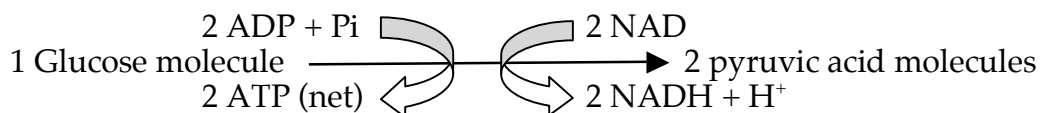


Fermentation and Cellular Respiration

Chemoheterotrophs such as animals, fungi, protozoa and many bacteria use preformed organic compounds as their source of energy. Organic compounds carry potential energy in the covalent bonds holding their atoms together. When these bonds are broken, the energy released can be used to make ATP through **phosphorylation reactions**. Chemoheterotrophs can use either **substrate level** or **oxidative phosphorylation** (or sometimes both) depending on the type of metabolism they have.

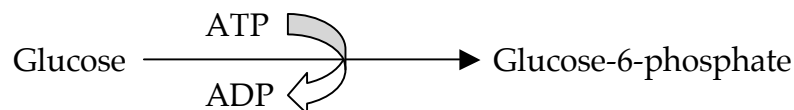
Since glucose is a compound abundant in the environment (most polysaccharides are glucose polymers), many types of organisms carry enzymes involved in glucose catabolism. Some types of cells use glucose exclusively, i.e., they are not capable of utilizing any other nutrient as a source of energy or carbon. It is not surprising then that the enzymes involved in glucose catabolism are **constitutive** in most types of cells.

Glycolysis – The term glycolysis (glyco = carbohydrate, lysis = to split) means breakdown of sweets, but glycolysis as presented here refers to a specific metabolic pathway or series of chemical reactions catalyzed by enzymes (also known as the Embden-Meyerhof pathway). Glycolysis may be presented diagrammatically as shown below.



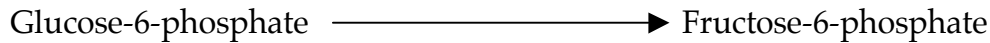
Glycolysis is the catabolism of glucose into two molecules of pyruvic acid with the associated production of two molecules of ATP (net yield), and the reduction of two molecules of NAD to NADH + H⁺. The ATP made during glycolysis is the result of **substrate-level phosphorylation**, i.e., the energy required comes directly from the breaking of covalent bonds within glucose molecules. As mentioned above, the enzymes associated with glycolysis are often constitutive and are **found in the cytoplasm** of both eukaryotic and prokaryotic cells.

Although glycolysis is a catabolic pathway, and yields energy in the form of ATP, it also requires energy for initiation (**activation energy**). Each glucose molecule entering the glycolysis pathway must be **phosphorylated**, i.e., must have a phosphate group bound to it. **ATP** provides both the phosphate group and the energy required to bind it to glucose. Enzymes catalyzing chemical reactions resulting in the transfer of phosphate groups between organic compounds are called **kinase** enzymes (recall kinesis = movement). The enzyme catalyzing the reaction binding a phosphate group to glucose at the beginning of glycolysis is called **phosphohexokinase**. The reaction can be diagrammed as shown.

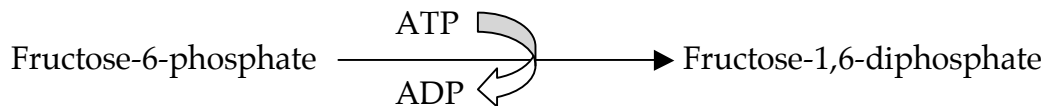


The glucose molecule now has a phosphate group bound to its number-6 carbon atom, and one ATP molecule has been converted to ADP (adenosine diphosphate).

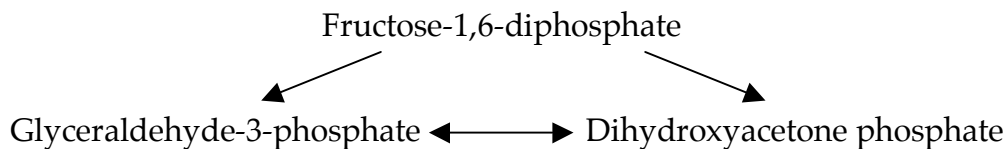
The next reaction in the pathway requires an **isomerase enzyme**. An isomerase catalyzes a reaction converting a molecule (in this case glucose), into its **chemical isomer** (in this case fructose). **Glucose and fructose are isomers**; they have the same molecular formula ($C_6H_{12}O_6$) and have the same types and number of bonds. The enzyme **phosphohexoisomerase** catalyzes the following reaction.



Since no energy is required for, or released during this reaction, ATP is not involved. During the next reaction, a second phosphate group is added to the sugar molecule. Once again, ATP provides the energy and phosphate, and a kinase enzyme is involved. The reaction catalyzed by **phosphofruktokinase** can be diagrammed as shown.

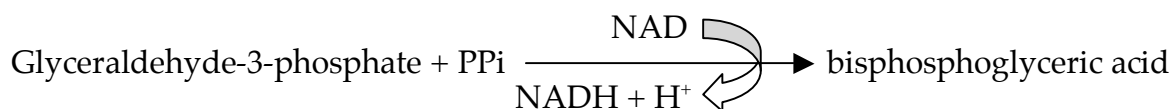


The fructose molecule now has a phosphate group bound to each end, i.e., one on the number-6 carbon and another on the number-1 carbon. Following this step, the fructose can be split into two, three-carbon molecules as shown.

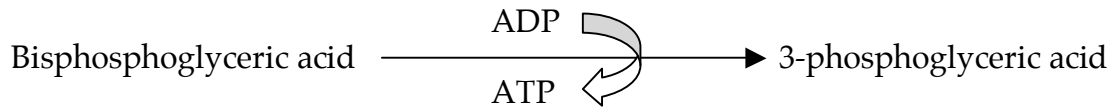


A new enzyme is required for this reaction, but once again energy is not required nor released. The double-arrow between the two, three-carbon compounds shown (**Glyceraldehyde-3-phosphate** and **Dihydroxyacetone phosphate**) indicates that these two compounds are in equilibrium, i.e., their concentrations will be maintained as equal by the activity of another **isomerase enzyme** (**triose phosphate isomerase**). Glyceraldehyde-3-phosphate (G3P) can also be called 3-phosphoglyceraldehyde (PGAL) or triose phosphate, and is a 3-carbon compound with a phosphate group bound to its number-3 carbon. Dihydroxyacetone-phosphate (DHAP) has the same molecular formula ($C_3H_6O_7P$), so is a chemical isomer of G3P.

During the next step in the glycolysis pathway, 2 molecules of Glyceraldehyde-3-phosphate are phosphorylated and oxidized to form 2 molecules of **bisphosphoglyceric acid**. The electrons and hydrogen protons removed from each Glyceraldehyde-3-phosphate are passed to **NAD**, reducing it to **NADH + H⁺**. The phosphate added during this reaction is provided by **inorganic pyrophosphate (PPi)**.



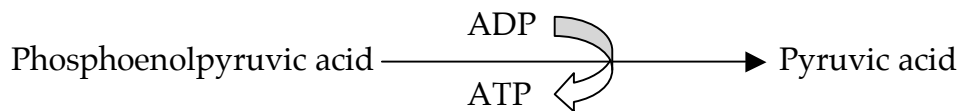
Next, another kinase enzyme catalyzes a reaction transferring one phosphate group from each bisphosphoglyceric acid molecule to ADP forming ATP and 3-phosphoglyceric acid.



The enzyme involved is called **phosphoglycerate kinase**, and the ATP formed is the result of **substrate level phosphorylation**. Since two molecules of bisphosphoglyceric acid were formed from each fructose-1,6-diphosphate catabolized, two molecules of ATP are formed at this step. Following this reaction, the 3-phosphoglyceric acid is converted to **2-phosphoglyceric acid** by a **mutase enzyme**, and then to **phosphoenolpyruvic acid** by an **enolase enzyme**.

Note – The glycolysis diagram provided in the lecture syllabus combines some of the reactions occurring between glyceraldehyde-3-phosphate and phosphoenolpyruvic acid. Although the information provided here is considerably more accurate, students are required to know only kinase and isomerase enzymes.

The last reaction in the glycolysis pathway involves the transfer of a phosphate group from each molecule of phosphoenolpyruvic acid to ADP, yielding ATP and pyruvic acid.



The kinase enzyme involved here is called **pyruvate kinase**, and once again the ATP formed is the result of **substrate level phosphorylation**. Since two molecules of phosphoenolpyruvic acid are donating phosphate groups to ADP, two molecules of ATP are formed.

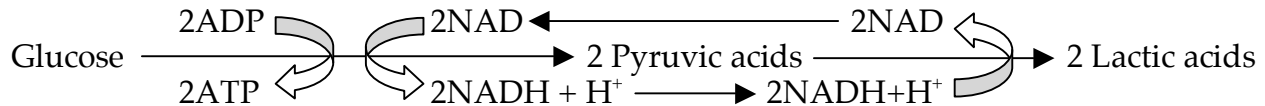
Two molecules of ATP were required toward the beginning of the glycolysis pathway, but four molecules of ATP are ultimately formed. This means glycolysis has a **net yield of two ATP** molecules for each glucose molecule catabolized. Since oxygen is not required, glycolysis can occur under anaerobic conditions; however, it cannot continue unless it is linked to one or more additional reactions, i.e., **glycolysis cannot "stand alone"**.

Why not?

Recall that living organisms can be divided into two categories based on their type of metabolism, **fermentative** organisms and **respiratory/oxidative** organisms. Fermentative organisms typically use some type of organic compound (pyruvic acid in the case of eukaryotic cells), as their **final electron acceptor**, while respiratory/oxidative organisms use some type of inorganic compound (e.g., molecular oxygen). In either case, a final electron acceptor is required, and this explains why glycolysis cannot continue unless linked to one or more additional reactions. Glycolysis does not involve a final electron acceptor. Without a final electron acceptor, the NAD reduced to NADH + H⁺ during glycolysis cannot be oxidized, and without NAD, glycolysis will stop.

Fermentation:

Fermentation – Fermentation can be defined as the anaerobic decomposition of organic compounds (usually carbohydrates) involving an organic compound (usually pyruvic acid) as the final electron acceptor. The fermentation of glucose to yield lactic acid can be diagrammed as shown below.



As explained above, glycolysis is used to catabolize glucose into two pyruvic acid molecules, but the pathway does not stop there. Instead, the **pyruvic acids serve as final electron acceptors**, the two molecules of NADH+H⁺ are oxidized to NAD and the two pyruvic acid molecules are converted into **lactic acid** molecules.

Homofermentative – Organisms that yield lactic acid as the only end product of their fermentation processes are called **homofermentative organisms (homo = same)**. Bacteria such as *Lactococcus lactis*, *Leuconostoc mesenteroides* and the *Lactobacillus* species associated with sauerkraut production (*Lactobacillus brevis* and *L. plantarum*), are homofermentative. Since lactic acid is the only fermentation product these organisms can make, they are often referred to as **lactic acid bacteria**, and are used commonly in food processing, i.e., in the production of sauerkraut, cheese, sourdough bread and other sour-flavored foods.

Heterofermentative – Organisms capable of producing a variety of different fermentation products are called heterofermentative organisms (hetero = different). These organisms contain different enzymes, and although they can still use pyruvic acid as a final electron acceptor, they can convert it into multiple different compounds, e.g., acetic acid, butyric acid, acetoin, acetone, acetaldehyde, 2, 3-butylene glycol, ethanol, carbon dioxide and hydrogen gas. The **Saccharomyces cerevisiae** used to make wine and rootbeer, and the Gram-negative, fermentative organisms used in Physiological Unknown No. 1 are all heterofermentative.

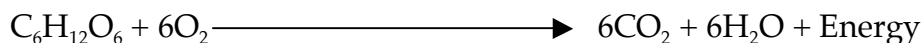
Fermentation allows organisms to produce ATP under anaerobic conditions, but it is not efficient in terms of capturing the energy potentially available in glucose molecules (only about 2% of the energy available is captured). During glycolysis, only one covalent bond is broken, and considerable energy remains in each three-carbon pyruvic acid molecule formed. This energy is typically lost as pyruvic acid is converted into various fermentation products and released from the cells. Another disadvantage of fermentation is the production of potentially toxic waste. Acids formed via fermentation can lower the pH of the environment and disrupt enzyme function, and alcohols can coagulate cellular proteins.

Cellular Respiration:

Organisms capable of cellular respiration can capture more of the energy potentially available in glucose, and release only carbon dioxide and water as waste materials.

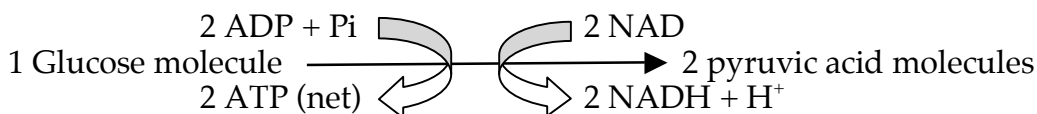
Cellular respiration – Cellular respiration is a multi-step process allowing organisms to completely catabolize glucose into carbon dioxide. If molecular oxygen serves as the final

electron acceptor, water is also produced. Some references represent cellular respiration with the following chemical formula.



This indicates that glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) is reacting with molecular oxygen (O_2) to form carbon dioxide (CO_2) and water (H_2O), and that energy is being released. Though handy and easily memorized, this formula is **not accurate**. Within living organisms, glucose does not interact with oxygen, oxygen is not converted to carbon dioxide, and water is not always produced. Some respiratory organisms (e.g., *Pseudomonas aeruginosa*) can use inorganic compounds other than oxygen as their final electron acceptors. If they do this, the formula shown above would not apply.

Cellular respiration as it occurs in many organisms **involves three stages** or three separate metabolic pathways; these are **glycolysis**, the **Krebs cycle** and the **electron transport chain** (also called the respiratory chain). Glycolysis, as presented earlier, can be represented diagrammatically as shown below.

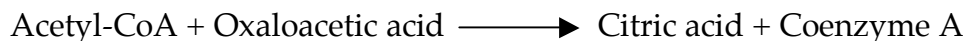


Since respiratory organisms do not use pyruvic acid as a final electron acceptor, alternative enzymes are employed and the fate of pyruvic acid is quite different. Typically, an enzyme complex called the **pyruvate decarboxylase complex (PDC)** removes a **carboxyl group** from each pyruvic acid molecule formed through glycolysis, then binds the remaining 2-carbon acetyl group to **coenzyme A** forming **acetyl-CoA** (a high energy compound). During this process the coenzyme **NAD is reduced to $\text{NADH} + \text{H}^+$** , and **carbon dioxide is released as a waste gas**. The overall process can be diagrammed as shown below.



Note - The pyruvate decarboxylase complex is composed of at least three different quaternary proteins with three different functions. Though handy, the diagram above is not entirely accurate. Details of the process can be found at the following site.
http://www.brookscole.com/chemistry_d/templates/student_resources/shared_resources/animations/pdc/pdc.html.

The **decarboxylation** of pyruvic acid and formation of **acetyl-CoA** serves as an **intermediate step linking glycolysis to the Krebs cycle**. Following this set of reactions, the energy stored in acetyl-CoA can be used to bind the 2-carbon acetyl group to **oxaloacetic acid** (4-carbons), forming **citric acid** (6-carbons), and beginning the Krebs cycle.

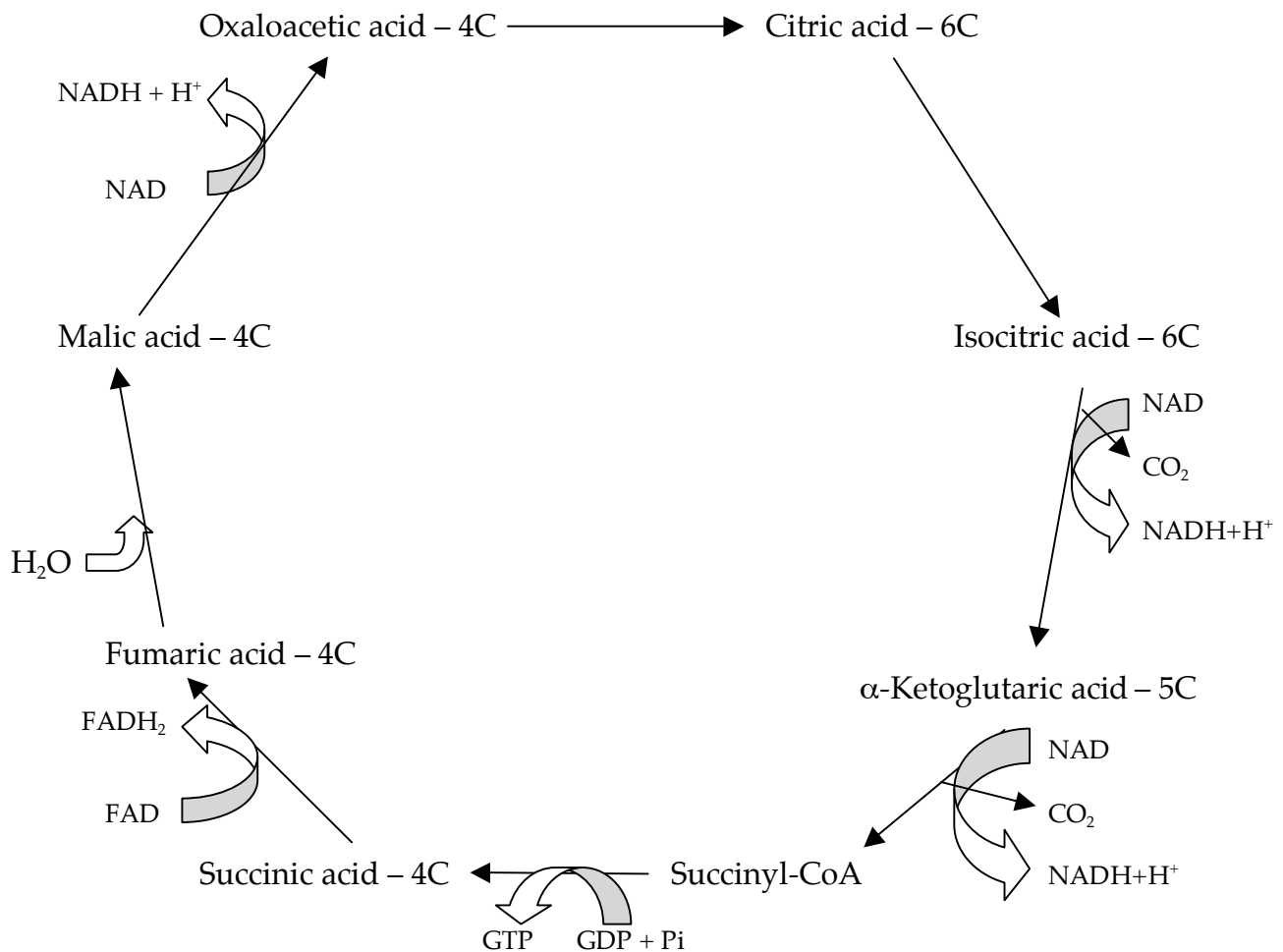


Citric acid is a tricarboxylic acid, i.e., a 6-carbon acid with three carboxyl groups in its structure. Since the Krebs cycle begins with citric acid, it is sometimes called the **Citric**

acid cycle, or **Tricarboxylic acid cycle** (TCA cycle). Although the chemical reactions associated with glycolysis occur within the cytoplasm of both eukaryotic and prokaryotic cells, the decarboxylation of pyruvic acid and chemical reactions of the Krebs cycle do not (or at least not usually). The pyruvate decarboxylase complex (PDC) and enzymes associated with the Krebs cycle occur within the **cytoplasm** of prokaryotic cells, but occur within the **matrix of mitochondria** in most eukaryotic cells (with the exception of succinate dehydrogenase, which is bound to the inner mitochondrial membrane).

Krebs Cycle:

Krebs cycle – The Krebs cycle (citric acid cycle or tricarboxylic acid cycle) is a cyclic metabolic pathway allowing organisms to **decarboxylate organic acids** and release the potential energy stored within them. Most of the energy released is captured in the form of **reduced coenzymes**, $\text{NADH} + \text{H}^+$ and FADH_2 ; however one molecule of ATP (or GTP) is also formed during each cycle. The carboxyl groups removed from the organic acids are converted into **carbon dioxide**, a waste gas. The various reactions occurring during the Krebs cycle are illustrated in the crude diagram shown below.



In this diagram, each arrow represents a chemical reaction being catalyzed by a specific type of enzyme. Three of these enzymes require the coenzyme NAD as a helper, and in

each reaction, the NAD is reduced to $\text{NADH} + \text{H}^+$. We can see here that coenzymes are less specific than are enzymes relative to the chemicals they interact with. During one reaction, the coenzyme FAD is reduced to FADH_2 . The reduced forms of these coenzymes have a **higher energy potential** than do the oxidized forms, and this is of considerable significance. The acids being **decarboxylated** during the Krebs cycle include isocitric acid and α -ketoglutaric acid. Some of the energy released during decarboxylation is captured as GTP (guanidine triphosphate), which is equivalent to ATP, but contains a different base.

Each 3-carbon pyruvic acid molecule formed during glycolysis is completely catabolized during the reactions described above. The first carbon is removed during the intermediate step prior to the formation of acetyl-CoA, the second is removed from isocitric acid and the third is removed from α -ketoglutaric acid. Each of these carbon atoms is associated with oxygen in the form of a **carboxyl group** (COOH^-). When **two electrons and one hydrogen proton** are removed from each carboxyl group and passed to NAD, carbon dioxide is released as a waste gas. Respiratory organisms such as humans and other animals release carbon dioxide when they exhale, and this is how it is formed. Notice that it has nothing to do with molecular oxygen being taken in, i.e., **we DO NOT take in O_2 , slap a carbon atom on it and release it as CO_2** (and neither does *Pseudomonas*).

The Electron Transport Chain/System:

The third set of chemical reactions associated with cellular respiration involves enzymes that are bound to membranes. In the case of prokaryotic cells, these membranes are **cell membranes**, while within most eukaryotic cells, the membranes involved are the inner folded membranes or **cristae of mitochondria**.

The electron transport chain or system involves a series of membrane-bound proteins that pick up electrons and hydrogen protons from coenzymes ($\text{NADH} + \text{H}^+$ and FADH_2) and pass them from one molecule to the next (through a series of oxidation/reduction reactions) until they are picked up by a final electron acceptor (often molecular oxygen). The number and types of proteins involved in electron transport systems is variable; however, all involve **cytochromes**, pigmented enzymes with iron prosthetic groups.

The diagram provided in the lecture syllabus represents an electron transport system involving a flavoprotein (a protein bound to flavin mononucleotide or FMN), a protein bound to coenzyme Q (ubiquinone) and a series of cytochromes. As electrons are passed along the chain (initially between different coenzymes, and then to a series of prosthetic groups), the integral proteins pump hydrogen protons through the membrane generating a **concentration and electrical gradient** known as the **proton motive force**. The gradient then provides the "force" necessary to move hydrogen protons back across the membrane through an enzyme complex called **ATP-synthase**. Since the hydrogen protons are flowing **down their concentration and electrical gradients**, their movement through ATP-synthase is **passive**. As the hydrogen protons flow through ATP-synthase, they provide the energy necessary to convert ADP plus inorganic phosphate into ATP. This process is called **oxidative phosphorylation** and requires that three hydrogen protons pass through the membrane for each molecule of ATP generated.

For each $\text{NADH} + \text{H}^+$ oxidized by passing its electrons to the electron transport chain, nine hydrogen protons are pumped across the membrane, and for each FADH_2 oxidized, around eight hydrogen protons are pumped across. Obviously the protons are not coming from the coenzyme, so presumably they are taken from hydronium ions (H_3O^+) within the matrix of the mitochondrion or within the cytoplasm of a prokaryotic cell. The protons moved across the inner membrane of a mitochondrion accumulate within the **intermembrane space** (the potential space between the inner and outer mitochondrial membranes). Protons moved across the cell membrane of a prokaryotic cell will accumulate within the periplasmic space. In either case, a transmembrane electrical potential is generated, and this represents potential energy (a bit like water behind a dam). **For each $\text{NADH} + \text{H}^+$ oxidized** by passing its electrons to the electron transport chain, nine protons will eventually flow back through ATP-synthase, and **3 molecules of ATP will be formed**. **For each FADH_2 oxidized**, less than nine protons flow back across the membrane and only **2 molecules of ATP can be formed**. Because there are ten NAD molecules and two FAD molecules reduced during the catabolism of each glucose molecule (as described in the pathways above), 34 molecules of ATP can be generated through oxidative phosphorylation. Since cellular respiration also generated 2 ATP in association with glycolysis, and the equivalent of 2 ATP in association with the Krebs cycle (via substrate level phosphorylation), the total number of ATP molecules potentially produced is 38 (max).

Though prokaryotic cells often produce close to 38 molecules of ATP for each glucose molecule they catabolize, eukaryotic cells are not as efficient, and generally produce only **36 ATP per glucose** (or less). This is because in eukaryotic cells, glycolysis occurs outside the mitochondria while the reactions of the Krebs cycle and passage of electrons to the electron transport chain occur inside. Pyruvic acid must be transported into the mitochondrion and the $\text{NADH} + \text{H}^+$ formed outside cannot as readily pass its electrons to the proteins bound to the inner mitochondrial membrane as can $\text{NADH} + \text{H}^+$ formed inside.

If molecular oxygen serves as the final electron acceptor at the end of the electron transport chain, water is formed as a by-product of cellular respiration ($1/2 \text{O}_2 + 2$ electrons and 2 hydrogen protons = H_2O). The electrons and hydrogen protons picked up by oxygen are those passed along the electron transport chain (not the protons flowing through ATP-synthase).

The advantages of cellular respiration over fermentation are multiple and significant, and can be summarized as follows:

- 1) More of the energy potentially available in glucose molecules can be captured in the form of ATP, so the process is energetically more efficient (about 40% as opposed to 2%).
- 2) The waste products of cellular respiration are considerably less toxic than those of fermentation (carbon dioxide and water as opposed to organic acids, solvents and alcohols).
- 3) The water formed at the end of the electron transport chain is potentially available for other metabolic processes. Organisms such as lichens, lizards and kangaroo rats can live in environments that are extremely dry, because they are able to reduce water loss due to evaporation/excretion and because they make metabolic water through respiration.

- 4) Respiration involving oxygen as the final electron acceptor effectively eliminates a potentially toxic substance, i.e., molecular oxygen. Oxygen is a powerful oxidizing agent and is potentially damaging to cells and tissues (it is lethal to obligate anaerobes). Respiratory organisms can eliminate the threat posed by oxygen by converting it into water.