP**
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding


Ischemia
Blockage of blood flow -> less O2 to cell -> less oxidative phosphorylation
Mitochondrion
Clumping of nuclear chromatin caused by a more acidic pH generated by increased anaerobic glycolysis (since ox phos isnt happening
ER swelling due to Ca and Na, H2O influx due to inability to maintain gradient via Atp dependent pump
↓ Oxidative phosphorylation
↓ ATP
↓ Na+ pump
↑ Anaerobic glycolysis
Detachment of ribosomes
from ER
into cytosol
↑ Influx of Ca2+ H2O, and Na+
↑ Efflux of K+
↓ Glycogen
↑ Lactic acid
↓ pH
↓ Protein synthesis
Need proteins to transport lipids
ER swelling
Cellular swelling
Loss of microvilli
Blebs
problem in lots of enzyme fucntion
Clumping of nuclear chromatin
Lipid deposition
in all the wrong places
lipid deposition in bad places occurs as a result of decreased protein synthesis needed to transport them to the right place
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Anaerobic versus Aerobic ATP Production

You should remember this from Mol and Cells. Make Newgaard proud!

Anaerobic Glycolysis:

Glucose + 2 ADP ---> 2 Lactate + 2 ATP

Glycogen + 3 ADP ---> 2 Lactate + **3 ATP**

Aerobic Oxidative Phosphorylation:

Glucose + $6O_2$+ 36 ADP --> $6CO_2$ + $6H_2O$ + **36 ATP**

Depletion of ATP

pp17-18

- **ATP depletion and decreased ATP synthesis are common with both hypoxic and toxic (or chemical) injury**

interferes with oxphos somehow

- **Na^+, K^+- ATPase pump activity is reduced**
- **Cellular energy metabolism is changed**
- **Failure of Ca^{++} pump**

Can't get the Ca out of the cell

na/ca exchanger?

yes

- **Reduced protein synthesis**

Mechanisms of Cell Injury

pp 18-19

- Depletion of ATP
- **Mitochondrial Damage**
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding

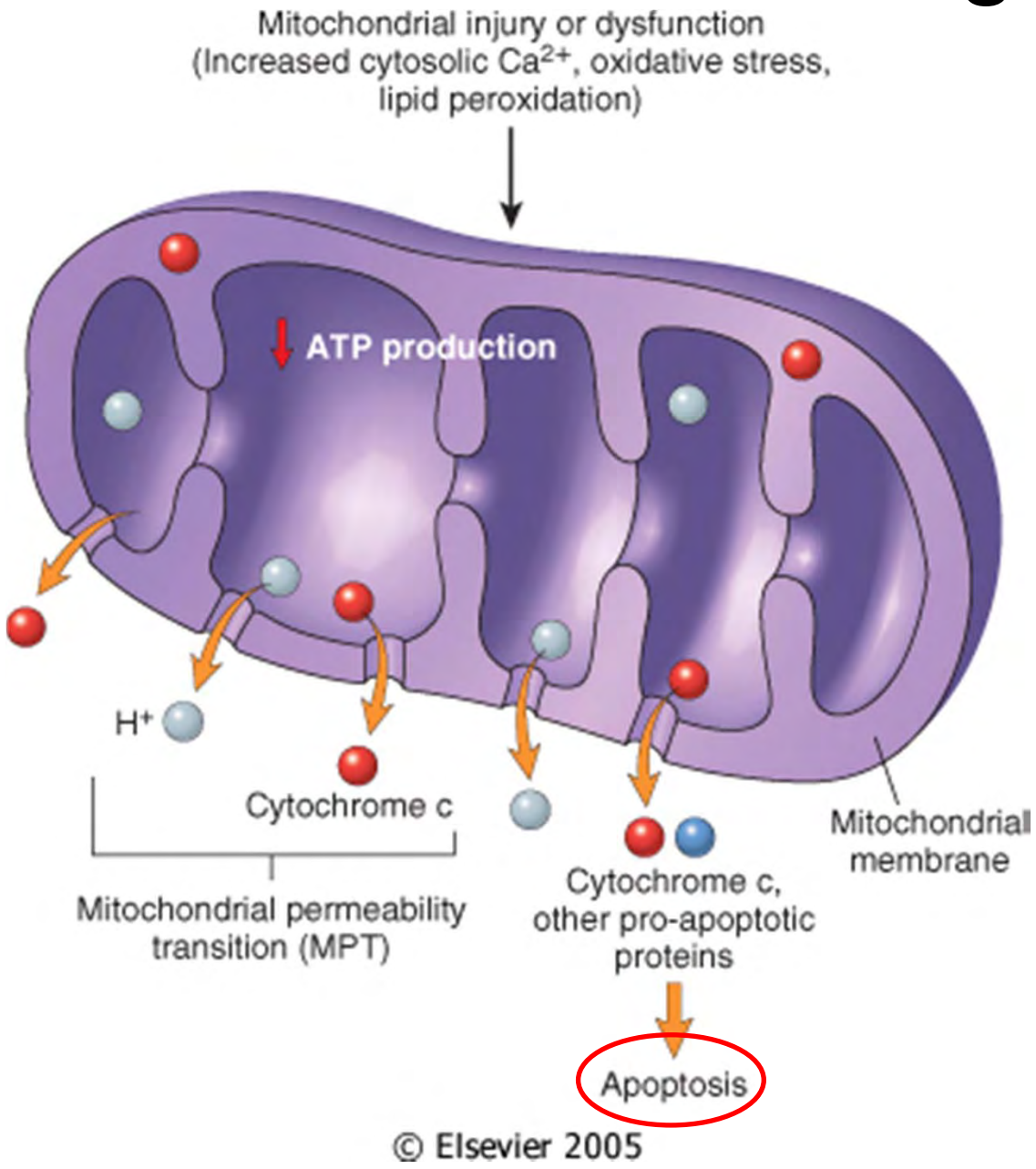
Often coincident w/ or cell damage

Consequences of Mitochondrial damage

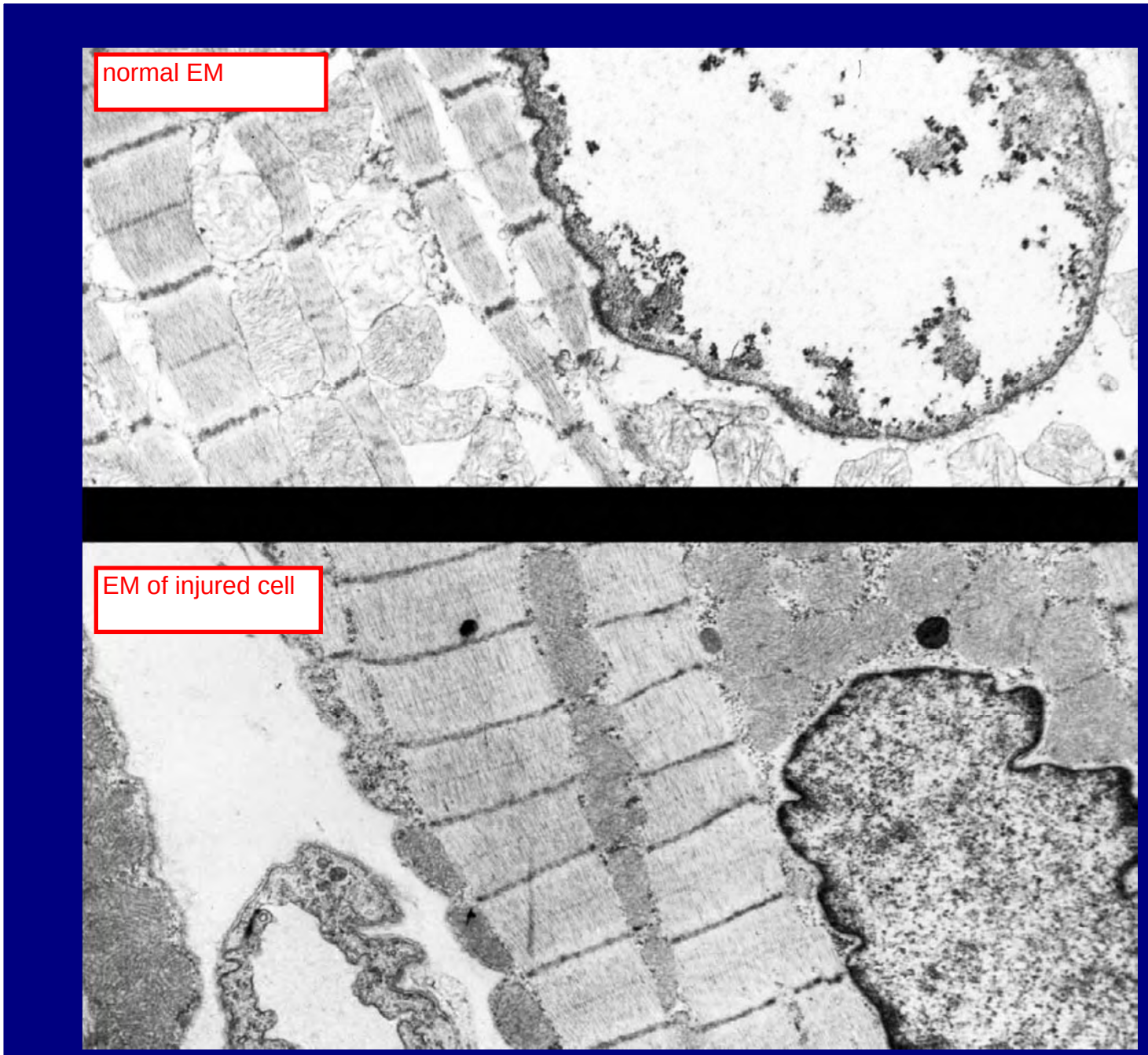
- **Loss of membrane potential via membrane permeability transition**
- **Results in failed oxidative phosphorylation and loss of ATP**
- **Membrane damage leads to leakage of Cytochrome c and other proteins which activate apoptotic pathways**

Mitochondrial Damage

Has secondary effects on other organelles and the cell itself



MPT-->apoptosis so not only is the MT injured but the cell and other organelles are injured because of the "toxic" stuff released in the cell.


normal EM
EM of injured cell

Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- **Entry of Calcium into the cell**
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding

Entry of Calcium into the cell

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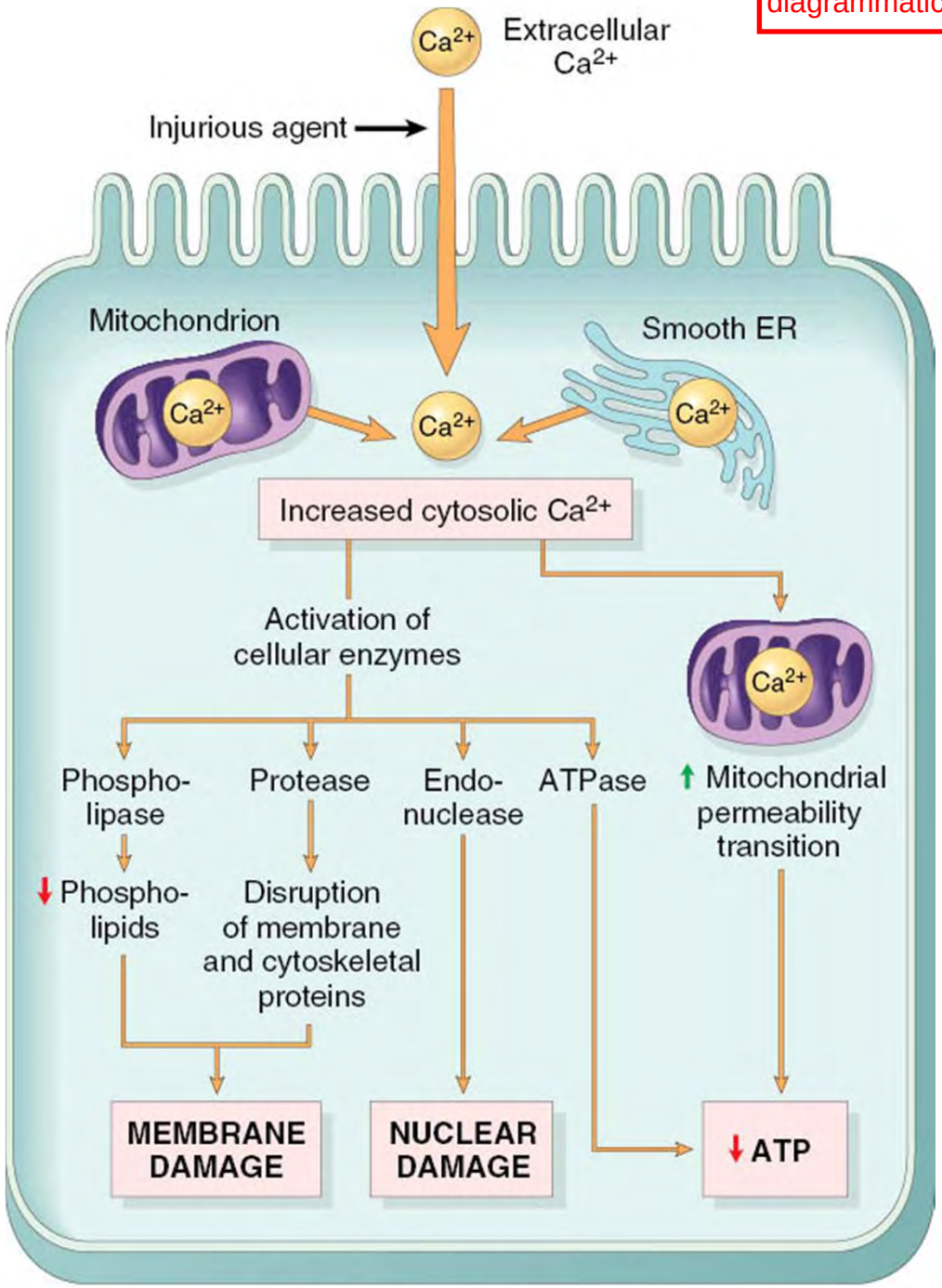
- **Intracellular Ca^{++} is low and is sequestered in mitochondria and endoplasmic reticulum**
- **Extracellular Ca^{++} is high**
- **Gradients are maintained by Ca^{++} Mg^{++} ATPases**
- **Increased cytosolic Ca^{++} activates enzymes: ATPases, phopholipases, proteases, endonucleases.**

starts breaking apart ATP, lipid membrane, cellular proteins, and DNA, respectively

tight regulation is needed!!!

Same thing in diagrammatic form



What cellular processes consume the most energy on an ongoing basis? Answer on next page

A. protein synthesis

B. DNA synthesis

C. DNA repair

D. phospholipid synthesis

E. ion transport

Ca++ is important!!!

E. Ion transport
You have to keep Ca++ out of the cell!

Mechanisms of Cell Injury

pp 20-22

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- **Increase reactive oxygen species (ROS)**
- Membrane Damage
- DNA damage, Protein misfolding

Not all ROS are free radicals

Accumulation of oxygen-derived free radicals (Oxidative stress)

- Reactive oxygen species (ROS)
- Biologically Important ROS
- Generation of ROS

normal

- Function of ROS
- Removal of ROS
- Pathologic effects of ROS
- Cellular defense against ROS

Reactive Oxygen Species

- **React with and modify cellular constituents.**
- **Initiate self perpetuating processes when they react with atoms and molecules.**
- **Electrons are frequently added to O_2 to create biologically important ROS.**

Biologically Important ROS

3 major ROS to remember:

- **Superoxide anion radical $O_2 + e^- \rightarrow O_2^-$**
- **Produced by phagocyte oxidase, damages lipids, proteins and DNA.**

in neutrophils and macrophages

Super**O**xide Dismutase

inflammatory response

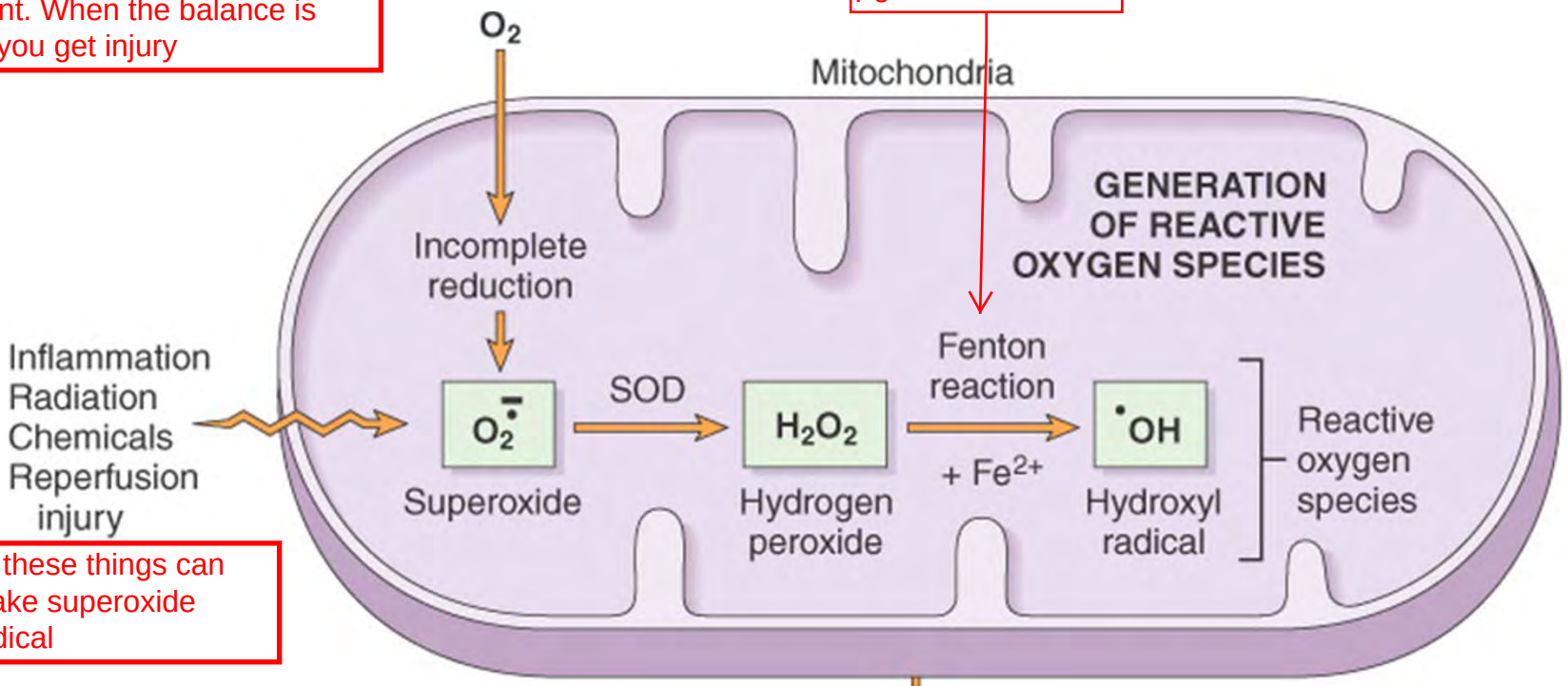
- **Hydrogen peroxide H_2O_2**
- **Generated by SOD and by oxidases, destroys microbes, may act at distant sites.**

H2O2 is relatively stable compared to the other 2 ROS

- **Hydroxyl radical $\cdot OH$**
- **Generated from H_2O by hydrolysis, most reactive, damages lipids, proteins and DNA.**

Normally this is all maintained in balance; they are all biologically important. When the balance is tipped, you get injury

Fenton reaction in the presence of Fe++



all these things can make superoxide radical

Can be a good thing or a bad thing depending on if you wanted to kill the cell

PATHOLOGIC EFFECTS OF ROS: CELL INJURY AND DEATH

ROS react with:

- Fatty acids → oxidation → generation of lipid peroxidases → disruption of plasma membrane, organelles
- Proteins → oxidation → loss of enzymatic activity, abnormal folding
- DNA → oxidation → mutations, breaks

REMOVAL OF FREE RADICALS

Antioxidant mechanisms:

- SOD (in mitochondria) converts $O_2^{\bullet-}$ → H_2O_2
- Glutathione peroxidase (in mitochondria) converts $^{\bullet}OH$ → H_2O_2 → $H_2O + O_2$
- Catalase (in peroxisomes) converts H_2O_2 → $H_2O + O_2$

Generation of ROS

- **Free radical is unpaired electron which makes the atom or molecule extremely reactive.**
- **When a free radical reacts with another atom or molecule, the result is usually another free radical.**
- **H_2O_2 is not a free radical but it is reactive, thus the term reactive oxygen species. It is generated by SOD from O_2^- and by oxidases.**
- **Common oxidases are P450 in the ER and NADPH oxidase in the plasma membrane.**

normal Function of ROS

- **Normal metabolism and respiration**
- **Absorption of radiant energy**
- **Inflammation** you could not fight off an infection w/o ROS
- **Enzymatic metabolism of chemicals or drugs** Necessary to make the doctor's Rx work
- **Nitric oxide synthesis**

Removal of free radicals

- **Antioxidants- Vitamins A and E, glutathione and ascorbic acid.**
- **Iron and Copper ions catalyze formation of ROS and are bound to transport proteins - transferrin, ferritin, ceruloplasmin**
- **Enzymes scavenge free radicals- Catalase in peroxisomes; Superoxide dismutase in mitochondria and cytosol; Glutathione peroxidase in cytosol.**

for Fe

Fe/Cu linkage to transport proteins keeps them in check

for Cu

Pathologic effects of reactive oxygen species (ROS)

- **Fatty acids- lipid peroxidation of plasma membranes and organelles**
- **Proteins- oxidation with loss of enzyme activity, protein misfolding**
- **DNA – oxidation, mutations, breaks**

Lipid damage

- **Plasma membranes and organelles have a high lipid content.**
- **Double bonds of unsaturated fatty acids are attacked by O_2- derived free radicals.**
- **This yields peroxides which are unstable and propagate the injury which leads to membrane injury.**

Protein damage

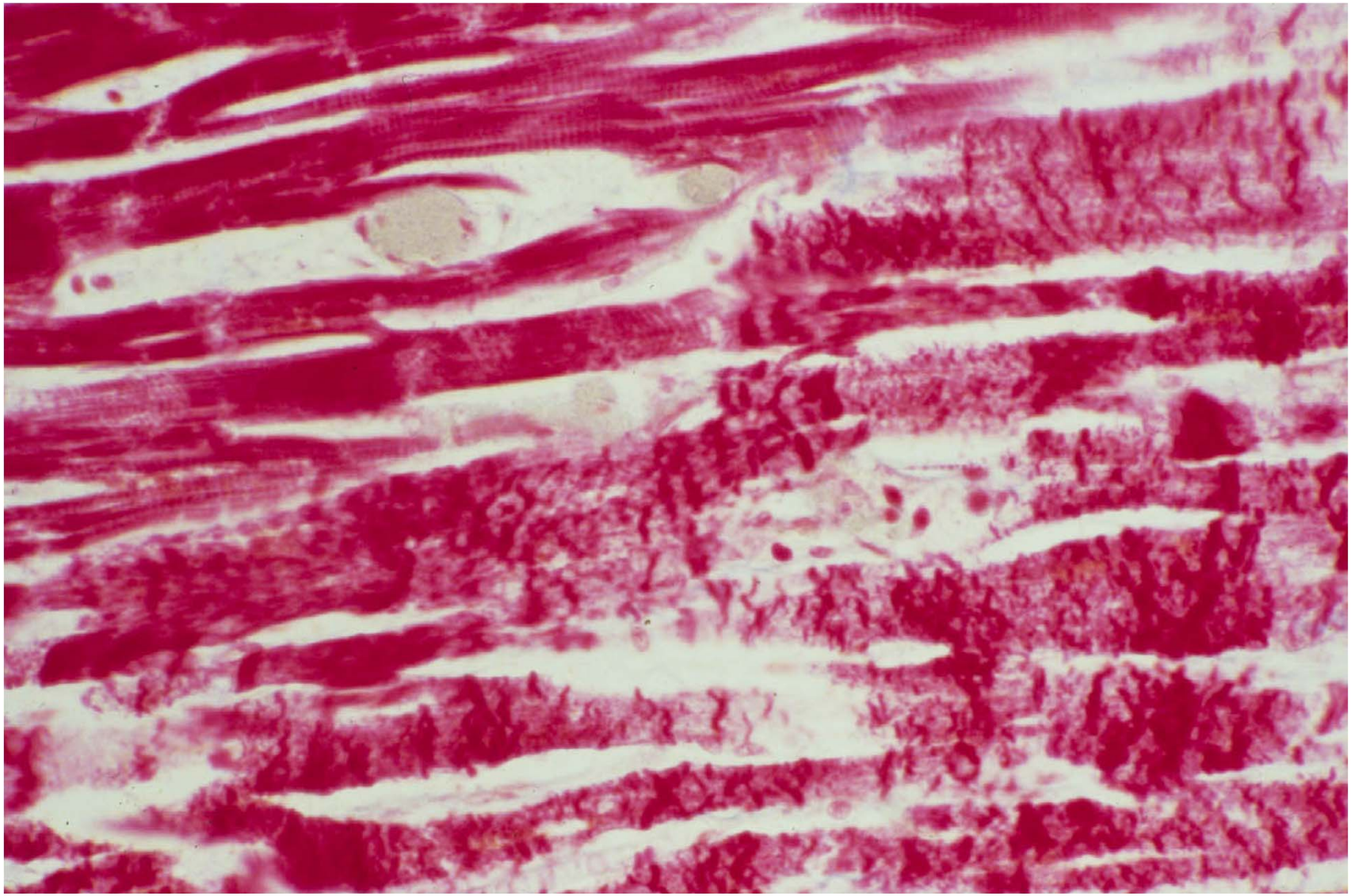
ROS can attack cyst residues and lead to the formation of disulfide bonds and that changes conformation of protein
- this alters activity
-causes cross linking

Cysteine residues (with SH groups) in proteins can be oxidized, resulting in the formation of disulfide (S--S) bonds

This results in conformational changes in proteins, loss of enzyme activity, and protein cross linking

Pathology-speak for abnormally cross-linked proteins; myosin in this case

Protein cross linking – Contraction band Necrosis



Injury to DNA

- **Free radicals cause single and double strand breaks.**
- **Free radicals cause cross-linking of DNA strands.**
- **Free radicals cause adducts.**
- **Cells may be able to repair DNA injury.**
- **These changes are implicated in cellular aging and malignant transformation.**

DNA moieties that can be bonded to a chemical that induces further injury

if damage is minor

Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- **Membrane Damage**
- DNA damage, Protein misfolding

Mechanisms of Membrane Damage

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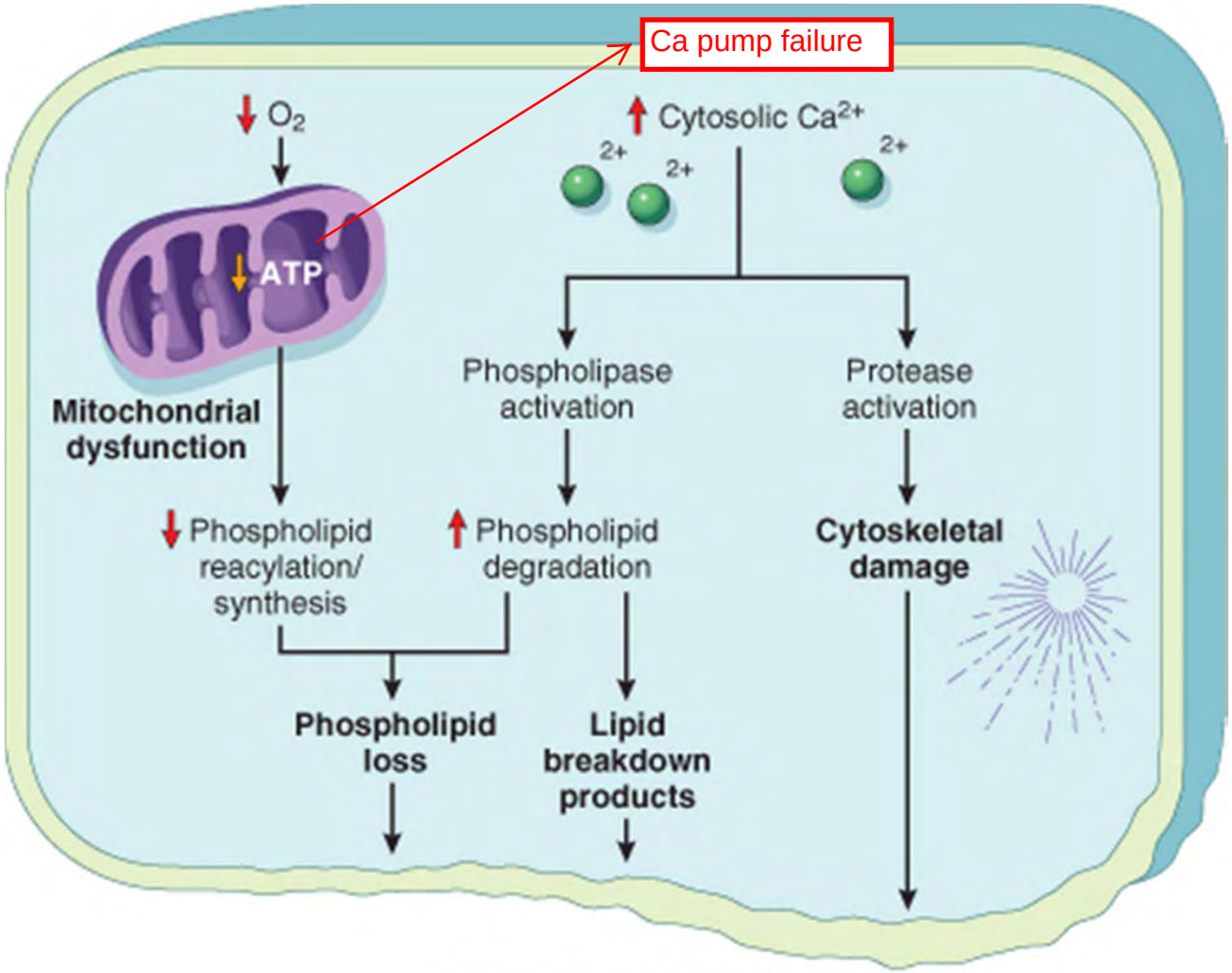
- Reactive oxygen species
- Decrease phospholipid synthesis
- Increase phospholipid breakdown
- Cytoskeletal abnormalities

Membrane Damage



MEMBRANE DAMAGE

Consequences of Membrane Damage

previously seen

- **Mitochondrial membrane damage causes increased cytosolic Ca^{++}, oxidative stress, lipid peroxidation, phospholipase activity, loss of membrane potential, leakage of Cytochrome c**
- **Plasma membrane damage causes loss of osmotic balance, loss of proteins, enzymes and nucleic acids.**
- **Injury to lysosome membranes causes leakage of enzymes with destruction of cellular components.**
- **Leading to Cell Death**

Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- **DNA damage, Protein misfolding**

DNA damage
Protein misfolding

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- If DNA damage to cell is too severe, apoptosis is initiated.
- Improperly folded proteins can initiate apoptosis.
- Cell Injury 3

tomorrow's lecture. we'll worry about it then

Examples of Cell Injury

- **Ischemic and Hypoxic Injury**
- **Ischemia-Reperfusion Injury** Only recently understood in the last 15-20 yrs
- **Chemical Injury**
- **Radiation injury**

Ischemic and Hypoxic Injury

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- **Most common type of injury in modern medical practice**
- **Hypoxia = reduced oxygen availability**

KNOW THE DIFFERENCE!

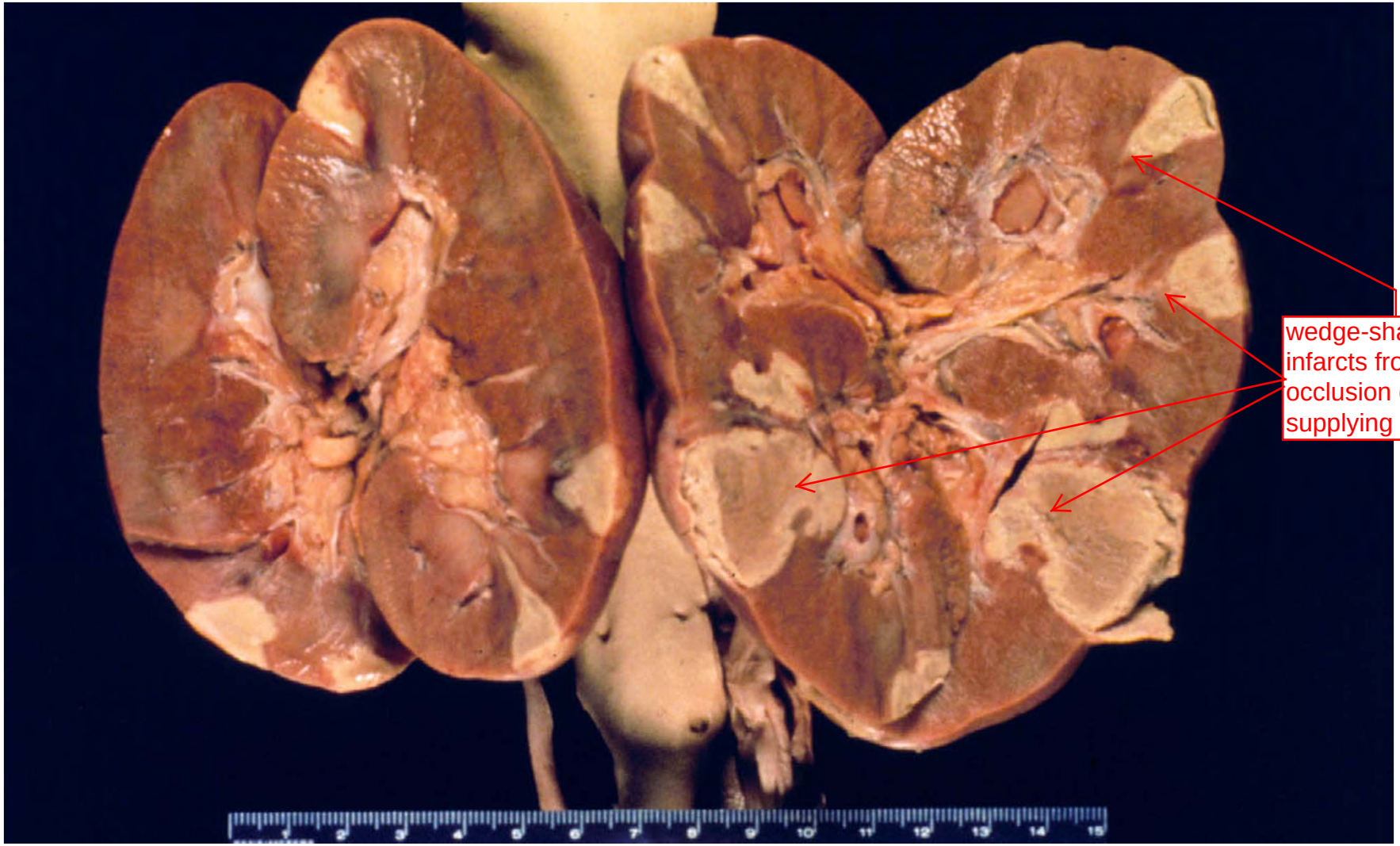
- **Ischemia = reduced blood flow usually due to atherosclerosis**

Less O2 and also less nutrients are delivered

- **Ischemia may also be caused by reduced venous return**

Less common cause, but know it too

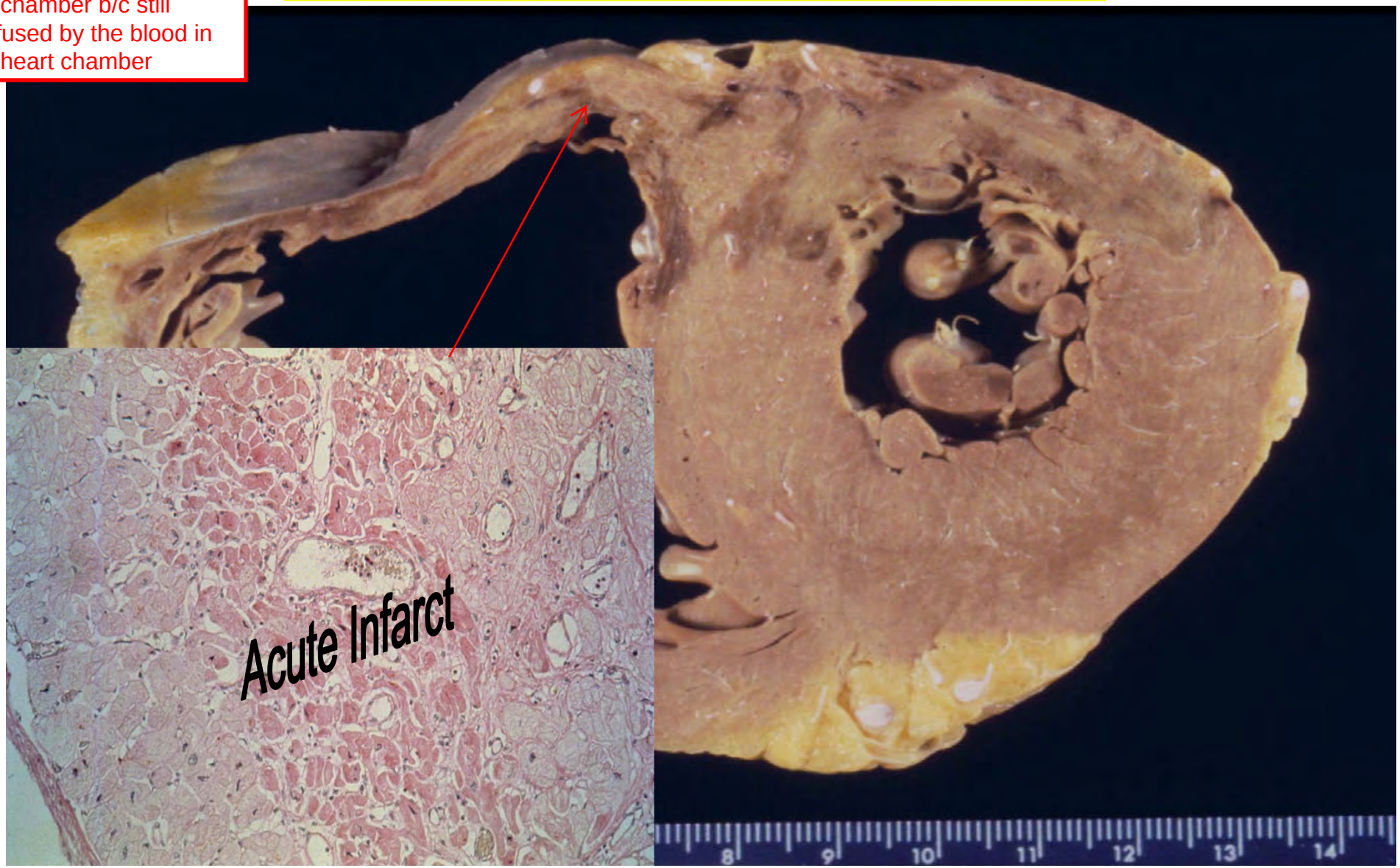
Ischemic injury
Kidney with multiple embolic infarcts



Ischemic injury

Acute Myocardial infarct

Less damage in the tissue immediately adjacent to the chamber b/c still perfused by the blood in the heart chamber



Ischemia-Reperfusion Injury

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- **Blood flow restored to ischemic cells which are injured but have not died.**
- **Injured cells may die when they are re-perfused.**
- **Other dead cells will release cellular contents into the restored blood stream.**
- **New damaging processes mediated by ROS become activated.**
- **Inflammation and complement activation.**

Common in corobary bypass grafts or stints

you'd think they'd be happy, but noooo. Mechanism not well understood

add to damage

Found in myocardium

A 53 year old man has had marked chest pain for the past 3 hours. Laboratory findings include elevated serum creatine kinase-MB. He is given a thrombolytic drug and the CK-MB rises further. Which of the following is the most likely biochemical basis for this observed rise in CK-MB?

answer on next page

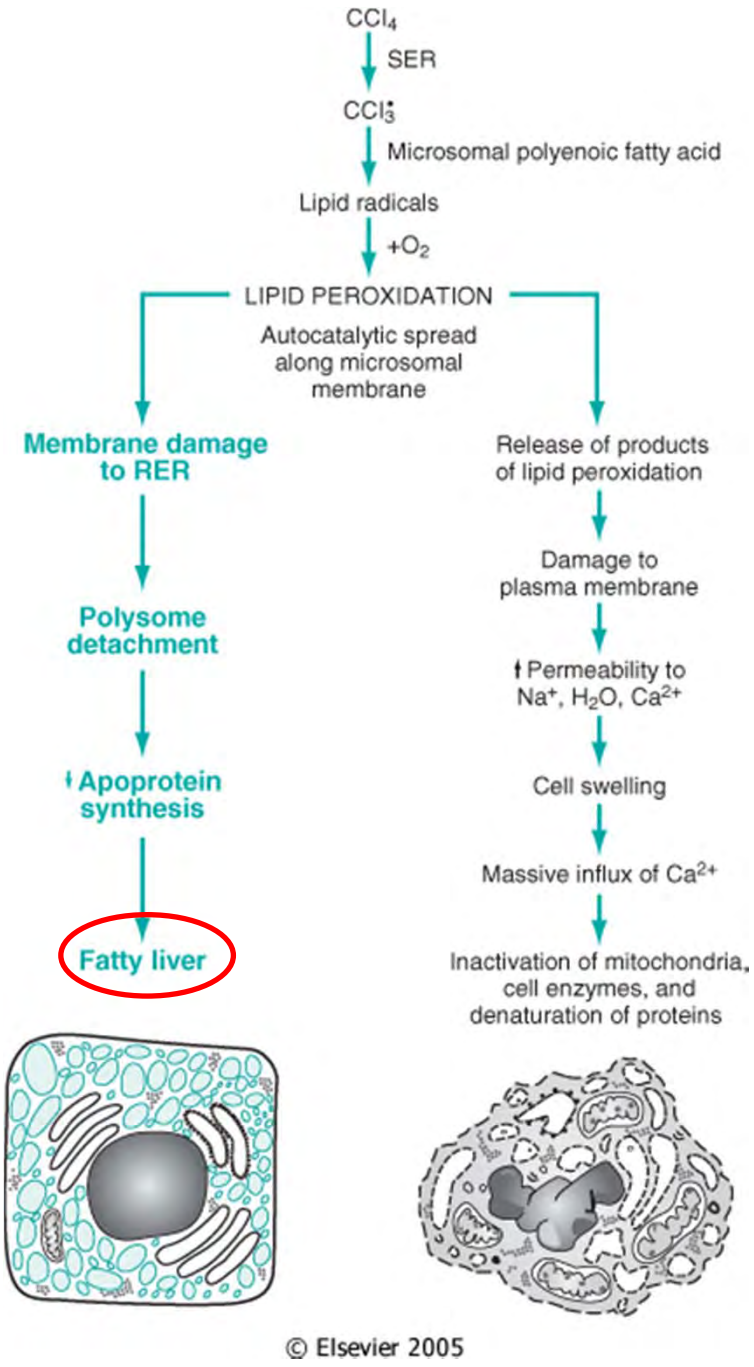
A. Reduced protein synthesis
B. Generation of reactive oxygen species
C. Increased activity of Catalase
D. Reduced oxidative phosphorylation
E. Release of calcium from endoplasmic reticulum

B. Generation of ROS
Delivery of drug causes reperfusion -> further injury -> generation of ROS

Chemical Injury

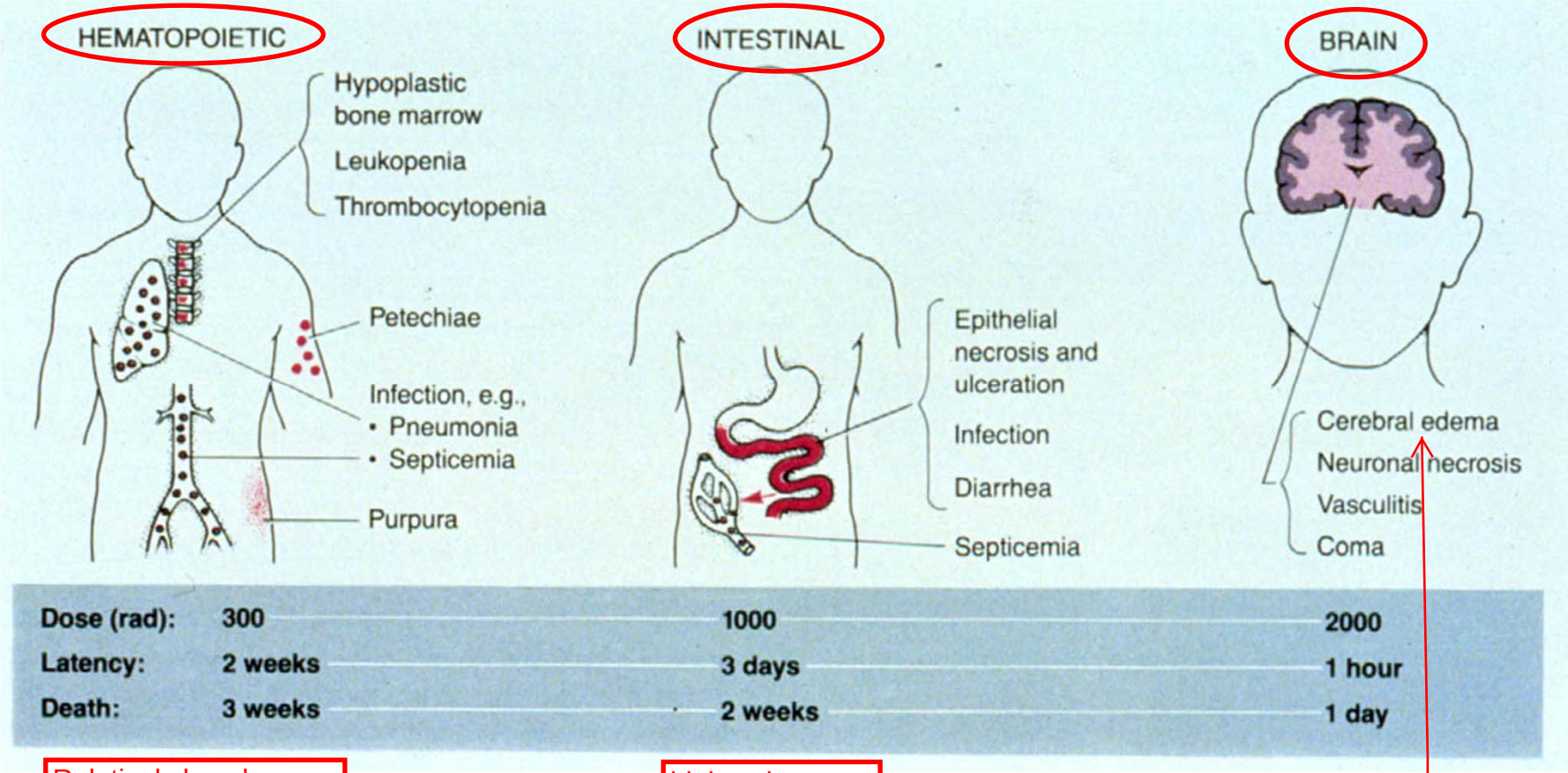
p 24-25

- **Direct injury** by combining with a critical molecule or organelle
  - Mercuric chloride — binds -SH groups of cell membrane proteins -> increased permeability and inhibits ion transport. Especially hurts GI and kidneys
  - Arsenic
- **Indirect injury** by conversion to toxic metabolites via P-450 mixed function oxidase
  - Direct covalent binding
  - Reactive free radicals and lipid peroxidation



Radiation injury

Will be discussed more thoroughly later in the course



Relatively low dose

higher dose

Really high doses

Mechanisms and Types of Cell Injury

- **Depletion of ATP**
- **Mitochondrial Damage**
- **Entry of Calcium into the cell**
- **Increase reactive oxygen species (ROS)**
- **Membrane Damage**
- **DNA damage, Protein misfolding**

- **Ischemic/Hypoxic injury**
- **Chemical Injury**
- **Radiation Injury**

Q: Radiation injury causes double strand breaks and ROS generation?
A: Yes