

## *Chapter 9 (Continued)*

### *The Pentose Phosphate Pathway*

- *The Hexose Monophosphate Shunt*
- *Can be used at the same time as the glycolytic pathway or the ED.*
- *A pathway that is, in its entirety, is **seldom used by anaerobic microorganisms**, but can be operated both aerobically and anerobically.*
- *Aerobic organisms can use the complete pathway to produce **Ribulose 5-phosphate**:*
  - *which is used in the **biosynthesis of nucleic acids** and to produce **reduced NADP***
- *The **NADPH** produced by HMP is used for **anabolic – biosynthesis, rather than catabolic pathways**.*
- *In addition to the 5 carbon sugars, HMP also produces 4-C sugars - **erythrose 4-phosphate**:*
  - *Needed for **synthesis of aromatic amino acids**.*
- *If the microorganism is using a 5 carbon sugar as its sole carbon source, the HMP can serve as a source of 6-carbon sugars:*
  - ***glucose is required for peptidoglycan synthesis***

- *Thus the HMP has both catabolic and anabolic functions, this is a good example of an **AMPHIBOLIC** pathway.*
- *Two unique enzymes of this pathway:*
  1. ***Transketolase:** catalyzes the transfer of a **2-carbon ketol group***
  2. ***Transaldolase:** transfers a three-carbon group from sedoheptulose 7 phosphate to glyceraldehydes 3 P.*
- *Glucose 6P  $\rightarrow$  Fructose 6P + G3P*
- *G3P enter 3 Carbon stage of Glycolytic pathway*
- *Then converted:*  
*G3P  $\rightarrow$  ATP + Pyruvate.*  
*Production of NADPH, which can be converted to NADH yielding ATP when oxidized by electrons transport chain.*

### ***Entner-Doudoroff Pathway***

- *Some microorganisms lack the embden meyerhoff pathway for the metabolism of glucose. These organisms use a different pathway, which was discovered by Entner and Doudoroff.*
- *The initial substrate in the ED and EMP pathway is **glucose**, but the two pathways differ in the final **electron acceptor molecule and the total energy yield.***

- *The cell expends one ATP to make glucose 6-phosphate and then converts it to 6 phospho-gluconate using NADP<sup>+</sup> to produce NADPH.*
- *6 phospho-gluconate is dehydrated to form a 6 carbon unit the key **intermediate in this pathway**, 2 keto-3-deoxy-6-phosphogluconate or **KDPG**.*
- ***KDPG** is then cleaved by an aldolase (KDPG aldolase) to yield pyruvate and G3P.*
- *G3P → to pyruvate with the production of ATP and NADH (Same as the bottom part of the glycolytic pathway).*
- *If ED pathway each glucose metabolized to pyruvate the yield of energy is:*
  - ***1 ATP***
  - ***1 NADPH***
  - ***1 NADH***

*Calculate: used ATP to phosphate glucose  
G6P to 6-Pgluconate (make NADPH)*

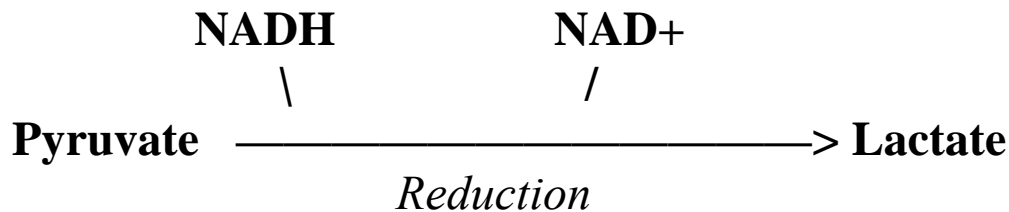
- *The ED is pathway is not used by all microorganisms, it is frequently used by aerobic microorganisms, which rely on respiration for the majority of their ATP synthesis.*
- *Anaerobic microorganisms, as a rule will not use ED, but will use HMP because they can generate more ATP.*

- *Mostly seen in the gram negative bacteria. Very few gram positive have with the exception of *Enterococcus faecalis*.*

## ***Fermentaion***

- *Pyruvic acid is readily available from the breakdown of glucose in a variety of metabolic pathways.*
- *Many cells use **pyruvate, or molecules derived from pyruvate as a terminal electron acceptor** to produce waste products that are exported out of the cell.*

## ***Lactic acid fermentation***



*This fermentation pathway is found in many bacteria and is exploited in the production of yogurt, cheese, buttermilk, sourcream, and that large clump that forms in your milk carton.*

## ***Two Groups of Lactic Acid Fermenters:***

### ***1. Homolactic Fermenters:***

- *Organisms which produce **only** lactic acid*

### ***2. Heterolactic Fermenters:***

- *organisms which can produce other products in*

*addition to lactate ie EtOH and/or Lactate, CO<sub>2</sub>*

### ***Alcoholic fermentation:***

- *Alcohol is produced by a two step reaction process.*
  - *First pyruvate is decarboxylated to acetaldehyde which is in turn reduced to Ethanol with NADH as the electron donor.*
- *Fungi, some bacteria, algae, and protozoa.*

### ***Formic acid fermentation:***

- *Many bacteria (Enterobacteriaceae) are able to split the 3 carbon pyruvate into formic acid (1-c) and a variety of other metabolic products.*
- *The pathways used to produce formate is a very useful tool in identifying the enteric bacteria. There are essentially two routes to format...*

### ***Mixed Acid Fermentation:***

- *Ethanol is produced and secreted along with a complex mixture of other fermentation products, particularly **acetic, lactic, succinic and formic acids.***
- *If the organism possesses the enzyme complex **formic hydrogenylase**, it will convert formic acid into CO<sub>2</sub> and H<sub>2</sub> gas.*
- *Mixed acid fermentation **produces considerably more acid than the other fermentation pathways, and consequently results in a significant change in pH***
- *This fermentation pattern is seen with *Escherichia*, *Salmonella*, *Proteus*, and other genera.*

### ***Butanediol fermentation:***

- *Pyruvate is converted to acetoin, which is subsequently reduced to 2,3-butanediol*
- *This pathway produces a large amount of alcohol, CO<sub>2</sub> and H<sub>2</sub>, and very little acid via the mixed acid pathways.*
- *It is important to emphasize that the cells are not committed to a single pathway... some of the pyruvate is still being converted to formate via alternate routes, **but the vast majority is being converted to butanediol***
- *Butanediol fermentation is a characteristic property of bacteria belonging to **Enterobacter, Serratia, Erwinia genera, and some species of Bacillus.***
- *The butanediol fermenters produce neutral end products while mixed acid fermenters produce a significant amount of acid end-products (4 times) which can drop the pH lower than 4.4.*
- *The acid end products can easily be identified by adding a pH indicator to the growth medium, (ie methyl red (red @ acid)) but the neutral end products will not react*
- *The voges proskauer test is designed to identify butanediol fermenters by detecting acetoin, the precursor of butanediol.*

## ***The Tricarboxylic Acid Cycle – Citric Acid Cycle – (Krebs Cycle):***

*In this cycle:*

- *Energy is released from the breakdown of glucose to pyruvate, much more energy is released when pyruvate is degraded aerobically to CO<sub>2</sub>.*
- ***TCA is a 3 stage Catabolic process***
  1. *Attachment of a acetyl group to the acetyl carrier, oxaloacetate to form citrate.*
  2. *Begins with citrate and end in the formation of succinly CO-A: acetyl carrier portion of citrate loses two carbons when oxidized to CO<sub>2</sub>.*
  3. *Convert succinyl-Co back to oxaloacetate, the acetyl carrier, so that it can pick up another acetyl group.*

***The TCA cycle consists of a set of 8 enzymes that further break down the two acetyl Co-A molecules into 4 CO<sub>2</sub>***

*TCA figure:*

- *2 (3-carbon) pyruvate molecules are broken down into 2 (2 carbon) acetyl coenzyme A molecules (acetyl-CoA) (an energy-rich molecule) and 2 CO<sub>2</sub> molecules by*
- ***Pyruvate Dehydrogenase****(multi-enzyme system) an enzyme reaction which links the Embden Meyerhoff pathway and the Citric Acid Cycle.*

***Isocitrate oxidized and decarboxylated (2x) → alpha ketoglutarate.***

- 2 NADHs
- 2 Carbons are lost as CO<sub>2</sub> maintaining the balance because 2 carbons were added at the beginning.

***Succiny CoA → Succinate***

- substrate level phosphorylation, where GTP (high energy molecule equivalent to ATP) is formed

***Succinate → Fumarate (Four carbon stage)***

- 1 FADH<sub>2</sub>
- 1 NADH

***TCA cycle generates:***

- 2 CO<sub>2</sub>s
- 3 NADHs
- 1 FADH<sub>2</sub>
- 1 GTP

*For each acetyl CoA molecule oxidized.*

***Electron Transport Chain***

- *The reduction of O<sub>2</sub> occurs in the cell membrane and is mediated by a series of proteins which are collectively called the electron transport chain.*
- *The electron transport chain consists of:*
  - ***Cytochromes:*** *a series of heme containing proteins that transport electrons.*

- *a series of nonheme iron sulfur proteins and quinones*
- *The electron transport system is essentially a proton pump which establishes a proton gradient across the cell membrane*
- *As electrons flow through the ETS, they are periodically moved from the inside to the outside of the cell membrane*
  - *Electron Transport Chain carriers are on the inside of the **inner membrane of the mitochondrion or the bacterial plasma membrane***
- *This translocation of proton drops the pH of the periplasm to ~ 5.5 while the cytoplasm (inside) remains at ~8.5, a difference of 3 pH units or 1000X concentration difference. This charge differential represents potential energy which is stored up in the proton gradient.*
- *This energy is referred to as the **proton motive force***

### **Proton Motive Force:**

- *The force arising from a gradient of protons and a membrane potential that is thought to power ATP synthesis and other processes.*

*Since the membrane is impermeable to protons, the only way that these protons can re-enter the cell is through a transport protein.*

- ***ATP synthase** is a protein complex which forms a channel that will let protons pass through the membrane.*

- *As the protons push through the channel, there is energy used to convert ADP and Pi to ATP*
- *Oxidative Phosphorylation or chemi-osmotic phosphorylation (Peter Mitchell 1961) is when the energy from electron transport is used to make ATP.*
- *Substrate level phosphorylation refers to the synthesis of ATP by phosphoryl group transfer directly catalyzed by catabolic enzymes.*

*The second way that a cell can store energy is by maintaining a proton (or charge) gradient across the cell membrane.*

*The phospholipid bilayer that composes the cell membrane is impermeable to protons. The cell uses a set of transport proteins to pump protons across the membrane, out of the cell*

*This creates a proton gradient across the membrane, High proton outside...low proton inside.*

*(the proton gradient is a reservoir of potential energy that can be harnessed in a controlled fashion to generate high energy bonds in the form of ATP)*

*The cells can then allow the protons to re-enter the cell in a controlled fashion, and the energy derived from this movement can be used to do work.*

*This is analogous to water stored behind a dam* **Respiration**

In respiration, the terminal electron (or H) acceptor molecules are generally inorganic.

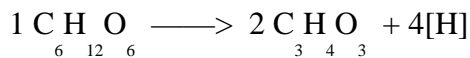
There are some exceptions, such as with fumarate and trimethylamine oxide)

In aerobic respiration, molecular oxygen is used as the terminal electron acceptor

In anaerobic respiration, compounds such as  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$  (sulfate) and other compound

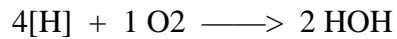
substitute for oxygen as the final H acceptor molecule

The equation for aerobic respiration can be expressed as follows:



1 molecule of glucose is oxidized to 2 molecules of pyruvate with an excess of 4 hydrogen atoms

The hydrogens are subsequently converted to HOH via the electron transport chain



(eq 1-1)

Why is it so advantageous for the cell to use O<sub>2</sub> as the terminal electron acceptor?

(eq1-2)

Because as oxygen is reduced, 4 ATP are produced

If oxygen is not a limiting nutrient even more ATP can be produced by breaking down each pyruvate molecule into CO<sub>2</sub>

This is achieved through a reaction pathway known as the Citric Acid Cycle (and a linking reaction)

### **Photosynthesis**

Photosynthetic pathways use light, rather than chemical compounds as a source of energy

While respiration can be expressed by the equation:

Photosynthesis is summarized by the equation.

Photosynthetic organisms use chlorophyll to trap sunlight and extract the energy to drive ATP synthesis

Chlorophyll has a chemical structure that is similar to heme, but it contains a magnesium ion instead of iron

Light energy is channeled to a chlorophyll molecule contained within a pigment complex called a reaction center.

light hits the reaction center, and excites electrons

The excited electron is then passed to a series of electron carriers, each one removing some of the energy, until the electron returns to its ground state where it is passed back to the reaction center complex.

The energy derived from the excited electrons by the electron carrier molecules is used to synthesize ATP.

This process of light driven ATP synthesis is called cyclic phosphorylation

In order to reduce CO<sub>2</sub> into more complex compounds, the cells require H atoms (or protons) and the reaction center complex does not have the strength required to strip H away from HOH

Therefore the Photoautotroph increases the energy by coupling a second reaction center to the first one

Electrons are stripped from HOH and passed to RCII

which is in turn stimulated to an excited state, and passed to RCI

Which is stimulated, as the electron is passed through the carrier systems, they are passed to NADP<sup>+</sup> to generate NADPH + H<sup>+</sup> which in turn is used to reduce CO<sub>2</sub>

This light driven reduction of  $\text{NADP}^+$  to  $\text{NADPH} + \text{H}^+$  is called non cyclic phosphorylation

In higher plants and algae, reaction centers I and II are found in the chloroplast membranes, if the cyanobacteria, they are found in the cell membrane

The purple and green photosynthetic bacteria do not have reaction center II complexes, only RCI

Therefore they are incapable of using water as an electron donor and must use other compounds, such as  $\text{H}_2\text{S}$

There are three essential questions about metabolism that we need to consider through the course of this discussion

- 1 How does the cell store its energy
- 2 How does the cell generate the energy for storage
- 3 How does the cell dispose of the extra hydrogen which is produced through the course of these reactions

### ***How does the cell store energy ?***

The cell stores its energy in chemical bonds, especially in highly ionizable anhydride bonds.

Examples of energy storing molecules are ATP and GTP

{Fig 4.10 Stx of ATP and anhydride bonds}

***ATP has two roles inside the cell,***

- (1) a precursor for the synthesis of nucleic acids***
- (2). that of a high energy carrier molecule***

*The charges in the phosphate groups repel each other, therefore it is very easy to push one or two of the Phosphate groups away from the rest of the molecule*

*ATP + HOH  $\longrightarrow$  ADP + Pi has a  $\Delta G$  of -7300 kCal/mole  
(energy released (exergonic))*

*ADP + HOH  $\longrightarrow$  Amp + Pi has a  $\Delta G$  of -6800 kCal/mole*

*In order to make ATP, the cell must supply more than 7300 kCal (~8000)*

*ATP is used within the cell as an energy donor for many of the anabolic, or biosynthetic, reactions. The energy released from the hydrolysis is coupled with other chemical reactions so that the overall  $\Delta G$  is negative, and therefore will proceed spontaneously*

*The cell can also use CTP and UTP as high energy carrier molecules.*

*These pools of nucleotide triphosphate molecules can be interchanged by the appropriate enzymes*

*The second way that a cell can store energy is by maintaining a proton (or charge) gradient across the cell membrane.*

*The phospholipid bilayer that composes the cell membrane is impermeable to protons. The cell uses a set of transport proteins to pump protons across the membrane, out of the cell*

*This creates a proton gradient across the membrane, High proton outside...low proton inside.*

*(the proton gradient is a reservoir of potential energy that can be harnessed in a controlled fashion to generate high energy bonds in the form of ATP)*

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*This is analogous to water stored behind a dam*

**How does the cell eliminate excess H?**

Each hydrogen atom is composed of 1 proton and 1 electron

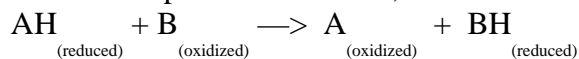
consequently, a transfer of H atoms is essentially a transfer of electrons

These electron transfer reactions are called **oxidation - reduction (redox)** reactions

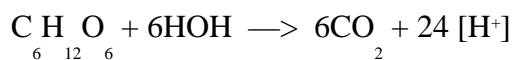
an **oxidation** reaction occurs when a compound experiences a **loss of e-** (H)

a **reduction** reaction occurs when a compound experiences a **gain of e-** (H)

As one compound is oxidized, another is **always** reduced



Under aerobic growth conditions, the cell can take a compound, such as glucose, and break it down, through a series of oxidation reactions to carbon dioxide and electrons



The brackets indicate that the H atoms are not free but are attached to other, undefined molecules.

A few of the hydrogens are consumed in various biosynthetic reactions, but the vast majority of these are excess.

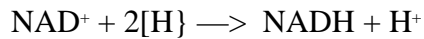
This presents the cell with a problem, if the H are allowed to accumulate, they will blow the proton gradient, and rob the cell of a useful source of energy.

Consequently, the cell has to have a way of disposing of the hydrogen

This occurs in a two step process

Extra hydrogen molecules are initially picked up by a hydrogen carrier molecule such as **nicotinamide adenine dinucleotide NAD, or nicotinamide adenine dinucleotide phosphate NADP**

{INSERT STRUCTURE}



NADH ultimately dumps the H atoms onto some other molecule as a final hydrogen acceptor, usually a metabolic waste product. This compound is then excreted by the cell

Excess [H]  $\longrightarrow$  NADH  $\longrightarrow$  Final H acceptor

Trash  $\longrightarrow$  garbage truck  $\longrightarrow$  landfill

An example of such a process occurs under anaerobic growth conditions

{Insert lactic acid Rx}

Which compound is oxidized and which compound is reduced in the reaction involving pyruvic acid

Other examples of redox carriers are FAD which carries 2H

Ubiquinone (Coenzyme Q) which carries 2H

and Heme groups in the cytochromes which only carry 1 electron

{structures}

*How does the cell generate energy for storage?*

There are three types of energy-generating metabolism, fermentation, respiration, and photosynthesis

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**Photosynthetic** metabolism occurs when the energy generating reactions are driven by light.

### ***Fermentation reactions***

- *Fermentation is the breakdown of sugar into organic acids, alcohols.*

3