MECHANICS OF RESPIRATION

Refers to the process by which organic molecules such as glucose are broken down into smaller inorganic molecules such as carbon dioxide and water and the energy stored within the bonds of the organic molecules is used to synthesize ATP (Adenosine triphosphate).

Respiration can be seen at two levels: (1) internal respiration: refers to the chemical process of energy release from organic compounds such as glucose (2) External respiration/breathing, refers to the process of exchanging oxygen and carbon dioxide between the organism and its surroundings.

ATP

ATP is an energy rich compound which acts as a link between cellular exergonic and endergonic reactions. It's a triphosphate ester compound of adenine ribonucleoside which is formed by union of one molecule of purine base adenine, one molecule of pentose sugar, ribose and three molecules of phosphoric acid. Adenine and ribose combine to form a nucleoside called adenine ribonucleoside. Three phosphate esters of adenine ribonucleoside are found which is called adenosine monophosphate, adenosine diphosphate (ADP) and adenosine triphosphate (ATP). In all these three compounds the CH_2OH group of ribose forms an ester-link with the phosphate group of phosphoric acid

ATP AS A SOURCE OF ENERGY

Small water-soluble molecule; easily transported around the cell/mobile/energy carrier; and immediately releases energy to body cells while glucose has to undergo series of biochemical changes to release energy;

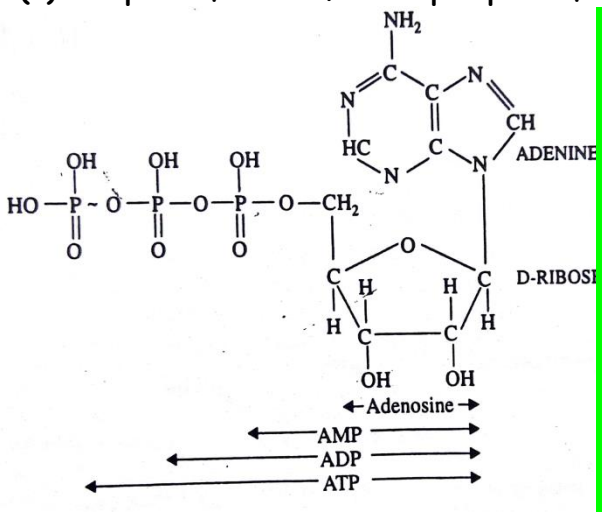
ATP releases little amount of energy in a given period of time to avoid burning out of the body cells while metabolism of glucose releases a lot energy and if not handled well cells may burn out;

Temporarily stores energy for the cell;

MOLECULAR STRUCTURE OF ATP

ATP is a phosphorylated nucleotide made up of the following parts

(1) Adenine; nitrogen containing organic base belonging to purines
(2) Ribose; sugar molecule with 5-carbon ring structure/pentose sugar; functions as a backbone to which other parts are attached;
(3) Phosphates; chain of three phosphates;



ATP HYDROLYSIS IS FAVOURED BY THE FOLLOWING

The repulsion between the four negative charges in the ATP^{4-} is reduced when ATP is hydrolysed because two negative charges are removed with phosphates

The H^+ ion which is released when ATP is hydrolysed reacts with OH^- ions forming water- this s a highly favoured reaction

The charge distribution on ADP + P is more stable than that on ATP

Hydrolysis of ATP yields energy that s utilized in the various metabolic pathways

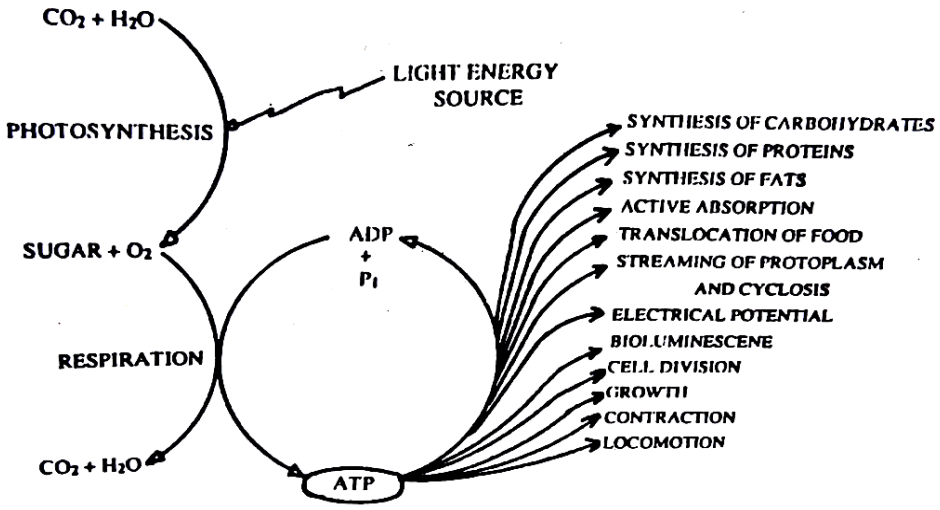
(1) Protein synthesis (2) fat synthesis (3) protoplasmic streaming (4) cell division

CAUSES OF ENERGY RICHNESS OF ATP

-In each of the phosphate groups of ATP oxygen atoms acquire electrons and so assumes a negative charge which induces positive charge on the neighboring atoms; thus, all the three phosphates become positively charged due to which electrostatic repulsion is created among phosphorous atoms. The energy required to overcome this electrostatic repulsion of like positive charges on phosphorous atoms and hold together, this energy is stored in these phosphates and when one phosphate group is removed by hydrolysis energy is released.

-ATP possesses lesser number of resonance forms than ADP and for this reason ATP is less stable. Comparatively ADP possesses lesser resonance forms than AMP and there AMP possesses very many resonance forms in all the three adenosine phosphates and is hydrolysed with great difficulty

The hydrolysis of ATP yields energy used in the following activities:

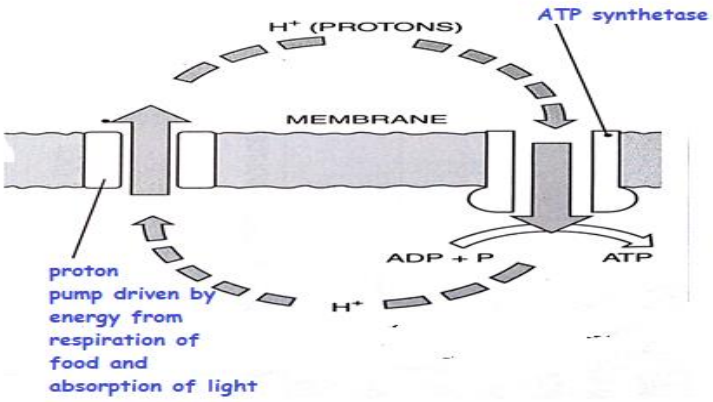


FORMATION OF ATP

ATP is produced during photosynthesis and respiration by Chemiosmosis.

Chemiosmosis involves generation of a proton gradient across an impermeable membrane and dissipated with release of energy that drives the phosphorylation of ADP to ATP catalyzed by ATP synthase/synthetase.

SCHEMATA SHOWING CHEMIOSMOSIS

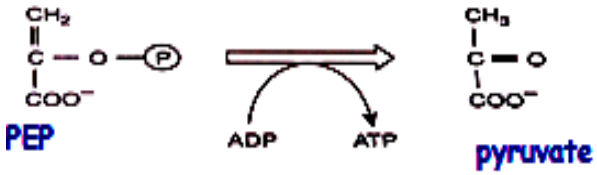


There are three types of phosphorylation

(1) **Oxidative phosphorylation**: refers to the synthesis of ATP during oxidation of coenzymes in the electron transport system but the initial stage involves oxidation of the substrate releasing pair of hydrogen atoms which later dissociate to release electrons (source of hydrogen atoms is organic molecules). It occurs on the inner mitochondrial membrane of plant and animal cells and the cell surface membrane of the bacterial cells.

(2) **Photophosphorylation:** formation of ATP using sunlight in green plants. Occurs in the grana of the chloroplasts.

(3) **Substrate level phosphorylation:** A phosphate group is transferred from phosphorylated compound to ADP. These compounds include phosphoenol pyruvate and 1, 3-bisphosphoglyceric acid.



DIFFERENCES BETWEEN OXIDATIVE AND PHOTOPHOSPHORYLATION

similarities

Both involve generation of proton electrochemical gradients across the membrane;
Both results into the formation of ATP molecules;
In both formation of ATP from ADP and Pi is catalyzed by ATpase enzyme;
In both proton pumps are powered by the energy released as electrons pass along chain of carriers;
In both protons flow back through chemiosmotic channels along PEG
Both occur in specialized organelles;
Both involve pigments i.e. chlorophyll and cytochromes

Differences

Oxidative phosphorylation	Photophosphorylation
Occur during respiration;	Occur during photosynthesis;
Occurs in the mitochondria;	Occur within the chloroplast;
Occurs on the inner membrane of cristae;	Occurs in the thylakoid membrane;
Molecular oxygen is needed during terminal oxidation;	Molecular oxygen is not required;
Energy released during electron transfer due to oxidation-reduction reaction during is used ATP formation;	Source of energy for conversion of ATP from ADP and Pi is external;
Process takes place in electron transport system involving cytochromes;	Pigment system I and II are involved during the process;
ATP molecules are released in the cytoplasm available for different metabolic reactions;	The produced ATP molecules are used up for carbon dioxide assimilation in the dark reaction of photosynthesis;
Main hydrogen acceptor is NAD;	Main hydrogen acceptor is NADP;

Independent of light;	Dependent on the light;

PHOTOPHOSPHORYLATION

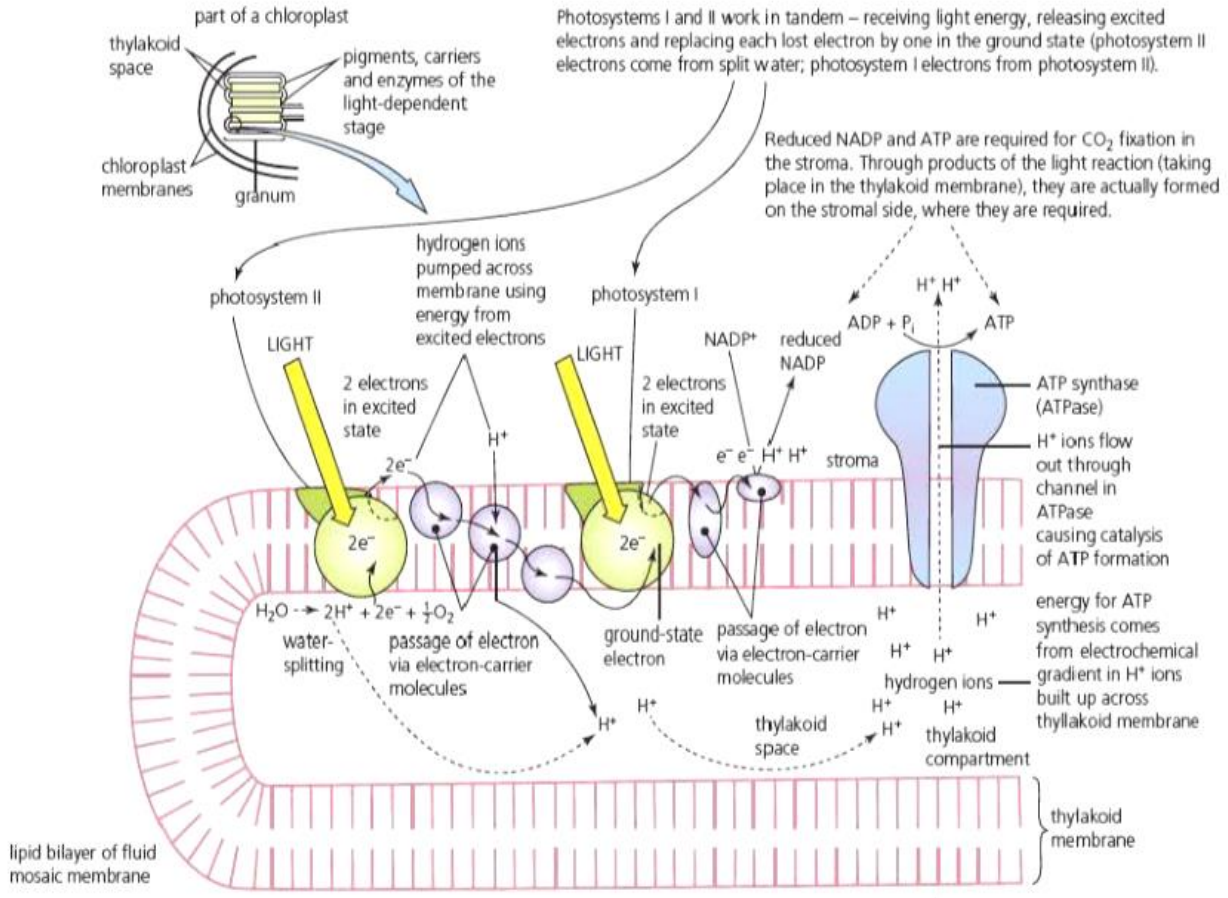
Refers to the generation of ATP from ADP and inorganic phosphate, in presence of sunlight
There are two types of photophosphorylation (1) Non-cyclic which involves PSI and PSII forming ATP, reduced NADP oxygen (more quantities of ATP formed)
(2) Non-cyclic photophosphorylation which involves PSI and formation of ATP and reduced NADP (less quantities of ATP formed)

MECHANICS OF NON-CYCLIC PHOTOPHOSPHORYLATION

When photon of light strikes PSII (P680); its energy is channeled to the primary pigment reaction centre; light excites a pair of electrons inside chlorophyll molecule; electrons escape from chlorophyll molecule and captured by electron carriers (protein with iron in the centre) embedded in the thylakoid membrane. Electrons replaced by the electrons derived from photolysis; when iron combines with the electrons it becomes reduced; it can now donate electrons to the next carrier in the chain that it becomes oxidized;
As the electrons flow along the electron carriers embedded in the thylakoid membrane they release the energy they are associated with at each step; energy is used to pump protons across the thylakoid membrane into the thylakoid space; electrons are then captured by another chlorophyll a in PSI and these are used to replace the electrons lost due to excitation of electrons from PSI;
Photolysis of water by light; releases electrons and hydrogen ions/protons; Protons are actively pumped out of the stroma across thylakoid membrane into the thylakoid lumen/compartment; the energy used for the pumping of protons is released from the excited electrons as they pass down the electron carrier system/photophosphrylation; protons accumulate in the thylakoid lumen creating an electrochemical gradient/concentration gradient; this gradient enables electrons to diffuse back into the stroma through chemiosmotic channels; because the inner membrane is impermeable to hydrogen ions the potential energy is lost by protons which is used to generate ATP from ADP and inorganic phosphate (pi) and this is catalyzed by ATP synthetase/ATpase/ATP synthase;

N.B

Remember the formation of reduced NADP is catalysed by NADP-reductase enzyme and hence chemicals such as DCMU (Dichloro-phenyl dimethyl urea) and other herbicides can interfere with its formation and the light dependent stage too as a whole.
Fig.1 Outlines what happens in non-cyclic photophosphorylation



OXIDATIVE PHOTOPHOSPHORYLATION

Uses electrons associated with reduced NAD and FAD released in the Krebs cycle and glycolysis to synthesised ATP with water produced as a by-product.

The respiratory break down of glucose and intermediates such as phosphoglyceraldehyde, pyruvic acid, iso-citric acid, alpha-ketoglutarate, succinic acid and malic acid oxidized, each oxidative state involves releases of pair of hydrogen atoms which dissociate into two protons and two electrons; the pair of electrons and hydrogen atoms formed do not combine directly with oxygen but pass through a series of coenzymes and cytochromes, which form the electrons transport system, before reacting with oxygen to form water; ETS is made up of the following coenzymes and proteins.

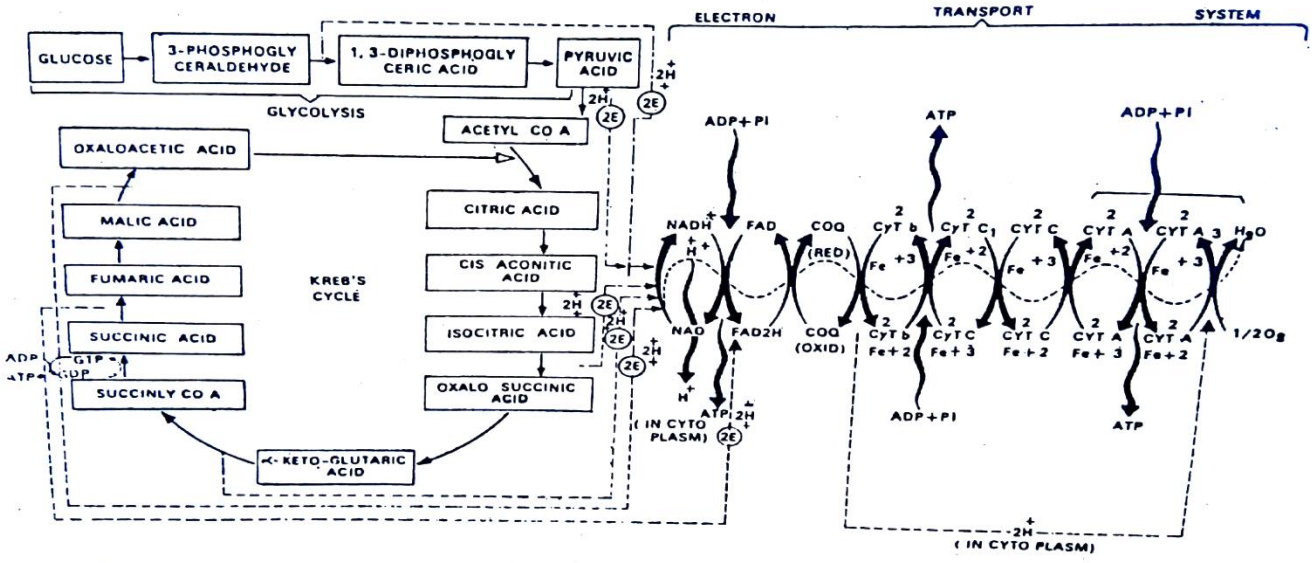
A COMPLETE SET OF ELECTRON CARRIERS IN AEROBIC RESPIRATION SHOWING THE TRANSFER OF ELECTRONS AND PROTONS BOTH OR ONLY ELECTRONS AS IN CASE OF CYTOCHROME

Nicotinamide adenine dinucleotide (NAD), Flavo-proteins (FMN) or FAD; Fe-S- protein complex; Coenzyme Q or ubiquinone (UQ), Cytochrome b (Cyt.b), Fe-S protein, Cytochrome C_1 (Cyt C1), Cytochrome C (Cyt. C), Cytochrome a (Cyt. a), Cytochrome a_3 (Cyt. a_3)

STEPS/SERIES OF THE ELECTRON TRANSPORT SYSTEM

(1) The pairs released from different substrates of Krebs cycle except succinic acid reacts with matrix oxidized NAD. Two electrons and one proton are transferred to oxidized NAD causing reduction and one proton is released to the medium.
(2) Now, two electrons and one proton are transferred from reduced NAD to Flavo-protein (FMN) causing oxidation of NAD and reduction of FMN into $FMNH_2$, one hydrogen atom is picked up from the hydrogen pool to complete this reaction.
(3) The free energy released at this step is stored during oxidative phosphorylation and one molecule of ATP is synthesised from ADP and inorganic phosphate.
(4) The hydrogen pair from succinic acid is first transferred to FAD to form $FADH_2$. The $FADH_2$ transfers electrons to ubiquinone through Fe-S and from UQ the electrons pass to cytochromes arranged in normal series on the basis of the redox potential. Thus during oxidation of succinic acid only 2ATP molecules are generated
(5) The oxidation of $FMNH_2$ takes place by transferring electrons to Fe-s protein to form reduced Fe-S and oxidized FMN. The protons are released in space.
(6) The reduced Fe-S protein transfers the two electrons to ubiquinone or CoQ one by one. The two protons are picked up from matrix to form UQH_2
(7) The reduced ubiquinone then transfers electrons to cytochrome b one by one while the two hydrogen ions are released in the medium. Thus UQH_2 is oxidized while Cyt.*b* becomes reduced;
(8) Cyt b transfers its electrons to Fe-S protein causing oxidation of Cyt b and reduction of Fe-S protein
(9) The reduced Fe-s protein transfers electrons to Cyt C_1 to reduce it. The energy released at this stage is coupled to form ATP from ADP and Pi
(10) The reduced Cyt C_1 transfers a pair of electrons to Cyt.C causing its reduction
(11) Reduced Cyt.C transfers electrons to Cyt.a causing its reduction
(12) Pair of electrons are transferred from Cyt.a to a3. Thus Cyt.a_3 is reduced. The energy released at this step is coupled to form ATP
(13) Reduced Cyt.a3 loses a pair of electrons which are accepted by molecular oxygen along with two hydrogen ions from medium to form water catalysed by cytochrome oxidase.

<u>SUMMARY OF THE STEPS ARE SHOWN BELOW</u>



CHEMIOSMOSIS (Mitchell's hypothesis)

Proposed by peter Mitchell, a British biochemist, in 1961

The hypothesis is explained through the following points:

Inner mitochondrial membrane possesses three kinds of flow

(i) Electron transport (ii) proton translocation (iii) ATP synthesis

Energy released when electrons flow along the electron transport chain is used to pump hydrogen ion actively across the impermeable inner membrane into the intermebranal space; from the mitochondrial matrix; generating an electrochemical proton gradient across the membrane/generates sufficient electrochemical energy. This exerts a proton motive force; protons move back into the mitochondrial matrix down electrochemical gradient through specific sites/stalked particles; operating the ATPase system; energy released by the protons is used to drive formation of ATP synthesis

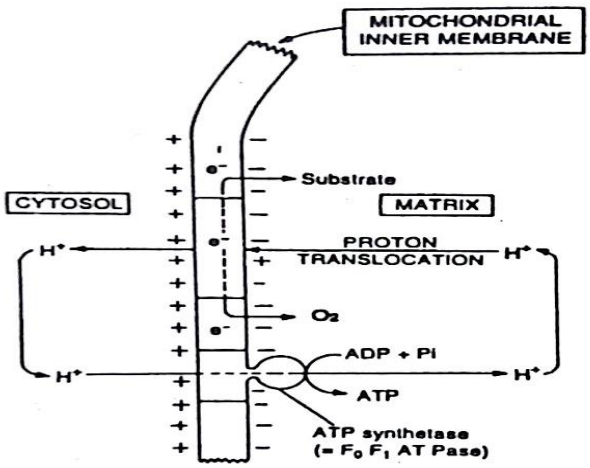
EVIDENCES FOR CHEMIOSMOTIC THEORY

The use of 2,4-dinitrophenol during photophosphorylation studies. Chemicals destroyed the proton gradient across the mitochondrial membranes preventing ATP synthesis but ATP synthesis was done with imposed PH gradient.

The inner membrane is impermeable to hydrogen ions, potassium ions and chloride ions

If the vectorial organisation of the respiratory chain and ATPase in the coupling is changed, oxidative phosphorylation does not take place

SCHEMATA OF THE CHEMIOSMOTIC COUPLING HYPOTHESIS



RESPIRATORY INHBITORS

1. Inhibitors of electron transport include Rotenone, antimycins, cyanides, azides, hydrogen sulphide and carbonmonoxde.
2. Inhibitors of oxidative phosphorylation include Oligomycin, Rutamycin, Atractylate and Bongkrekate
3. Uncouplers of oxidative phosphorylation include 2,4-dinitrophenol, DIcumarol and Chlocarbonyl and phenylhydrazone
4. Ionophores of oxidative phosphorylation, Ionophores promote the transport of cations other than hydrogen ions through the membrane Valinomycin, Gramicidin and Nigericin

CYANIDE RESISTANT RESPIRATION PLANTS(CCR)

Respiration is cyanide sensitive because cytochrome oxidase which catalyses the transfer of electrons between cyt. a3 and oxygen is rapidly inactivated by the reaction with cyanide leading to inhibited oxygen uptake and ATP generation. However, respiration of a number of higher plants such as potato, arum fungi and bacteria are cyanide resistant and its believed that these organisms have alternative insensitive non-cytochrome terminal pathway which allow transfer of electrons and in this pathway the electrons are transferred from CoQ to oxygen and in this transfer, heat is evolved not ATP

NOTE

The concentration of the cyanide decreases with increasing age due to its break down in order to stop the inhibition of the enzymes involved in respiration forming energy used to form new tissues of the highly growing plant

CHEMIOSMOSIS IN CHLOROPLASTS AND MITOCHONDRIA

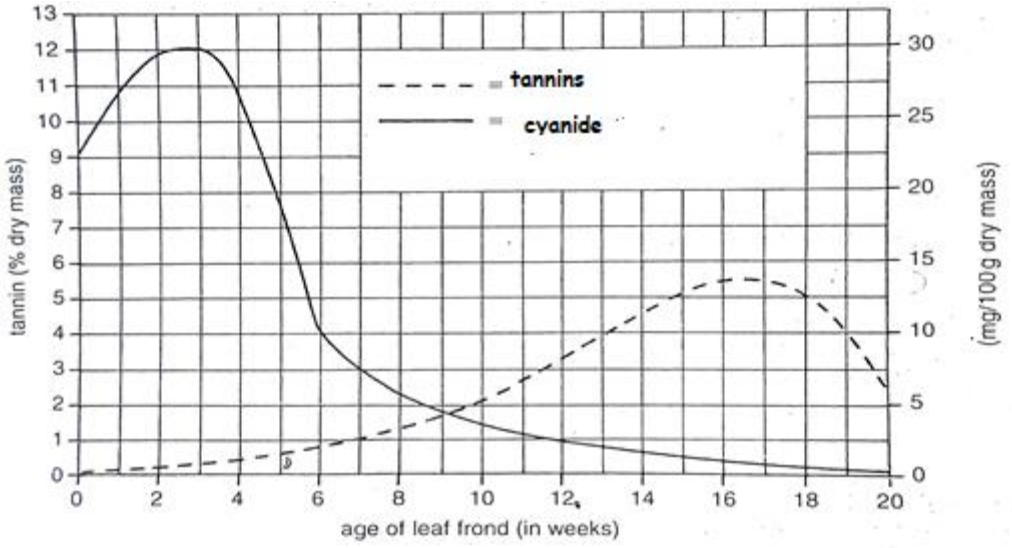
Similarities

in both there is creation of an electrochemical proton gradient across membrane;
in both electrons are passed via chains of carriers at different energy levels to form energy;
in both hydrogen ions are pumped along using energy formed from electron carriers;
In both ATpase is used to combine ADP and inorganic phosphate to form ATP;
in both hydrogen ions diffuse via chemiosmotic channels;

Differences

Chloroplasts	*Mitochondria*
Hydrogen ions are obtained from photolysis;	*Hydrogen ions are got from dissociation of hydrogen atoms from reduced NAD;*
Hydrogen ions are actively pumped via thylakoid membranes;	*They are pumped via the inner membrane;*
Electrons are obtained from excitation of chlorophyll molecules using light energy;	*Obtained from dissociation of hydrogen atom from reduced NAD;*

THE GRAPH SHOWS VARIATION OF TANNINS AND CYANIDE WITH AGE



COMMENT(S)

From 0 week to 3 weeks the concentration of the cyanide increased rapidly; to a peak; because they are made by the leaves; in order to provide protection against herbivory;(Feeding on buds/young leaves) from 3 weeks to 20 weeks the concentration of cyanide decreased rapidly later gradually to a minimum; because cyanide was broken down by plant enzymes; to stop inhibition of the respiratory enzymes; adequate ATP required for rapid growth during this age. From 0 week to 17 weeks, the concentration of tannins increases gradually to a peak; due to increased secondary metabolism; forming tannins; for protection of the plant against herbivory/entry of pathogen; and tannins take up role of cyanides due to their lack of inhibitory effects on respiratory enzymes; From 17 weeks to 20 weeks, the concentration of tannins decreased gradually, due to fall in secondary metabolism; with increasing age.

MECHANISM OF RESPIRATION

1) Glycolysis 2) Krebs cycle 3) Electron transport system

GLYCOLYSIS (GK: Glycos=sugar, lysis= dissolution)

(EMBDEN, MEYERHOF AND PARNAS PATHWAY)

Definition: Oxidation of glucose or glycogen to pyruvate and lactate is called glycolysis.

This is the process by which glucose is oxidized to pyruvic acid in presence of many enzymes and coenzymes (NAD^+) in the cytoplasm; Eq. involves the activation, cleavage/splitting and oxidation of a 6C-sugar into a two 3C-pyruvate with direct formation of a net of two ATP molecules

This was described by Embden, Meyerhof and Parnas. Hence, it is also called as Embden Meyerhof pathway. Process of fermentation in yeast cells was similar to breakdown of glycogen in muscles. occurs virtually in all tissues. Erythrocytes and nervous tissues derive its energy mainly from glycolysis.

This pathway utilizes O2 if available (aerobic) and it can function in absence of O2 also (anaerobic).

Two Phases of Glycolysis

Aerobic phase: Oxidation is carried out by dehydrogenation and reducing equivalent is transferred to NAD+. Reduced NAD in presence of O2 is oxidized in electron-transport chain producing ATP.

Anaerobic phase: NADH cannot be oxidized in electron transport chain, so no ATP is produced in electron transport chain. But the NADH is oxidized to NAD+ by conversion of pyruvate to lactate, without producing ATP. Anaerobic phase limits the amount of energy per mol. of glucose oxidized. Hence, to provide a given amount of energy, more glucose must undergo glycolysis under anaerobic as compared to aerobic. Enzymes: Enzymes involved in glycolysis are extra mitochondrial

Steps of glycolysis

(i) **Activation (ii) cleavage (iii) oxidation**

ACTIVATION OF SUGAR

Glucose is phosphorylated to glucose-6-phosphate, where ATP donates an inorganic phosphate to hexose sugar ;(Hexokinase enzyme catalyses the reaction)

Glucose-6-phosphate undergoes isomerization to fructose-6-phosphate (enzyme: phosphohexoisomerase)

Phosphorylation of fructose-6-phosphate using ATP forming fructose-1,6-phosphate)

CLEAVAGE

Fructose-1,6-phosphate splits into two carbon 3C-compounds, 3C-phosphoglyceraldehyde and dihydroxyacetone phosphate (Enzyme aldoses), both compounds are termed as triose phosphates and interconverted into each other in presence of enzyme phosphotrioisomerase.

OXIDATION

3-phosphoglyceraldehyde reacts with inorganic phosphate forming 1,3-diphosphoglyceraldehyde;

1,3-diphosphoglceraldehyde is oxidized to 1,3-diphosphoglyceric acid in presence of coenzyme NAD (dehydrogenation) during which two molecules of hydrogen are released from diphosphoglyceraldehyde which reduces NAD to $NADH_2$

The 1,3-diphosphoglyceric acid loses one of its phosphate which combines with ADP forming ATP and 3-phosphogyceric acid

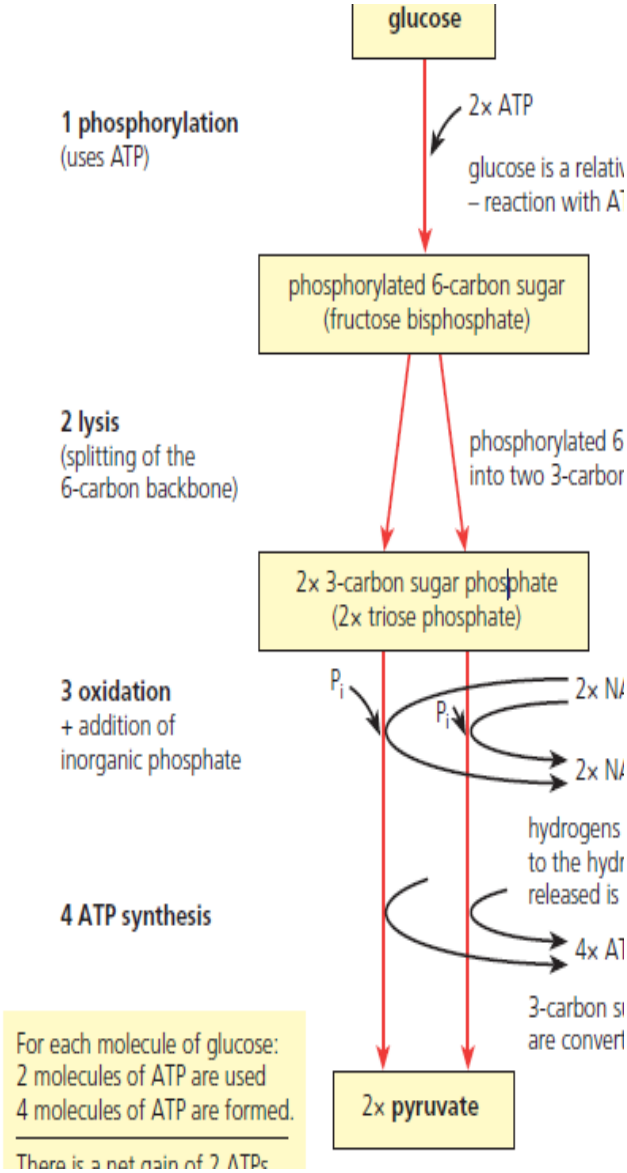
Isomeric change occurs resulting into the transfer of phosphate in the 3-phosphoglyceric acid from C3 to C2 forming 2-phosphoglyceric acid;

2-phosphoglyceric acid loses two molecules of water in presence of enolase and converted to PEP/phosphoenol pyruvic acid)

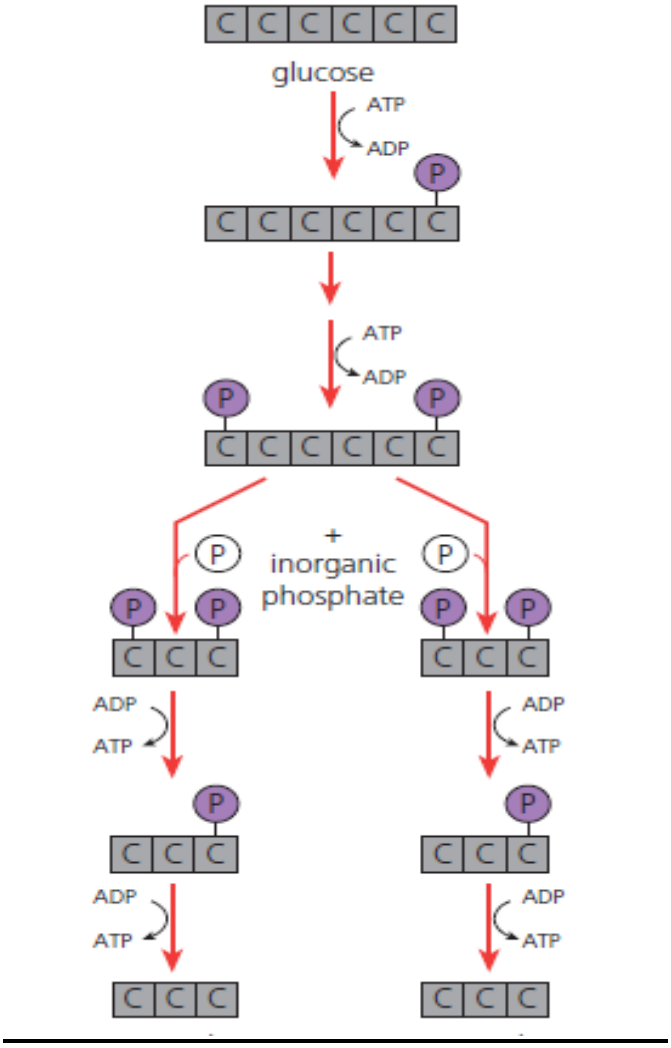
Now one phosphate group is removed from phosphoenol pyruvic acid with production of ATP (ADP accepts the phosphate group forming ATP)

STEPS/STAGES IN GLYCOLYSIS

PHASE ONE OF GLYCOLYSIS



CHANGES TO THE CARBON SKELETON



Significance of Glycolysis

Glycolysis is the principal route for glucose metabolism for the production of ATP molecules.

(1) Important biochemical significance is the ability of glycolysis to provide ATP in the absence of oxygen and allows tissues to survive anoxic episodes.

(2) It generates precursors for biosynthetic pathway, e.g. Pyruvate may be transminated to amino acid alanine. In the liver, pyruvate provides substrate, acetyl-CoA for fatty acid biosynthesis. Glycerol-3-phosphate, which is required for the synthesis of triacylglycerol, is derived from glycolytic pathway. In erythrocytes, glycolysis supplies 2,3-BPG which is required for the transport of oxygen by Hb.

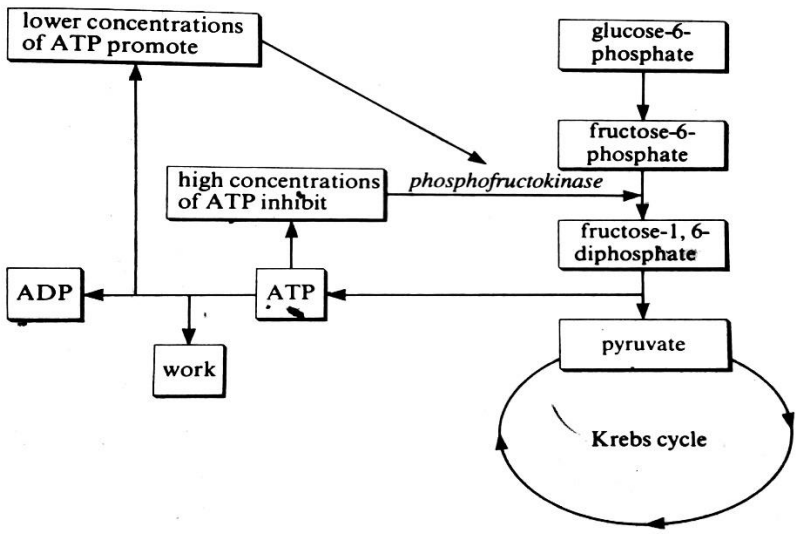
Formation of reduced NAD

Breakdown of glucose to pyruvate ready for Krebs cycle

REGULATION OF GLYCOLYSIS/RESPIRATION BY END-PRODUCT INHIBITION

Phosphofructokinase; catalyses phosphorylation of fructose-6-phosphate; to fructose-1,6-diphosphate; during glycolysis; when ATP concentration is high; as a result of higher rate of respiration/glycolysis; inhibits the enzyme; by binding onto the allosteric sites; active sites shape change; complementary substrate(fructose-6-phosphate) fails to bind; no phosphorylation; ATP produced reduced; when ATP concentration falls; due to increased usage in cell metabolism; inhibition is reversed; metabolic pathway comes into operation again;

SCHEMATA



SUMMARY OF GLYCOLYSIS

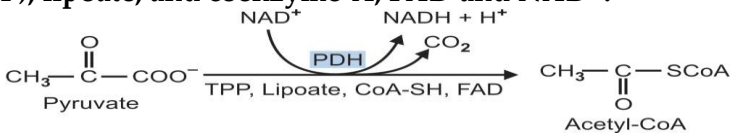
In glycolysis from one molecule of glucose, two molecules of pyruvic acid are formed
In these four molecules of ATP are formed but because two ATP molecules are consumed during activation of glucose, the net gain of ATP becomes Two;
Two molecules of NAD^+ are reduced to two molecules of reduced NAD which are later oxidized aerobically to yield six molecules of ATP.

STEP	GAIN/LOSS
Glucose to glucose-6-phosphate	*-1*
Fructose (6) to fructose-1,6-diphosphate	*-1*
2-molecules of 1,3-phosphoglyceraldehyde to 2-molecules of 1,3-diphosphoglyceric acid (NADH2 to NAD^+)	*+6*
2[1,3-diphosphoglyceric acid] to 2[3-phosphoglyceric acid]	*+2*
2[PEP] to 2-pyruvic acid	*+2*

CONVERSION OF PYRUVATE TO Acetyl-CoA

Pyruvate is converted to acetyl CoA by oxidative decarboxylation. This step occurs only in mitochondria.
This is an irreversible reaction catalyzed by a multienzyme complex known as pyruvate dehydrogenase complex (PDH)

The enzyme pyruvate dehydrogenase requires five coenzymes, namely thiamine pyrophosphate (TPP), lipoate, and coenzyme-A, FAD and NAD+.



Energetics in conversion of pyruvate to Acetyl CoA

As a result of oxidation of pyruvate to acetyl CoA catalyzed by pyruvate dehydrogenase, one molecule of NADH is produced for each molecule of pyruvate. Oxidation of NADH by electron transport chain results in synthesis of 3 ATP molecules.

Significance

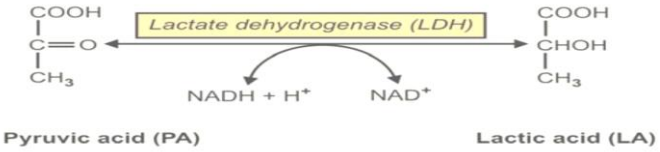
The conversion of pyruvate to acetyl-CoA is a central step, linking the glycolytic pathway with citric acid cycle.

ANAEROBIC RESPIRATION

FATE OF PYRUVIC ACID in absence of oxygen.

Conversion of Pyruvic Acid to Lactic Acid

It is an important reaction, because it occurs in skeletal muscles working under conditions of absolute or relative lack of O2. In anaerobic glycolysis, Pyruvate acts as a temporary H-store. It dehydrogenates (oxidises), the reduced NADH + H+ back to oxidised NAD+, so that glycolysis can continue even in absence of O2. Pyruvate is thus reduced to Lactic acid. In presence of O2, Lactic acid can be oxidised to pyruvic acid again.



FATE OF PYRUVIC ACIDS

- **Forms acetyl-CoA by oxidative decarboxylation (in presence of O2)**
- **Forms lactic acid by reduction (in absence of O2)**
- **Forms alanine by amination**
- **Forms glucose (gluconeogenesis)**
- **Forms malic acid → to OAA (oxaloacetic acid)**
- **Forms oxaloacetic acid (OAA) by CO2-fixation reaction**

FORMATION OF ETHANOL IN PLANTS AND SOME MICROORGANISM

Anaerobic respiration in plants leading to production of ethanol in organisms such as bacteria, fungi and higher plants, for example root cells under water logged conditions

Pyruvate molecule formed at the end of glycolysis loses a molecule of carbon dioxide and ethanal is formed catalyzed by pyruvate decarboxylase; ethanal accepts hydrogen atom from reduced NAD; to produce ethanol and oxidized NAD; catalyzed by alcohol dehydrogenase.

CITRIC ACID CYCLE

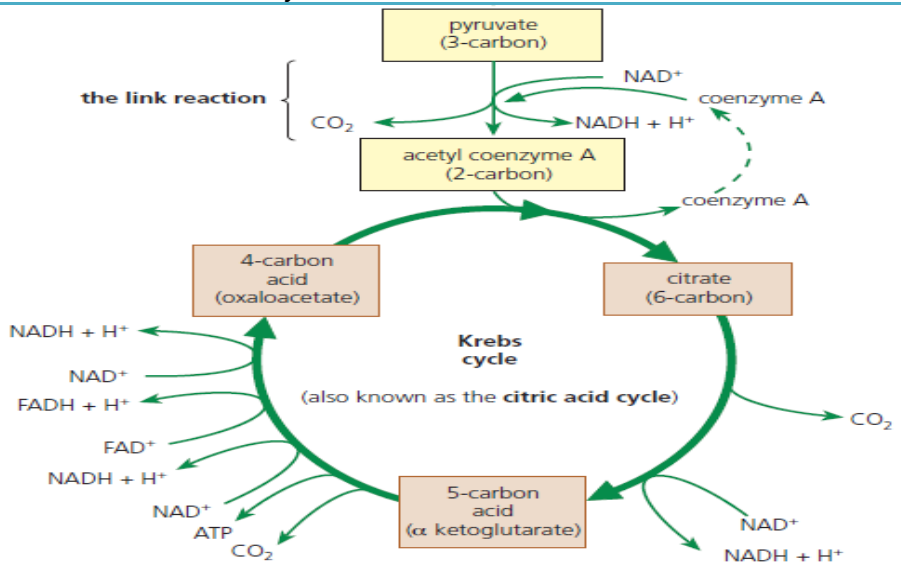
The citric acid cycle is a series of reactions in mitochondria that brings about the catabolism of acetyl-CoA to CO_2 and H_2O with generation of ATP.

Citric acid cycle is also called Krebs cycle or tricarboxylic acid (TCA) cycle.

• It is called citric acid cycle because citrate was one of the first compounds known to participate.
• It is called Krebs cycle, because its reactions were formulated into a cycle by Sir Hans Krebs.
• The most common name for this pathway is, the tricarboxylic acid or TCA cycle, due to involvement of the tricarboxylates—citrate and isocitrate.

MECHANISM

First reaction of the citric acid cycle is the condensation of acetyl-CoA with oxaloacetate to yield citrate, catalyzed by citrate synthase.
2. Citrate is converted to isocitrate by an enzyme aconitase. This conversion takes place in two steps:
– Dehydration to cis-aconitate
– Rehydration to isocitrate.
3. Isocitrate undergoes dehydrogenation in the presence of isocitrate dehydrogenase to form oxalosuccinate.
There follows a decarboxylation to -ketoglutarate, also catalyzed by isocitrate dehydrogenase.
The formation of NADH and liberation of CO2 occurs at this stage.
4. Next -ketoglutarate undergoes oxidative decarboxylation, catalyzed by a multi-enzyme complex, -ketoglutarate dehydrogenase, an
-ketoglutarate dehydrogenase complex requires thiamine pyrophosphate
(TPP), Lipoate, NAD, FAD and coenzyme- A and results in the formation of succinyl-CoA, a high energy compound, this reaction is physiologically irreversible. At this stage, second NADH is produced along with liberation of second CO2 molecule.
5. Succinyl-CoA is converted to succinate by the enzyme succinate thiokinase. This reaction is coupled with the phosphorylation of GDP to GTP. This is a substrate level phosphorylation. This GTP is converted to ATP.
6. Succinate is oxidized further by succinate dehydrogenase to fumarate with the production of FADH2.
7. Next, fumarase catalyzes the addition of water to fumarate to give malate.
8. Malate is converted to oxaloacetate by malate dehydrogenase, and requires NAD+. The synthesis of third NADH occurs at this stage. The oxaloacetate is regenerated which can combine with another molecule of acetyl-CoA and continue the cycle.



ENERGETICS OF CITRIC ACID CYCLE

• As a result of oxidation of acetyl-CoA to H_2O and CO_2 by citric acid cycle, three molecules of NADH and one FADH2 are produced.

• Oxidation of 3 NADH by electron transport chain results in the synthesis of 9 ATP, whereas FADH2 generates 2 ATP molecules.

• One molecule of ATP is generated at substrate level during the conversion of succinyl-CoA to succinate. Thus, a total of 12 ATP is generated from one molecule of acetyl-CoA

ATP producing steps during glycolysis

2{pyruvic acid} to 2{acetyl-CoA}	+6
2{isocitric acid} to 2{alpha-ketoglutarate}	+6
2{alpha-ketoglutarate} to 2{succinyl CoA}	+6
2{succinyl CoA} to 2{succinic acid)	+2
2{succinic acid} to 2{fumaric acid}	+
2(malic acid} to 2(oxaloacetic acid)	6

SIGNIFICANCE OF CITRIC ACID CYCLE

• The primary function of the citric acid cycle is to provide energy in the form of ATP.

• Citric acid cycle is the final common pathway for the oxidation of carbohydrates, lipids, and proteins as
glucose, fatty acids and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle.

• Citric acid cycle is an amphibolic process. Citric acid cycle has a dual function, it functions in both catabolism (of carbohydrates, fatty acids and amino acids) and anabolism. Some metabolic pathways end in the constituent of the citric acid cycle while other pathways originate from the cycle, such as:

– Gluconeogenesis
– Transamination
– Fatty acid synthesis
– Heme synthesis.

• Gluconeogenesis: All major members of the citric acid cycle from citrate to oxaloacetate are glucogenic. They can give rise to glucose by gluconeogenesis.

• Transamination: Oxaloacetate and -ketoglutarate respectively, serve as precursors for the synthesis
of aspartate and glutamate by transamination which in turn are used for the synthesis of other nonessential amino acids, purines and pyrimidines.

• Fatty acid synthesis: Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl-CoA for the biosynthesis of fatty acids and steroids.

ALTERNATIVE RESPIRATORY SUBSTRATES

Sugars are not the only substances oxidized by the cells to release energy. Both lipids and proteins may, in certain circumstances be used as respiratory substrates, without being converted to carbohydrates

RESPIRATION OF LIPIDS

Before being respired, they are converted to glycerol and fatty acids. Glycerol is then phosphorylated and converted to triose phosphate which enters glycolysis pathway and

subsequent Krebs cycle. Fatty acid component is broken down into 2-carbon fragments which are converted to acetyl coenzyme-A. this enters the Krebs cycle.

The oxidation of lipids produces 2-carbon fragments of carbohydrate and many hydrogen atoms. The hydrogen ions are used to produce ATP during oxidative phosphorylation. For this reason, lipids yield/release more than double the energy of the same mass of carbohydrate.

RESPIRATION OF PROTEINS

Protein is first hydrolyzed to is constituent amino acids, their amino-group is removed/deaminated before entering respiratory pathway at different points depending on the number of carbon atoms they contain. 3-carbon compound is converted to pyruvate, while 4- and 5-carbon compound are converted to intermediates in the Krebs cycle.

STARVATION

Starvation is the deprivation of the food and thereby deprivation of exogenous supply of calories to meet
the energy demands of the body for basal metabolism and other activities.

• Starvation is not always the result of unavailability or scarcity of food.

Starvation can be divided into two phases characterized by distinct metabolic changes. In starvation, the changes in metabolism are not abrupt but gradual

PHASES OF STARVATION

1. Short-term starvation, which covers the 12 hr overnight fast and can extend to 24 hr.
2. Prolonged starvation, which lasts longer than 24 hr and can extend to several days or weeks.

Changes in carbohydrate metabolism and role of liver

• The first phase of starvation begins four to five hours after a meal.
• Within about 1 hr after a meal, blood glucose levels begin to fall.
• Consequently, insulin levels decline and glucagon, epinephrine levels rise.
• The brain, the erythrocytes, the bone marrow, the renal medulla and peripheral nerves have to be supplied with glucose, for their energy needs.
• However, the tissues such as muscle can readily use free fatty acids, released from adipose tissue.

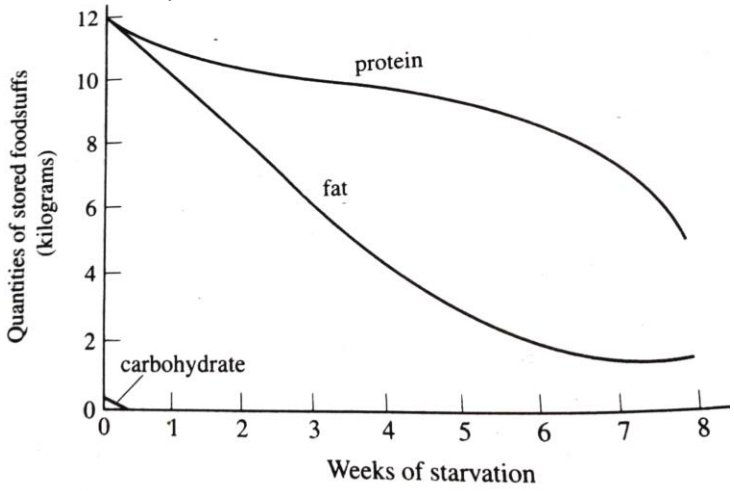
In this phase, the main source of blood glucose is liver glycogen.

• The liver first uses glycogenolysis (glycogen degradation) then gluconeogenesis to maintain blood glucose levels and provide sufficient glucose to the brain and other glucose requiring tissues.

The fall of blood glucose and insulin concentration and rise of glucagon stimulates glycogenolysis to maintain the blood glucose levels.

• Glucagon stimulates c-AMP formation, essential for degradation of glycogen as the liver glycogen store begins to be depleted, gluconeogenesis becomes active, which ensures a continuous supply of glucose to the brain and other tissues.
• The source of substrates for gluconeogenesis are: – Pyruvate – Lactate which is a product of glycolysis in red blood cell and exercising muscle – Glucogenic amino acids released from muscle – Glycerol released by degradation of triacylglycerols

CHANGES IN PROTEINS, FATS AND CARBOHYDRATES DURING STARVATION



Comment on the above graph

From 0 day to day three, carbohydrates decrease rapidly; first reserve to be used as energy source; glycogen stored in the liver and muscles decreases rapidly to depletion; glycogen is broken down into glucose for oxidation to release energy; to maintain vital processes;

During the first 6.5 weeks of starvation, amount of fats decreases rapidly because they are the second energy stores used after depletion of carbohydrates; since have high energy value; broken down in the liver into fatty acids used to produce energy; from week 6.5 to week 9, fat quantity reduced gradually since their great reduction in quantity at the stores led to gradual rate of lipid metabolism as energy stores; and also fats quantity very low; due to full depletion of fat stores and thus not used as respiratory substrates;

From week 0 to week 6.5 quantity of stored protein decreased gradually/slightly; because muscle proteins are source of energy through gluconeogenesis; where proteins are converted to glucose; and during this time the major source of energy are fats which quantity decreases rapidly to provide energy; from week 6.5 to week 9 quantity of proteins decreased very rapidly since fat stores are completely depleted; by then the body resorts to use of proteins as a respiratory substrate as the final phase of starvation;

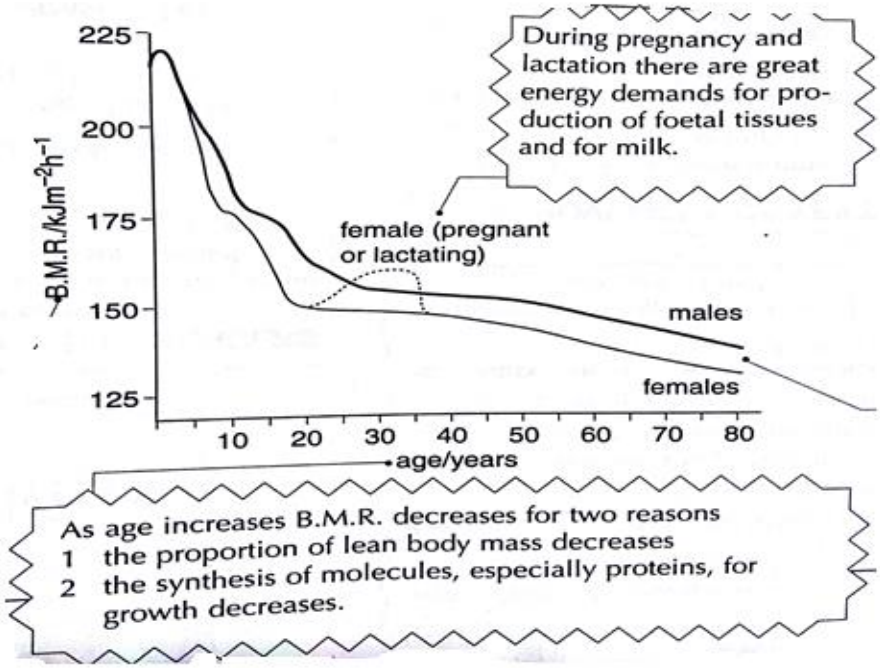
BASAL METABOLIC RATE (BMR)

Refers to the minimum rate of energy conversion required just to stay alive during complete rest or sleep.

B.M.R refers to the energy consumed by the body when at rest. Energy is required for breathing movements/heartbeat/maintenance of ionic gradient across membranes/synthesis of molecules such as proteins.

Metabolic rate refers to the energy consumed by the body in performance of its normal activities/ amount of energy expended by an organism in given time, and it's the measure of the speed at which energy yielding work reactions take place

GRAPH SHOWING VARIATION OF THE B.MR WITH AGE



RELEVANT POINTS TO NOTE

Factors decreasing B.M.R include fasting, malnutrition and sleep
Factors increasing B.M.R include Adrenaline secretion, altered environmental temperatures, exercise and stress.
Age of 19 to about 40 years BMR of females increases rapidly and peaks at 30 years because during pregnancy and lactation there is a great deal of energy demands for production of fetal tissues and for milk
As age increases B.M.R decreases proportion of lean body mass decreases.
Synthesis of molecules such as proteins for growth reduces.
Males have greater B.M.R than women because have a greater proportion of lean body mass (fat cells have a very low metabolic rate)

RESPIRATORY QUOTIENT

Is the measure of the ratio of carbon dioxide given out by an organism to the oxygen consumed over a given period of time

$$RQ = \frac{\text{volume of } CO_2 \text{ given out}}{\text{volume of } O_2 \text{ taken in}}$$

Although the term respiratory quotient is used, the ratio should more accurately be called respiratory exchange ratio (RER). This is because some of the gases being expired may come from non-respiratory sources. The RQ should not include these gases
Different RQ given is an indication of the type of substrate being respired. For example, a typical sugar such as glucose is oxidized accordingly to the following equation.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

The RQ is therefore: $\frac{6CO_2}{6O_2} = 1.0$

Lipids however, have less oxygen relative to carbon and hydrogen. A greater volume of oxygen is therefore required to oxidize lipids completely and their RQs are therefore lower than those of carbohydrates.

$$C_{18}H_{36}O_2 + 26O_2 \longrightarrow 18CO_2 + 18H_2O$$

The RQ is therefore: $\frac{18CO_2}{26O_2} = 0.7$

Proteins have a very varied structure depending on the number and types of each aminocids in the protein molecule. Their RQs are therefore equally varied but most have a value of 0.9

Critical thinking!

Why determining RQs to tell the type of substrate respired is not realistic

Substrates are rarely completely oxidized and partial oxidation gives a different value
Organisms rarely, if ever, respire a single food substance and the RQ therefore reflects the proportion of the different substrates being respired
Most resting animals have RQ of 0.8 and 0.9. although these values would suggest that proteins are respired, we know that protein is used only in extreme situation such as starvation, we must assume, therefore, that these values are due to a mixture of carbohydrates (1.0) and lipid (0.7) being respired

RECOLLECT!

High RQS

High RQs may indicate anaerobic respiration for example high RQ exceeding 1.0 are often obtained from organisms, or tissues, which are short of oxygen. Under these conditions they resort to anaerobic respiration, with the result the amount of carbon dioxide produced exceeds the amount of oxygen used.
They also result from conversion of carbohydrate to fat, because carbon dioxide is liberated in the process. Noticeable in organism laying down extensive food reserves-animals preparing to hibernate and fattening livestock

Low RQs

A very low RQ, on the other hand, may mean that some of carbon dioxide released in respiration is being put to some sort of use by organism, e.g. photosynthesis in plants and construction of calcareous shells

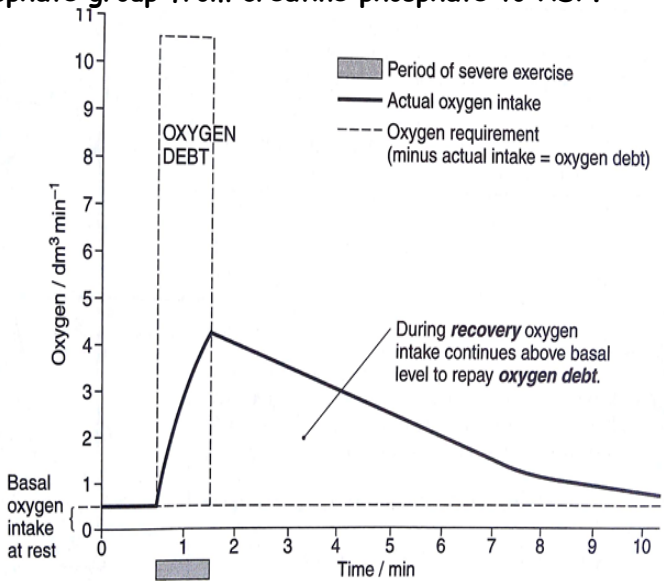
RQ OF GERMINATING SEED

TIME	RQ	POSSIBLE REASON(S)
Seeds soaked in water	*7.2*	*With little oxygen dissolved in water, respiration is a mixture of aerobic and anaerobic respiration*
After 14 hours in soil	*1.5*	*As oxygen becomes available, the amount of aerobic respiration increases while anerobic respiration decreases.*

After 48 hours in the soil	*0.7*	*A mixture of lipids (0.7) and carbohydrates (1.0) is respired. Conversion of lipids to carbohydrates (0.35) also occurs.*
After 14 days	*1.0*	*Leaves have emerged and the plant is producing carbohydrates that is respired (1.0)*

OXYGEN CONSUMPTION AND EXERCISE

The immediate source of energy for contraction of muscle for the formation of the acto-myosin bridges is adenosine triphosphate (ATP), a problem is encountered when stimulation is continuous, since there is only a low concentration of ATP in muscle cell. ATP is replaced by the transfer of a phosphate group from creatine phosphate to ADP.



Comments

From 0 minute to about 0.9 minutes, pre-exercise conditions, oxygen intake was constant and low; athlete was at rest; amount of oxygen taken in met the demands of aerobic respiration; needed to generate ATP; to maintain activities of muscles at rest (muscle tone)/maintaining posture etc. and the basal metabolism for survival;

From 0.9 minutes to 1.5 minutes, oxygen intake increased rapidly up to a peak; because ATP is used up in resting muscles when few twitches occur; and oxygen supply in the body by haemoglobin and storage by myoglobin depleted; on addition the phosphocreatine in muscles used to regenerated ATP by combing with ADP is depleted; so the breathing in increases to supply oxygen required for aerobic respiration to generate ATP; but due to high volumes of oxygen required by the body to cope up with ATP generation and requirement is not met by breathing in hence resulting into oxygen deficit.

aerobic respiration supplement by anaerobic respiration.

Later oxygen uptake decreases rapidly, post exercise condition, because the oxygen taken in is firstly used to oxidize lactic acid and aid the reconversion of some of it into glycogen in the liver.
Finally, gradual decrease in oxygen uptake because oxygen is needed to regenerate phosphocreatine; recharge myoglobin stores and recharge haemoglobin with oxygen

SUMMARY OF GENERAL CHANGES THAT OCCUR DURING RESPIRATION

Oxygen is taken in as a result of movement of ventilation muscles;
Oxygen is exhausted/utilized during oxidation of food materials; where it is a final acceptor of proton and electron in the ETS forming water;
Oxidation of food materials results into release of protons that are actively pumped into the Intermembrane space creating EPG which generates PMF driving protons back into matrix through chemiosmotic channels associated with ATPase catalyzing ATP formation;
Intermediates are formed during oxidation; for example, succinate/alpha-ketoglutarate/fumaric acid;
carbon dioxide and water are released as waste products which removed to outside the body;
Dry weight of the organism is reduced due to break down of organic matter and liberation of water and carbon dioxide;
Dephosphorylation of substrates/intermediates to directly form ATP; e.g. 1,3-phosphoglyceric acid to 3-phospoglyeric acid/succinyl-CoA to succinic acid;

ANAEROBIC RESPIRATION IS LESS EFFICIENT

Anaerobic respiration is wasteful of respiratory substrate. The useful product is a tiny quantity of ATP only, when compared with the yield of ATP from aerobic respiration of the same quantity of respiratory substrate. Also, the waste products ethanol or lactic acid contain much unused chemical energy. For example, ethanol is a very good fuel, in its own right. Lactic acid and ethanol may also be harmful to organisms if they accumulate. However, organisms may be able to tap the energy locked up in the waste products of fermentation by converting them back to sugar, which can then be respired. For example, in the vertebrate body, lactic acid is eventually converted back to glucose in the liver. Yeast, on the other hand, cannot metabolise ethanol, which makes it a useful organism in the industrial production of ethanol.

FACTORS AFFECTING RESPIRATION

Temperature: very low temperature slows down respiration in both homoiotherms and poikilotherms and high temperature increases respiration
Body size: Small organisms with a large surface area to volume ratio lose heat faster and therefore respire faster than large organisms.
Level of activity: Animals engaging in vigorous physical exercise require much energy and so experience faster respiration rate e.g. sprinting, flying, etc.
Growth: Actively growing organisms e.g. young animals and germinating seeds respire faster to generate much energy required to drive metabolic processes involved in cell division.
Dormancy during extreme cold and hot seasons: Respiration rate is always slow to avoid depleting food reserves before the unfavorable season ends.
Type of substrate- carbohydrates metabolized faster than lipids.

PHOTORESPIRATION AND DARK RESPIRATION

Photorespiration	Cellular respiration
Takes place only in the presence of light;	Takes place in both presence of light and dark;
Occurs in green cells;	Occurs in all cells;
Dependent up on concentration of oxygen;	Oxygen concentration has no effect;
Respiratory substrate is 2-carbon glycolic acid	Respiratory substrate may carbohydrate/fats/proteins;
Three different organelles involved namely chloroplast, mitochondria and peroxisomes;	One organelle is involved namely mitochondria
ATP molecules are not synthesized;	ATP molecules are synthesized;
Hydrogen peroxide is formed;	Hydrogen peroxide not formed;
Process of transamination occurs during photorespiration;	Does not occur;
Maximum rate between 25 to 35^0c;	Maximum rate between 35 to 40^0C;
Respiratory substrate immediately formed;	Respiratory substrate may have been previously stored or immediately formed;

COMPARISON OF RESPIRATION WITH PHOTOSYNTHESIS

Similarities Both:

(i) Involve converting energy from one form to another
(ii) Occur in living cells (iii) Form adenosine triphosphate (ATP) (iv) Require energy to occur
(v) Involve a series of multi-enzyme catalysed reactions (vi) Involve flow of electrons along electron carriers.

Differences

Photosynthesis Respiration Occurs in cells with chlorophyll while respiration Occurs in all cells
photosynthesis ocurs in the presence of light while respiration ooccurs all the time
Raw materials for photosynthesis are Carbon dioxide and water while Raw materials for respiration are lipids/glucose/proteins;
photosynthesis Forms Reduced carbon compounds, oxygen, and water while respiration Forms Carbon dioxide and water in photosynthesis
Light is a source of energy in photosynthesis while in respiration Chemical bonds are the source of energy stored

ECONOMIC / COMMERCIAL IMPORTANCE OF ANAEROBIC RESPIRATION

(i) In breweries, fermentation of sugars forms alcoholic drinks like wine, beer and spirits.
(ii) In bakeries, fermentation of starch by yeast is used in leavening of bread i.e. production of raised bread
(iii) Applied in the manufacture of milk products like sour milk, yoghurt and cheese
(iv) Applied in food industries for the manufacture of organic acids e.g. citric acid, oxalic acid and butyric acid.