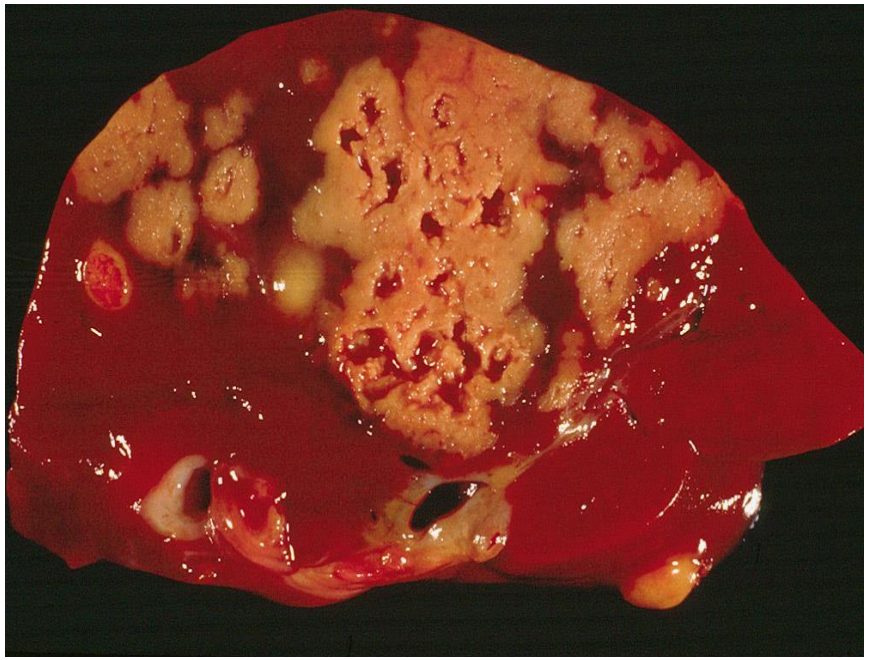
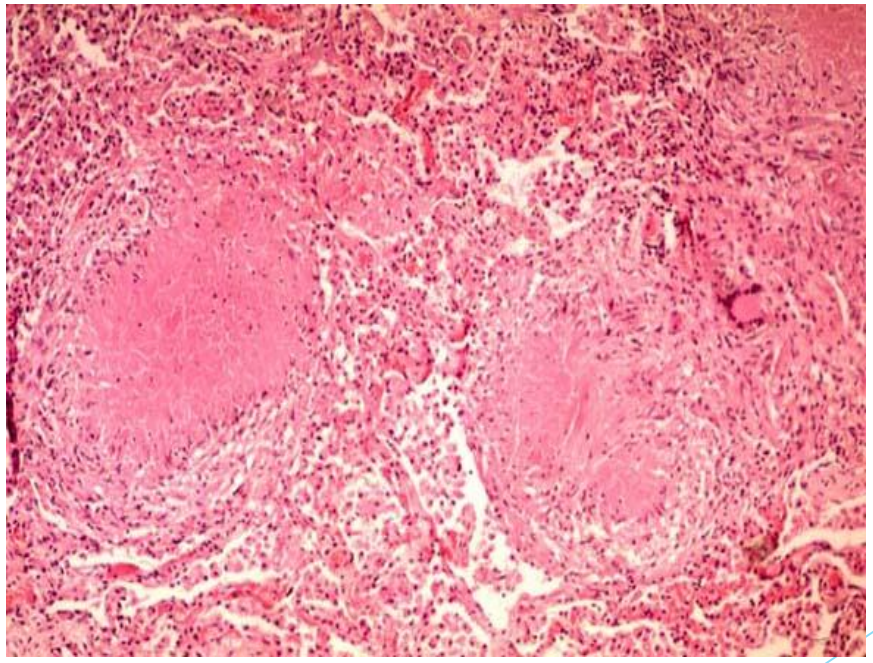
M.J.F. COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

DEPARTMENT OF VETERINARY PATHOLOGY





Cell Injury

Rudolph Virchow- 150 years ago-stated that Disease begins at cellular level.

This is established since then by progress made in molecular pathology

Today also it is true all forms of injury commence with molecular and structural changes in the cell.

-Normal cell lives in a hostile environment i.e. in a state of striking disequilibrium with its external environment. eg. Ca. conc. within and outside the cell-10000 times difference.

The plasma mem. of a cell maintains ionic composition against large chemical gradients between intra and extra cellular components by its selective permeability.

-Normal cell also maintain a state of homeostasis. It constantly modifies its structure and function in response to changing demands and stresses. It can undergo adaptation and acquire a steady state and preserve its health. eg. Bulging muscles of race horse- cellular response called hypertrophy , Atrophy,Hyperplasia, Metaplasia are adaptive responses

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If no adaptive response is possible or adaptive capability is exceeded- a sequence of retrogressive changes (to step back) follow collectively known as Cell injury previously termed as Degenerations

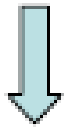
Within certain limits injury is reversible- morphological changes due to non lethal injury

With severe and persistent stress- the cell reaches a point of no return-suffers irreversible injury and dies

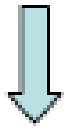
Adaptation, reversible injury, irreversible injury and cell death are progressive encroachment on cell's normal structure and function

Cell injury or Degenerative changes are retrograde/retrogressive changes due to non lethal injury in which protoplasm of a cell is converted back in to substances of inert nature i.e.CHO. protein, fat, minerals, water etc.

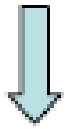
Homeostatic 'steady state'



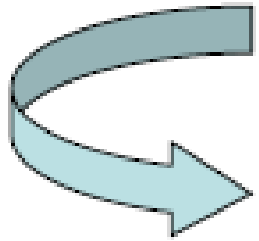
Cellular adaptations



Reversible cell injury



Irreversible cell injury



Cell death

Necrosis

- Molecular mechanisms for cell injury are complex.
- For injury to cells there are many causes and there are number of pathways to cell death that interact with each other
- The many macromolecules, enzymes and organelles within the cell are so closely interdependent that it is difficult to differentiate the primary target of injury . Point of no return is difficult to determine.
- Morphological changes of cell injury first develop at biomolecular level-this leads to structural changes first at ultrastructural level-then microscopic level and at gross level.
- Cellular response to injury depends on type, severity and duration of injury as well as type of the cell affected. Due to loss of blood supply

  Nerve cells die die 3-5 minutes

  Cardiac,hepatic and renal cells 30 mnts-2hours

  Skeletal muscles,fibroblasts and epidermis-many hours

Following intracellular systems are exposed when cellinjury occurs

1.Cell mem.-Integrity of cell/ organellar mem for ionic and osmotic homeostasis

2.Aerobic respiration-Oxidative phosphorylation and production of ATP

3. Synthesis of enzymic &structural proteins

4. Preservation of Integrity of genetic appratus of a cell

Injury at one site leads to wide ranging secondary effects

Common Biochemical Mechanisms

- For certain injuries biochemical mechanisms are well defined eg. Cynide inactivates cytochrome oxidase in mitochondria ; certain bact produce phospholipases that attack phospholipids in cell mem.

Common biochemical pathways in the mediation of cell injury and cell death

- **1. ATP depletion-** ATP is required for many synthetic and degradative processes within the cell. Like membrane transport, protein synthesis,

lipogenesis, phospholipid turnover

ATP is produced in two ways.

- The major pathway is oxidative phosphorylation of adenosine diphosphate. a reaction that requires oxygen
- The second is the anaerobic glycolytic pathway, which generate ATP in absence of oxygen using glucose derived from body fluids or from glycogen

Common pathway in ischemic and toxic injury.

Effects of depleted ATP

a) The activity of the plasma membrane energy-dependent sodium pump is reduced. It causes sodium to accumulate intra cellularly and potassium to diffuse out of the cell causing cell swelling, and dilation of the endoplasmic reticulum.

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- 2. Lack of oxygen or Generation of oxygen derived free radicals: Partially reduced activated oxygen species also cause cell injury and death. Cells generate energy by reducing molecular oxygen to water. During this O2 derived free radicals are formed which are highly toxic and cause damage to lipids, proteins, Nucleic acids of the cells

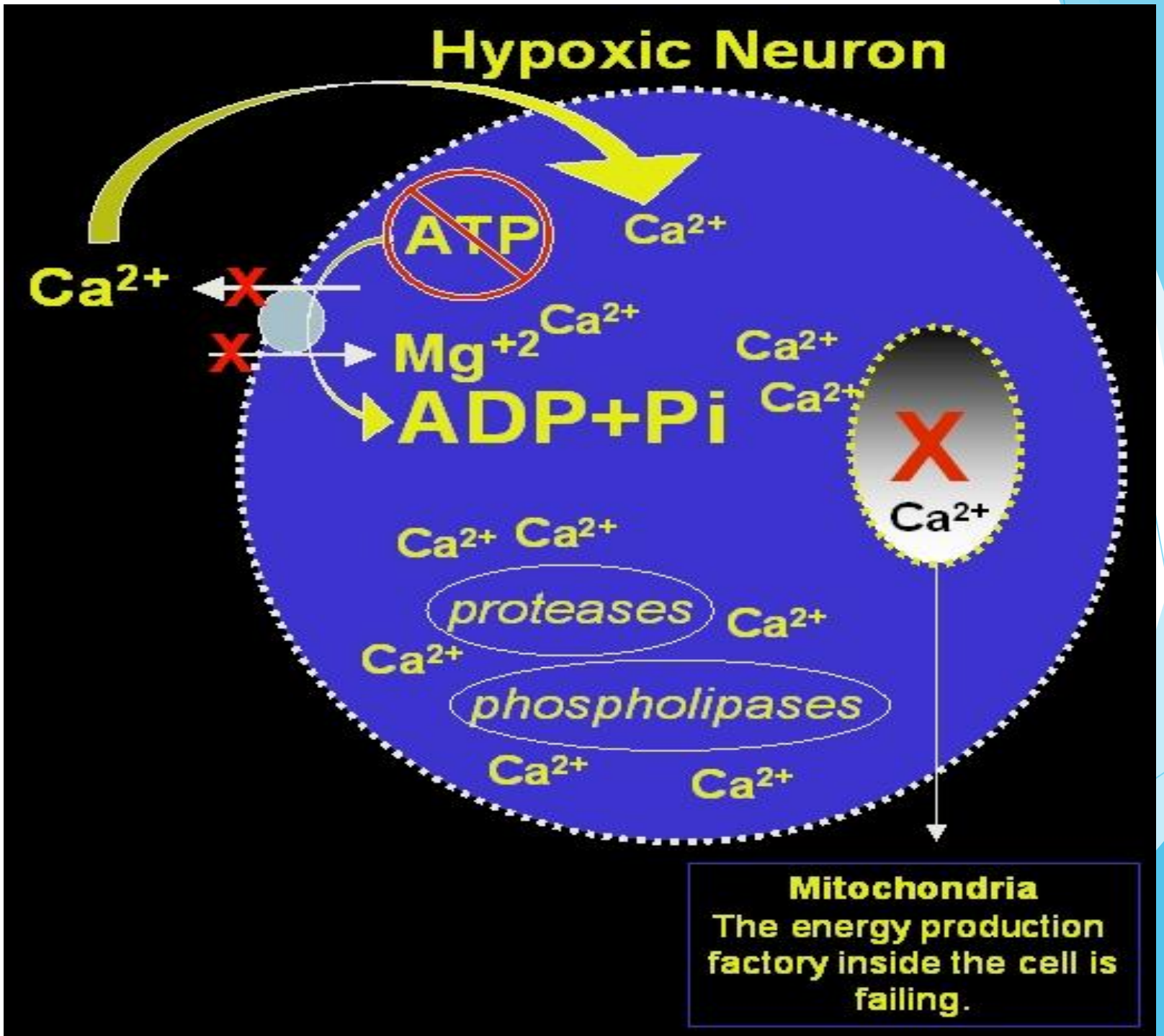
3. Loss of calcium homeostasis:

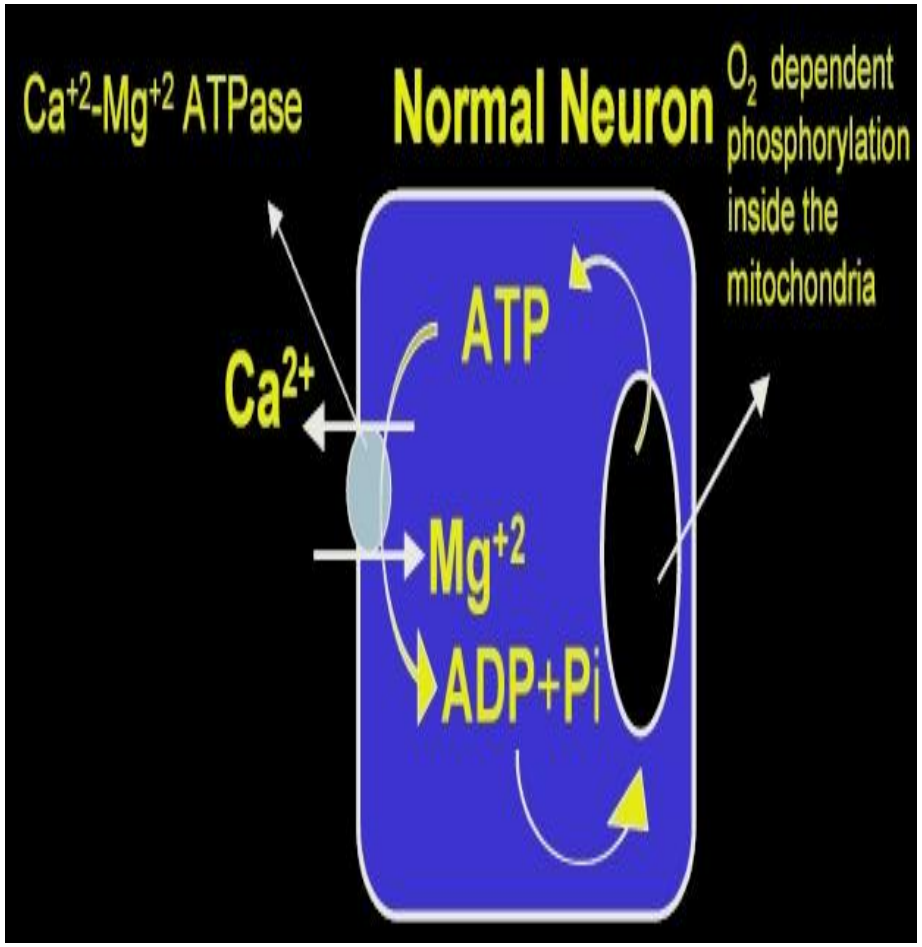
Ca. conc. in cytosol is extremely low than extra cellular. Intra cellular Ca. is sequestered within mitochondria & in E.R. This is maintained by energy dependent Ca,Mg-ATPse.

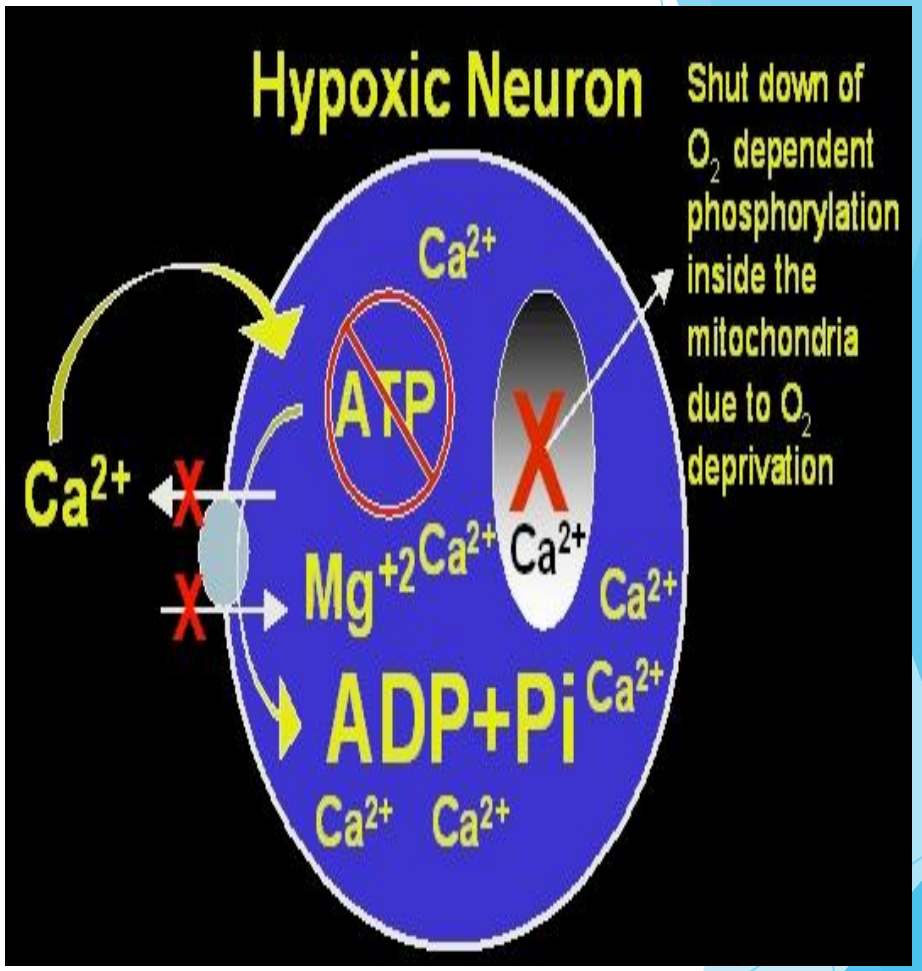
- Ischemia and toxins increase cytosolic Ca conc. due to influx of extra cellular calcium through plasma mem. and also because of release of Ca. from Mito. And E.R.
- Increse cytosolic calcium activates number of enzymes with harmful effects. The enzymes activated are

1. **Phospholipases**-cause phospholipid degradation of cell mem and leads to its damage
2. **Proteases**-cause breakdown or rupture of membrane (cytoskeletal) proteins- like microfilaments,microtubules and intermediate filaments

   Damage to microtubules leads defects in movement of leucocytes, sperms, cilia etc
3. **ATPases** –increases ATP depletion
4. **Endonucleases**-cause breakdown of nuclear chromatin


Hypoxic Neuron
ATP
Ca2+
Ca2+
Mg+2
Ca2+
ADP+Pi
Ca2+
Ca2+
Ca2+
Ca2+ Ca2+
proteases
Ca2+
Ca2+
phospholipases
Ca2+
Ca2+
Mitochondria
The energy production factory inside the cell is failing.


Ca+2-Mg+2 ATPase
Normal Neuron
O2 dependent phosphorylation inside the mitochondria
ATP
Ca2+
Mg+2
ADP+Pi


Hypoxic Neuron
Shut down of O2 dependent phosphorylation inside the mitochondria due to O2 deprivation
Ca2+
ATP
X
Mg+2
ADP+Pi

4.Defects in Membrane permiability:

The plasma mem. damaged by bact. toxins, viral proteins, complement components, cytotoxic lymphocytes, physical & chemical agents, secondary loss of ATP synthesis or ca. mediated activation of phopholipases

5. Mitochondrial damage:

Integrity of mito. mem is important for survival of cell.

Irreparable damage to mito. will ultimately kill the cell.

Mito. are damaged by increase cytosolic calcium, oxidative stress, breakdown of phospholipids etc.

Damage results in **Formation of high conductance channels** in inner mem. also called **Mitochondrial permeability transitions or pores are formed.** This results in

1. disappearance of proton gradient across the mito. mem preventing ATP generation.
2. Leakage of cytochome –c an imp soluble protein in electron transport chain in to cytosol which activates apoptotic death pathways.

- Two model systems to understand cell injury:
- 1. Ischaemic and Hypoxic cell injury
  - Reversible
  - Irreversible
- 2. Free radical mediated cell injury

Hypoxia- Glycolytic energy production can continue although less efficiently than aerobic pathway

Ischemia-Loss of blood supply so anaerobic energy generation will stop after glycolytic substrate present in blood are exhausted.

Ischemia injuries tissues faster than hypoxia

Ischemic and Hypoxic injury
(Reversible)

The first point of attack of hypoxia-Cell's aerobic respiration

i.e. oxydative phosphorylation by mitochondria

Production of ATP decrease or stops as O2 conc. within the cell decreases.This depletion of ATP cause widespread effects on many systems within the cell.

Normal cell- high intracellular osmotic pressure exerted by proteins

To balance this Na+ is maintained at higher conc outside the cell by sodium pump mechanism and K+ is also kept at higher conc inside the cell.

The decrease ATP conc due to acute hypoxia disrupts the sodium pump and results in accumulation of Na+ inside the cells and diffusion of K+ outside the cell.

The Na+ and protein inside the cell increases intracellular osmotic pressure so Water enters inside the cell- Acute cellular swelling

This is the first manifestation of almost all forms of cell to injury

The entry of water inside the cell is associated with early dilation of E.R.

The ↓ in cellular ATP leads to associate ↑ in cellular AMP

This stimulate the enzyme phosphofructokinase & phosphorylase → ↑ rate of anaerobic glycolysis to maintain cell's energy by generating ATP from glycogen

so glycogen is rapidly depleted. This results in accumulation of lactic acid and inorganic phosphates and intracellular pH is reduced.

The reduced pH → Early clumping of chromatin

detachment of ribosomes from rough ER

dissociation of polysomes in to monosomes with resultant reduction in protein synthesis

If hypoxia continues → increase mem permiability & decrease mito. function, bleds may form at cell surface,

myelin figures (concentric lamenations) derived from plasma or orgenellar mem may seen in cytoplasm or extracellularly.

AT this stage mito. usually swollen and E.R. is dilated & cell is swollen

All these disturbances are reversible if oxygen is restored

Ischemic and Hypoxic injury (Irreversible)

- In continuous hypoxic injury-at what point the cell actually die or event responsible for point of no return (Irreversibility)
- Two phenomena characterize irreversibility
- **A. Cell mem. damage-** profound disturbances in mem. function- central factor for irreversibility. It occurs due to
- 1. Progressive loss of phospholipids by loss of calcium homeostasis and activation of enzymes. Following phospholipid loss lipid breakdown products (unesterified free fatty acids, acyl carnitine, lysophospholipids accumulate within ischemic cells and cause further cell damage. Calcium is greedily accepted by the mitochondria and E.R and permanently poisons them.
- B. **Inability to reverse mitochondrial dysfunction-** Lack of oxidative phosphorylation and ATP production even after correction of original injury- mitochondrial permeability transitions and pores are formed causing permanent damage to the cells.

Organellar changes associated with Irreversible injury:

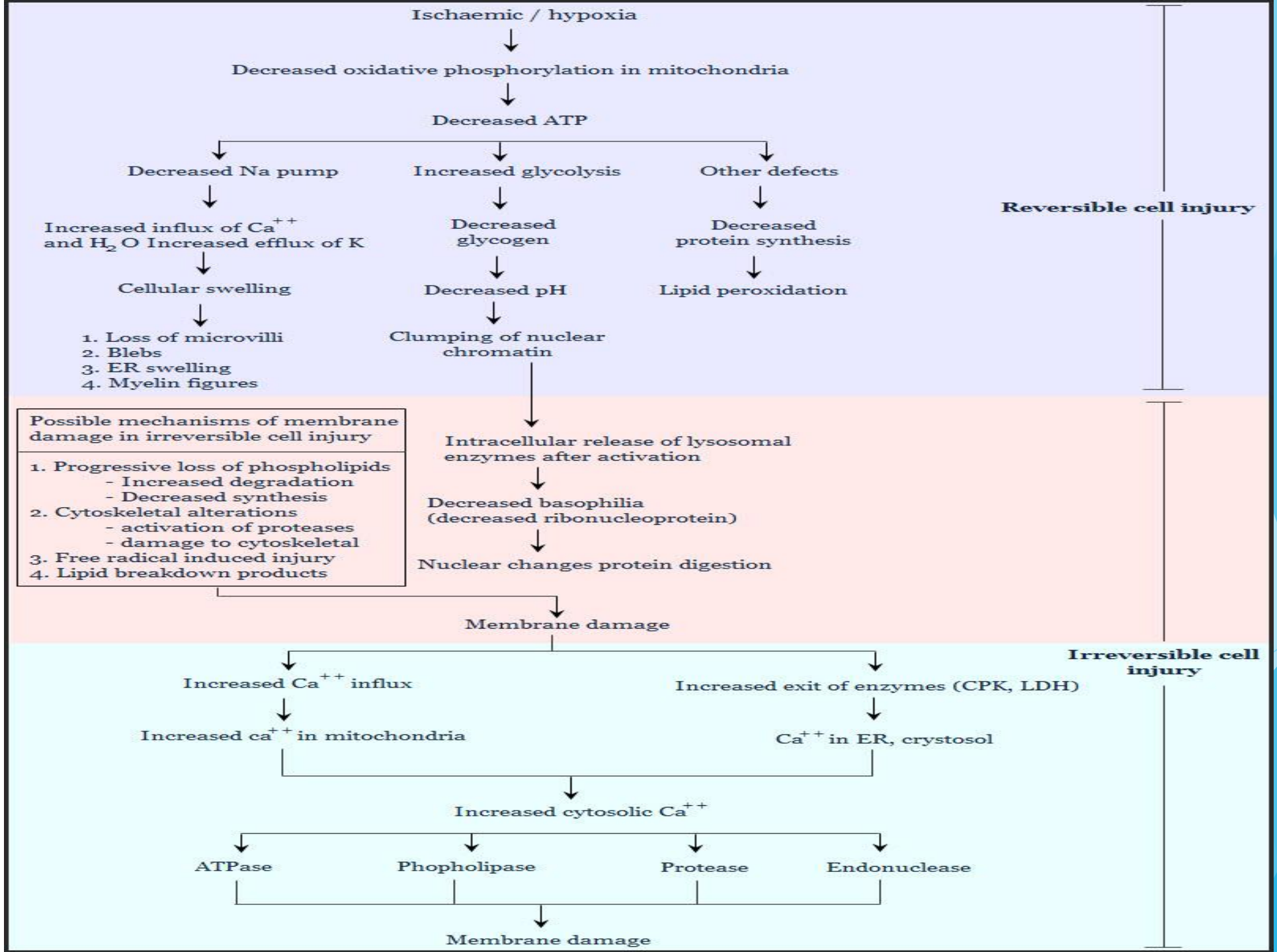
- Severe swelling & vacuolization of mitochondria including cristae
- extensive damage to plasma membrane
- Swelling of lysosomes
- Massive Ca influx within the cell with large amorphous rich densities in mito. Matrix

There is continued loss of proteins, essential coenzymes, metabolites and RNA from hyper permeable plasma mem. which further leads to depletion of ATP and fall in pH

Fall in pH leads to accumulation of lactic acid and inorganic phosphates- cause injury to the lysosomal mem- leakage of lysosomal enzymes-activation of acid hydrolases, RNases, DNases, proteases, phosphatases, cathepsins,glucoxidases- enzymic digestion of cytoplasmic and nuclear components of a cell

After death

- Cellular components are progressively digested by lysosomal enzymes
- There is leakage of potentially destructive cellular enzymes into extra cellular space and entry of extra cellular macromolecules into dying cells.
- Dead cell may be replaced by large masses composed of phospholipids called myelin.
- This is either phagocytosed by other cells or degraded into fatty acids.
- Calcification of such fatty acid residues occur with formation of calcium soaps


Ischaemic / hypoxia
Decreased oxidative phosphorylation in mitochondria
Decreased ATP
Decreased Na pump
Increased glycolysis
Other defects
Increased influx of Ca++ and H2O Increased efflux of K
Decreased glycogen
Decreased protein synthesis
Cellular swelling
Decreased pH
Lipid peroxidation
1. Loss of microvilli
2. Blebs
3. ER swelling
4. Myelin figures
Clumping of nuclear chromatin
Reversible cell injury
Possible mechanisms of membrane damage in irreversible cell injury
1. Progressive loss of phospholipids
- Increased degradation
- Decreased synthesis
2. Cytoskeletal alterations
- activation of proteases
- damage to cytoskeletal
3. Free radical induced injury
4. Lipid breakdown products
Intracellular release of lysosomal enzymes after activation
Decreased basophilia (decreased ribonucleoprotein)
Nuclear changes protein digestion
Membrane damage
Increased Ca++ influx
Increased exit of enzymes (CPK, LDH)
Increased ca++ in mitochondria
Ca++ in ER, crystosol
Irreversible cell injury
Increased cytosolic Ca++
ATPase
Phopholipase
Protease
Endonuclease
Membrane damage

Free radical induced injury

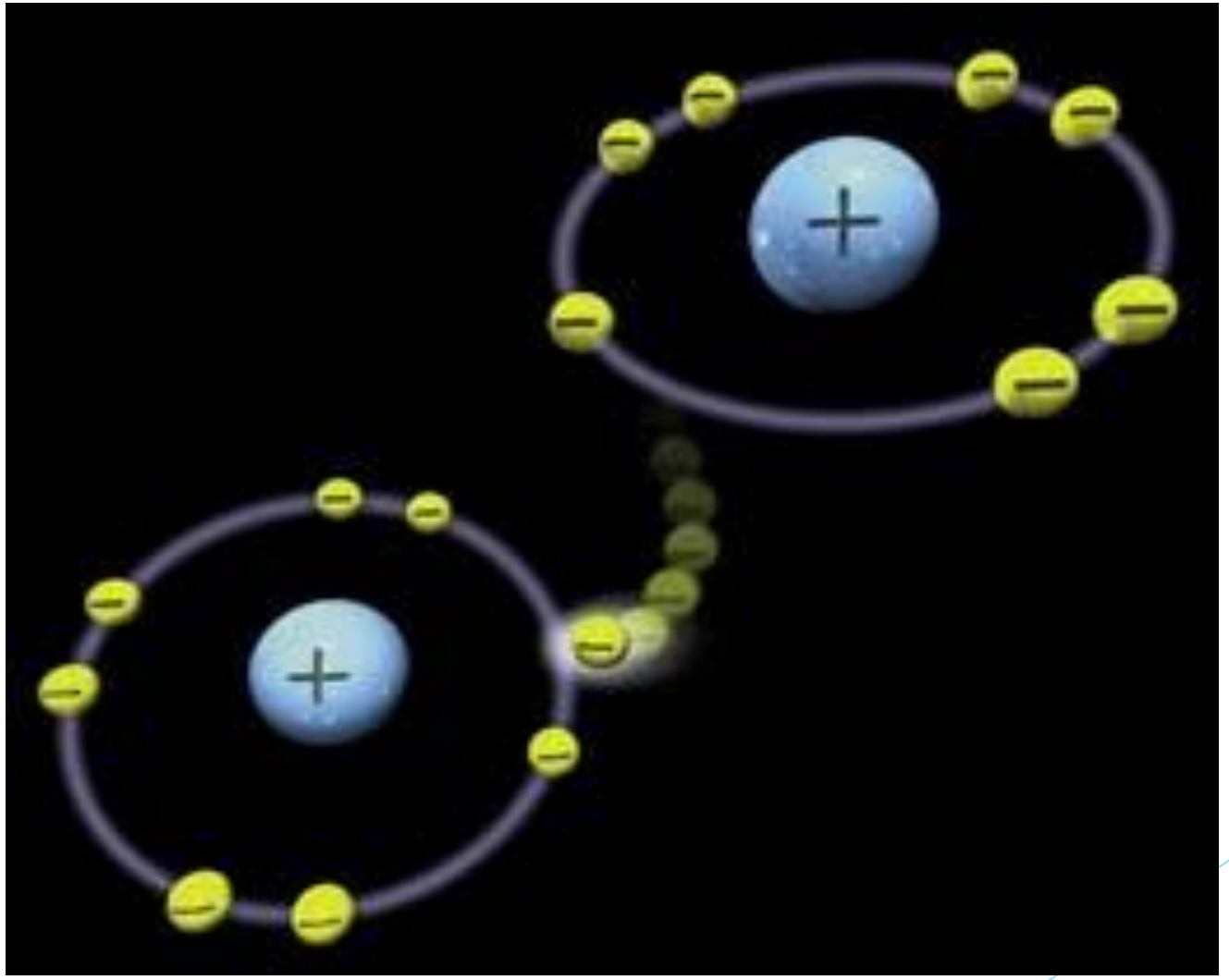
Injury induced by **free radicals** particularly by partially reduced activated oxygen species is imp. mechanism of cell damage.

Small amounts of partially reduced reactive oxygen forms are produced as a by-product of mitochondrial respiration.

- Free radical-mediated damage are seen in
- Chemical and radiation injury
- Oxygen and other gaseous toxicity
- Ischemia-reperfusion injury
- Inflammatory cell damage
- Cellular aging
- Tumour destruction by macrophages
- Microbial killing by phagocytes.

- Free radicals are chemical species that have single unpaired electron in an outer orbit.
- **Extremely unstable, highly reactive, chemical species with a single unpaired electron in an outer orbit.**

PHYSICAL CHEMISTRY OF ATOM



- Elements –Atoms –(p+, N, e^-)
- e^-- rotate around the nucleus (p+ = e^-)
- e^- arranged with in a series of shells or orbits and are in pairs and spin in opposite direction.
- The inner orbit fills first with e^- and it must have two e^- otherwise it will be unstable and highly reactive. eg. Atom of H has only one e^- in its orbital and so it is unstable and highly reactive. It reacts with another atom of H and by combining, two atoms share e^- and form a stable molecule of H2. Each pair of shared e^- is called covalent bond which holds two adjacent atoms together. When atoms combine to form molecules, electrons only in the outer orbital are involved.
- For atom to be stable outer orbital must have a group of 8 e^- (oclet)

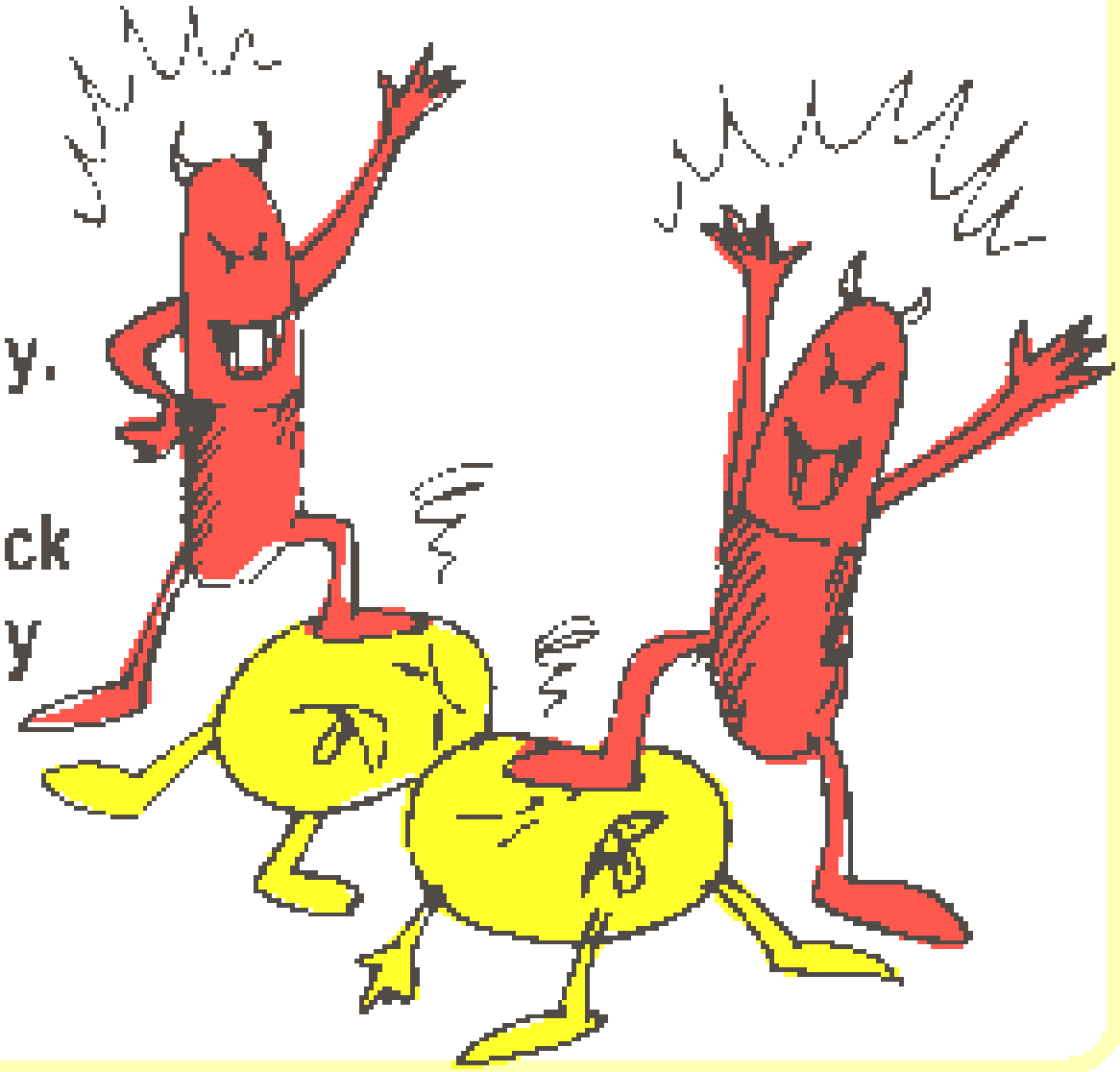
  otherwise atom will be unstable and will react until oclet is formed.

  eg. Atom of O2- has 8 e^- so 6 e^- in outer orbit, so it combine with another atom of O2 shares e^- through covalent bonds and fulfill its requirement of eight and becomes a stable molecule of O2 or react with two atoms of hydrogen and form a stable molecule of water

- So Free radicals is atom or group of atoms that have single unpaired electron in an outer orbit.
- In this state it is extremely reactive and unstable and react with proteins, lipids, CHO, nucleic acids present inside the cells.
- They also initiate autocatalytic reactions means molecules that react with free radicals are themselves converted into free radicals-chain of reactions occur.
- Unpaired electrons derived from oxygen are most imp in cell injury . Oxygen derived free radicals are formed during normal metabolic processes in redox reactions.
- Nitric oxide (NO) also formed in cells and act as free radical.
- Toxic metabolites formed during activity of oxidative enzymes in cells are Superoxide radicals,H2O2,hydroxyl radicals $OH^{\cdot}$

What are Free radicals ?

- Free radicals are like robbers which are deficient in energy.
- Free radicals attack and snatch energy from the other cells to satisfy themselves.



Superoxide (O_2^-)

- Molecule of O2 that has 13 e.
- It carries a negative charge and extremely reactive- Superoxide anion
- Generated during autooxidation or by the transfer of single electron to O2 in reactions catalysed by cytoplasmic enzymes such as cytochrome P450,NADPH oxidase in neutrophils.
- $O_2 + e^- \rightarrow$ (NADPH oxidase) $\rightarrow O_2^-$
- Rapid bursts of super oxide occur in activated neutrophils during inflammation.
- Super oxide once produced can be inactivated spontaneously or rapidly by the enzyme super oxide dismutase forming H2O2
- $2O_2^- + 2H \rightarrow$ (SOD) $\rightarrow H_2O_2 + O_2$

Hydrogen peroxide(H_2O_2)

- Produced either by dismutation of super oxide
- $2O_2^- + 2H \rightarrow (SOD) \rightarrow H_2O_2 + O_2$ or directly by oxidases present in peroxisomes (a cytoplasmic organelles contain enzymes both for production and degradation of H_2O_2)
- Catalase present in peroxisome decomposes H_2O_2 in to oxygen and water. $2H_2O_2 \rightarrow O_2 + 2H_2O$
- Glutathione peroxidase (GSH-Px) present in cytosol protects against injury caused by H_2O_2
- $H_2O_2 + 2\ GSH(Reduced) \rightarrow GSSG(Oxidised) + 2H_2O$ or $2OH^{\cdot}$
- $2OH^{\cdot} + 2GSH \rightarrow 2H_2O + GSSG$

Hydroxyl radicals($OH^{\cdot}$)

- Produced by interaction with transition metals (Fe,Cu) in the Fenton reaction where Fe and Cu donate or accept free electrons during certain intra cellular reaction
- $Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + 2OH^{\cdot} + 2OH^{--}$

- Also produced by hydrolysis of water by ionizing radiation due to absorption of radiant energy (U.V light, X rays)
- $H_2O \rightarrow OH^{\cdot} + H^{\cdot}$
- It has 7 electrons in outer orbital and most reactive in inducing cell damage.

- Singlet Oxygen : A form of oxygen in which one electron is shifted in to a high energy orbit. because of it distorted configuration it is unstable and reactive
- Nitric Oxide (NO) :Soluble free radical gas produced by endothelial cells, macrophages, neurons etc.it can be converted in to highly reactive nitrite species like peroxy nitrite anion as well as $NO_2^{\cdot}$ and NO_3^{-}

Mechanism of Free radical injury

1. Lipid peroxidation of membranes: In the presence of oxygen they cause peroxidation of lipids in plasma and organeller membrane at double bonds in unsaturated F.acids. Lipid radical interaction yield peroxides.they also initiate autocatalytic chain reaction causing severe injury
2. Cross linking of proteins :Act on sulfhydryl bonds(-SH-HS-) of proteins and form disulphide bonds(-S-S-) in methionine, lysine, histidine, cystine and cause extensive damage to the cell
3. DNA Fragmentation: reacts with thymine in nuclear and mitochondrial DNA and produced single stranded breaks and cause cell killing and malignant transformation

Cells have developed multiple mechanisms to remove free radicals and thereby minimize injury

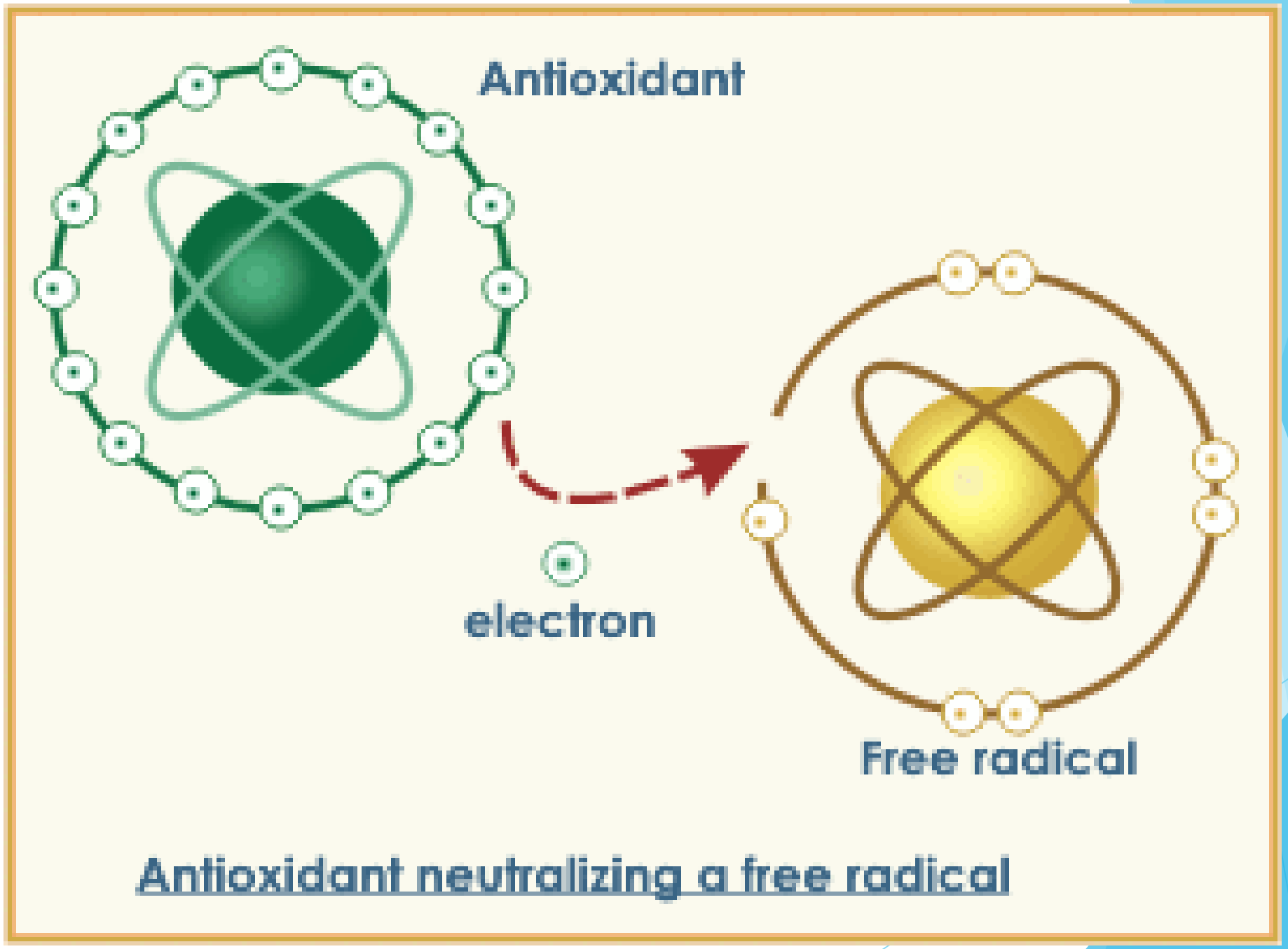
- ANTIOXIDANTS:

  antioxidants either block the initiation of free radical formation or inactive free radicals.

  e.g.: vitamin-E

  vitamin-A

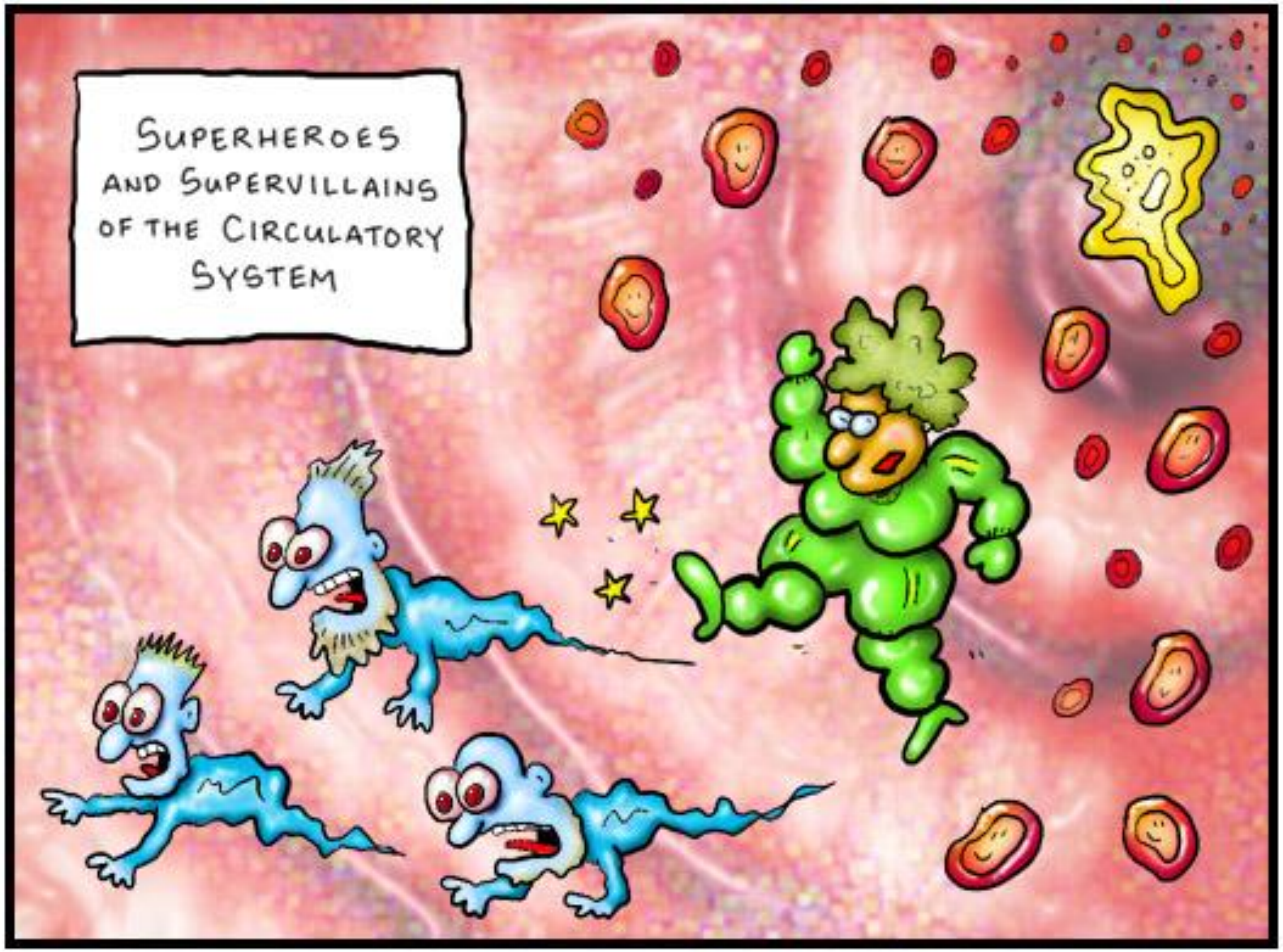
  Ascorbic Acid



Antioxidant neutralizing a free radical

DOCTOR FUN

23 May 2000



Auntie Oxidant kicks out the Free Radicals.

Source Of Antioxidant

I. In the form of Medicines:

- Vitamin A, C & E,
- Cystine, Glutathion, Melthionine,
- Bioflavines, Se, Zn.

II. Food sources:

- Green & yellow vegetables
- Herbs: -Turmeric, Garlic, Grape, Tea, Berries, Carrot, Spinach, Broccoli,
- Red Meat, Kidney, Liver & Lipoic Acid

- As we have seen, iron and copper can catalyze the formation of reactive oxygen species.
- The levels of these reactive forms are minimized by binding of the ions to storage and transport proteins.

  e.g:-transferrin

  -lactoferrin

  -ferritin