CHAPTER 9
CELLULAR RESPIRATION: HARVESTING CHEMICAL ENERGY

OUTLINE

I. Principles of Energy Conservation
   A. Cellular respiration and fermentation are catabolic (energy-yielding) pathways
   B. Cells must recycle the ATP they use for work
   C. Redox reactions release energy when electrons move closer to electronegative atoms
   D. Electrons "fall" from organic molecules to oxygen during cellular respiration
   E. The "fall" of electrons during respiration is stepwise, via NAD^+ and an electron transport chain

II. The Process of Cellular Respiration
   A. Respiration involves glycolysis, the Krebs cycle, and electron transport: *an overview*
   B. Glycolysis harvests chemical energy by oxidizing glucose to pyruvate: *a closer look*
   C. The Krebs cycle completes the energy-yielding oxidation of organic molecules: *a closer look*
   D. The inner mitochondrial membrane couples electron transport to ATP synthesis: *a closer look*
   E. Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes: *a review*

III. Related Metabolic Processes
   A. Fermentation enables some cells to produce ATP without the help of oxygen
   B. Glycolysis and the Krebs cycle connect to many other metabolic pathways
   C. Feedback mechanisms control cellular respiration

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Diagram energy flow through the biosphere.
2. Describe the overall summary equation for cellular respiration.
3. Distinguish between substrate-level phosphorylation and oxidative phosphorylation.
4. Explain how exergonic oxidation of glucose is coupled to endergonic synthesis of ATP.
5. Define oxidation and reduction.
6. Explain how redox reactions are involved in energy exchanges.
7. Define coenzyme and list those involved in respiration.
8. Describe the structure of coenzymes and explain how they function in redox reactions.

9. Describe the role of ATP in coupled reactions.
10. Explain why ATP is required for the preparatory steps of glycolysis.
11. Describe how the carbon skeleton of glucose changes as it proceeds through glycolysis.
12. Identify where in glycolysis that sugar oxidation, substrate-level phosphorylation and reduction of coenzymes occur.
13. Write a summary equation for glycolysis and describe where it occurs in the cell.
14. Describe where pyruvate is oxidized to acetyl CoA, what molecules are produced and how it links glycolysis to the Krebs cycle.
15. Describe the location, molecules in and molecules out for the Krebs cycle.
16. Explain at what point during cellular respiration glucose is completely oxidized.
17. Explain how the exergonic "slide" of electrons down the electron transport chain is coupled to the endergonic production of ATP by chemiosmosis.
18. Describe the process of chemiosmosis.
19. Explain how membrane structure is related to membrane function in chemiosmosis.
20. Summarize the net ATP yield from the oxidation of a glucose molecule by constructing an ATP ledger which includes coenzyme production during the different stages of glycolysis and cellular respiration.
21. Describe the fate of pyruvate in the absence of oxygen.
22. Explain why fermentation is necessary.
23. Distinguish between aerobic and anaerobic metabolism.
24. Describe how food molecules other than glucose can be oxidized to make ATP.
25. Describe evidence that the first prokaryotes produced ATP by glycolysis.
26. Explain how ATP production is controlled by the cell and what role the allosteric enzyme, phosphofructokinase, plays in this process.

KEY TERMS

fermentation
cellular respiration
redox reactions
oxidation
reduction
reducing agent
oxidizing agent
NAD+
glycolysis
Krebs cycle
oxidative phosphorylation
substrate-level phosphorylation
acetyl CoA
cytochrome (cyt)
ATP synthase
chemiosmosis
proton-motive force
aerobic
anaerobic
alcohol fermentation
lactic acid fermentation
facultative anaerobe
beta oxidation

LECTURE NOTES

I. Principles of Energy Conservation

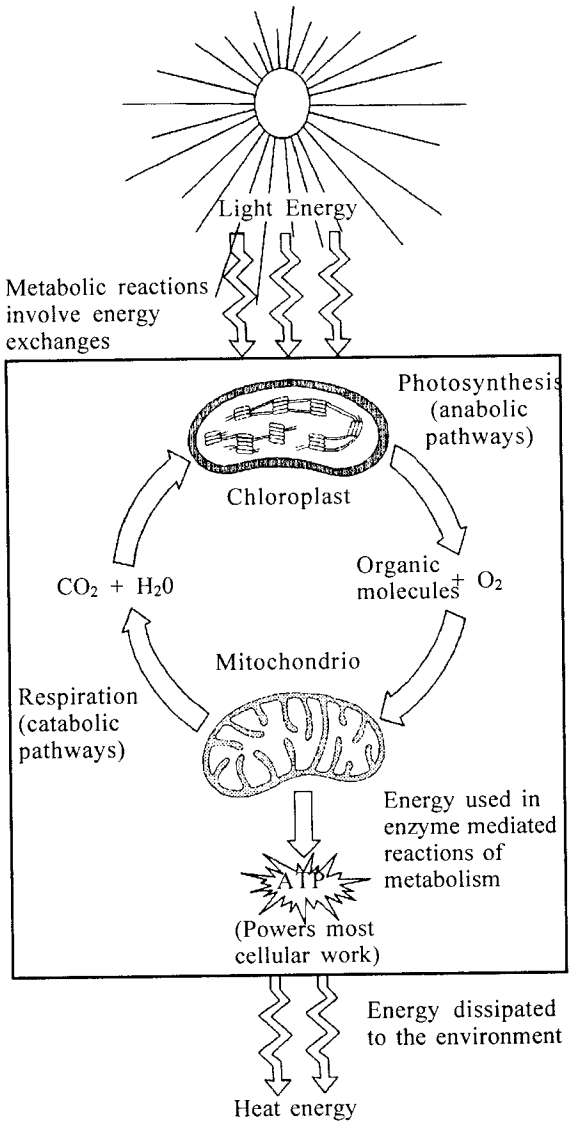
As open systems, cells require outside energy sources to perform cellular work (e.g., chemical, transport, and mechanical).

Energy flows into most ecosystems as sunlight.

Photosynthetic organisms trap a portion of the light energy and transform it into chemical bond energy of organic molecules. O_2 is released as a byproduct.

Cells use some of the chemical bond energy in organic molecules to make ATP–the energy source for cellular work.

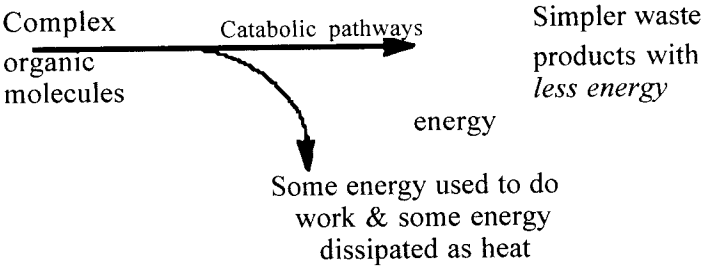
Energy leaves living organisms as it dissipates as heat.



The products of respiration (CO_2 and H_2O) are the raw materials for photosynthesis. Photosynthesis produces glucose and oxygen, the raw materials for respiration.

Chemical elements essential for life are recycled, but energy is not.

How do cells harvest chemical energy?



A. Cellular respiration and fermentation are catabolic (energy-yielding) pathways

Fermentation = An ATP-producing catabolic pathway in which both electron donors and acceptors are organic compounds.

- Can be an anaerobic process
- Results in a partial degradation of sugars

Cellular respiration = An ATP-producing catabolic process in which the ultimate electron acceptor is an inorganic molecule, such as oxygen.

- Most prevalent and efficient catabolic pathway
- Is an exergonic process ($\Delta G = -2870$ kJ/mol or -686 kcal/mol)
- Can be summarized as:

  Organic compounds (food) + Oxygen $\longrightarrow$ Carbon dioxide + Water + Energy
- Carbohydrates, proteins, and fats can all be metabolized as fuel, but cellular respiration is most often described as the oxidation of glucose:

  $C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$ + Energy (ATP + Heat)

B. Cells recycle the ATP they use for work

The catabolic process of cellular respiration transfers the energy stored in food molecules to *ATP*.

ATP (adenosine triphosphate) = Nucleotide with high energy phosphate bonds that the cell hydrolyzes for energy to drive endergonic reactions.

- The cell taps energy stored in ATP by enzymatically transferring terminal phosphate groups from ATP to other compounds. (Recall that direct hydrolysis of ATP would release energy as heat, a form unavailable for cellular work. See Chapter 6.)
- The compound receiving the phosphate group from ATP is said to be *phosphorylated* and becomes more reactive in the process.
- The phosphorylated compound loses its phosphate group as cellular work is performed; inorganic phosphate and ADP are formed in the process (see Campbell, Figure 9.2).
- Cells must replenish the ATP supply to continue cellular work. Cellular respiration provides the energy to regenerate ATP from ADP and inorganic phosphate.

C. Redox reactions release energy when electrons move closer to electronegative atoms

1. An introduction to redox reactions

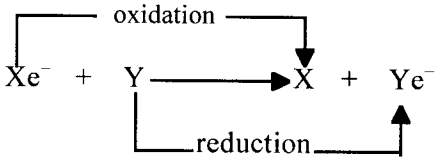
Oxidation-reduction reactions = Chemical reactions which involve a partial or complete transfer of electrons from one reactant to another; called *redox reactions* for short.

Oxidation = Partial or complete loss of electrons

Reduction = Partial or complete gain of electrons

Generalized redox reaction:

Electron transfer requires both a donor and acceptor, so when one reactant is oxidized the other is reduced.



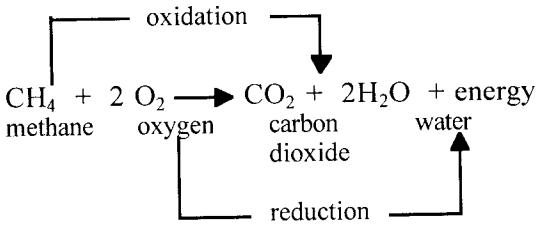
Where:

X = Substance being oxidized; acts as a *reducing agent* because it reduces Y.

Y = Substance being reduced; as an *oxidizing agent* because it oxidizes X.

Not all redox reactions involve a complete transfer of electrons, but, instead, may just change the degree of sharing in covalent bonds (see Campbell, Figure 9.3).

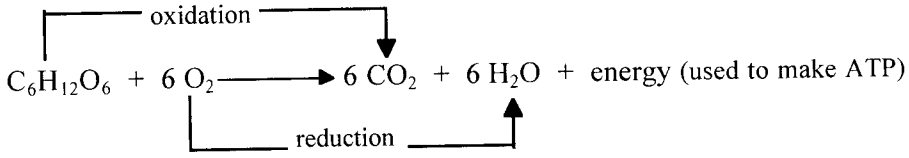
- Example: Covalent electrons of methane are equally shared, because carbon and hydrogen have similar electronegativities.



- As methane reacts with oxygen to form carbon dioxide, electrons shift away from carbon and hydrogen to the more electronegative oxygen.
- Since electrons lose potential energy when they shift toward more electronegative atoms, redox reactions that move electrons closer to oxygen release energy.
- Oxygen is a powerful oxidizing agent because it is so electronegative.

D. Electrons "fall" from organic molecules to oxygen during cellular respiration

Cellular respiration is a redox process that transfers hydrogen, including electrons with high potential energy, from sugar to oxygen.



- Valence electrons of carbon and hydrogen lose potential energy as they shift toward electronegative oxygen.
- Released energy is used by cells to produce ATP.
- Carbohydrates and fats are excellent energy stores because they are rich in C to H bonds.

Without the activation barrier, glucose would combine spontaneously with oxygen.

- Igniting glucose provides the activation energy for the reaction to proceed; a mole of glucose yields 686 kcal (2870 kJ) of heat when burned in air.
- Cellular respiration does not oxidize glucose in one explosive step, as the energy could not be efficiently harnessed in a form available to perform cellular work.
- Enzymes lower the activation energy in cells, so glucose can be slowly oxidized in a stepwise fashion during glycolysis and Krebs cycle.

E. The "fall" of electrons during respiration is stepwise, via NAD^+ and an electron transport chain

Hydrogens stripped from glucose are not transferred directly to oxygen, but are first passed to a special electron acceptor—*NAD^+*.

Nicotinamide adenine dinucleotide (NAD^+) = A *dinucleotide* that functions as a *coenzyme* in the redox reactions of metabolism (see Campbell, Figure 9.4).

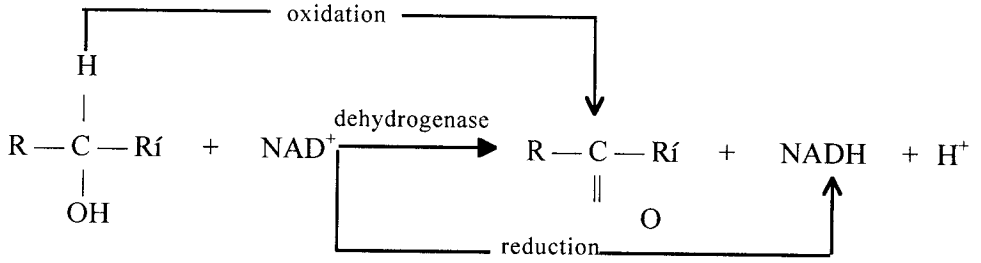
- Found in all cells
- Assists enzymes in electron transfer during redox reactions of metabolism

Coenzyme = Small nonprotein organic molecule that is required for certain enzymes to function.

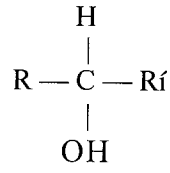
Dinucleotide = A molecule consisting of two nucleotides.

During the oxidation of glucose, NAD^+ functions as an oxidizing agent by trapping energy-rich electrons from glucose or food. These reactions are catalyzed by enzymes called *dehydrogenases*, which:

- Remove a pair of hydrogen atoms (two electrons and two protons) from substrate
- Deliver the two electrons and *one* proton to NAD^+
- Release the remaining proton into the surrounding solution



Where:



= Substrate that is oxidized by enzymatic transfer of electrons to NAD^+

NAD^+ = Oxidized coenzyme (net positive charge)

NADH = Reduced coenzyme (electrically neutral)

The high energy electrons transferred from substrate to NAD^+ are then passed down the *electron transport chain* to oxygen, powering ATP synthesis (*oxidative phosphorylation*).

Some instructors find it difficult to drive this point home. Surprisingly, some students can recall the intermediate steps of glycolysis or the Krebs cycle, but cannot explain in general terms how energy from food is transferred to ATP. Campbell, Figure 9.16 can be used to give students an overview when respiration is introduced; it is useful to refer to it here so students can place the process you are describing in context. It can be used again later as a summary to bring closure to the topic.

Electron transport chains convert some of the chemical energy extracted from food to a form that can be used to make ATP (see Campbell, Figure 9.5). These transport chains:

- Are composed of electron-carrier molecules built into the inner mitochondrial membrane. Structure of this membrane correlates with its functional role (form fits function).
- Accept energy-rich electrons from reduced coenzymes (NADH and $FADH_2$); and during a series of redox reactions, pass these electrons down the chain to oxygen, the final electron acceptor. The electronegative oxygen accepts these electrons, along with hydrogen nuclei, to form water.
- Release energy from energy-rich electrons in a controlled stepwise fashion; a form that can be harnessed by the cell to power ATP production. If the reaction between hydrogen and oxygen during respiration occurred in a single explosive step, much of the energy released would be lost as heat, a form unavailable to do cellular work.

Electron transfer from NADH to oxygen is exergonic, having a free energy change of –222 kJ/mole (–53 kcal/mol).

- Since electrons lose potential energy when they shift toward a more electronegative atom, this series of redox reactions releases energy.
- Each successive carrier in the chain has a higher electronegativity than the carrier before it, so the electrons are pulled downhill towards oxygen, the final electron acceptor and the molecule with the highest electronegativity.

II. The Process of Cellular Respiration

A. Respiration involves glycolysis, the Krebs cycle, and electron transport: *an overview*

There are three metabolic stages of cellular respiration (see Campbell, Figure 9.6):

1. Glycolysis
2. Krebs cycle
3. Electron transport chain (ETC) and oxidative phosphorylation

Glycolysis is a catabolic pathway that:

- Occurs in the cytosol
- Partially oxidizes glucose (6C) into two *pyruvate* (3C) molecules

The *Krebs cycle* is a catabolic pathway that:

- Occurs in the mitochondrial matrix
- Completes glucose oxidation by breaking down a *pyruvate* derivative (acetyl CoA) into carbon dioxide

Glycolysis and the Krebs cycle produce:

- A small amount of ATP by substrate-level phosphorylation
- NADH by transferring electrons from substrate to NAD^+ (Krebs cycle also produces $FADH_2$ by transferring electrons to FAD)

The *electron transport chain*:

- Is located at the inner membrane of the mitochondrion
- Accepts energized electrons from reduced coenzymes (NADH and $FADH_2$) that are harvested during glycolysis and Krebs cycle. Oxygen pulls these electrons down the electron transport chain to a lower energy state.
- Couples this exergonic slide of electrons to ATP synthesis or oxidative phosphorylation. This process produces *most* (90%) of the ATP.

Oxidative phosphorylation = ATP production that is coupled to the exergonic transfer of electrons from food to oxygen.

A small amount of ATP is produced directly by the reactions of glycolysis and Krebs cycle. This mechanism of producing ATP is called substrate-level phosphorylation.

Substrate-level phosphorylation = ATP production by direct enzymatic transfer of phosphate from an intermediate substrate in catabolism to ADP.

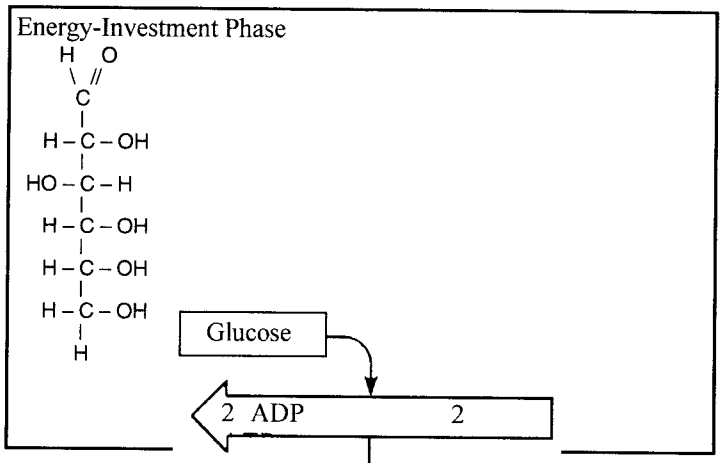
B. Glycolysis harvests chemical energy by oxidizing glucose to pyruvate: *a closer look*

Glycolysis = (Glyco = sweet, sugar; lysis = to split); catabolic pathway during which six-carbon glucose is split into two three-carbon sugars, which are then oxidized and rearranged by a step-wise process that produces two pyruvate molecules.

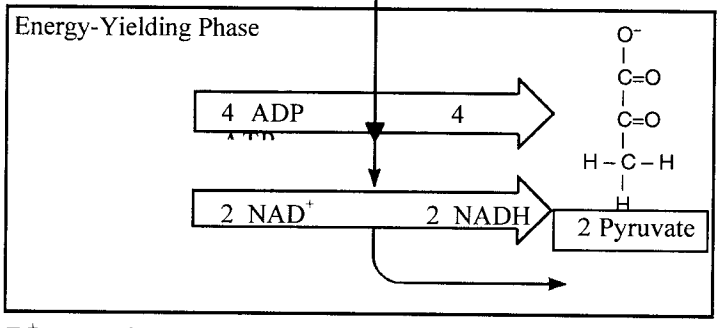
- Each reaction is catalyzed by specific enzymes dissolved in the cytosol.
- No CO_2 is released as glucose is oxidized to pyruvate; all carbon in glucose can be accounted for in the two molecules of pyruvate.
- Occurs whether or not oxygen is present.

The reactions of glycolysis occur in two phases:

Energy-investment phase. The cell uses ATP to phosphorylate the intermediates of glycolysis.



Energy-yielding phase. Two three-carbon intermediates are oxidized. For each glucose molecule entering glycolysis:



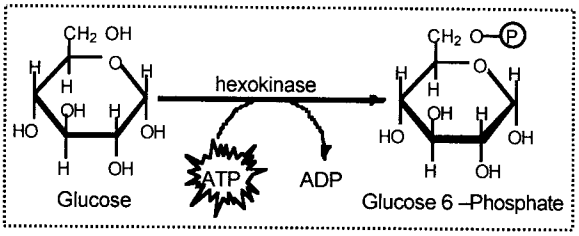
1. A net gain of two ATPs is produced by substrate-level phosphorylation.
2. Two molecules of NAD^+ are reduced to NADH. Energy conserved in the high-energy electrons of NADH can be used to make ATP by oxidative phosphorylation.

> You may not want students to memorize the structures or steps of glycolysis, but you should expect them to understand the process, where it occurs, and the major molecules required and produced. It may be helpful to summarize a lecture with an overhead transparency.

Energy-investment phase:

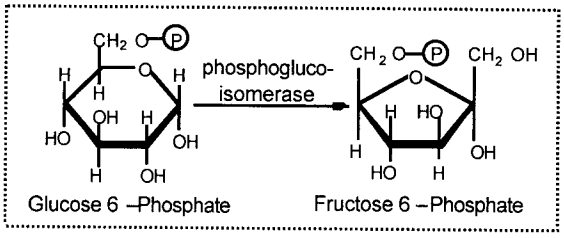
The *energy investment phase* includes five preparatory steps that split glucose in two. This process actually *consumes* ATP.

Step 1: Glucose enters the cell, and carbon six is phosphorylated. This ATP-coupled reaction:

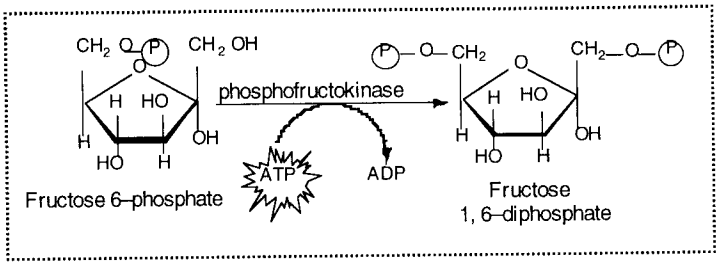


- Is catalyzed by *hexokinase* (*kinase* is an enzyme involved in phosphate transfer)
- Requires an initial investment of ATP
- Makes glucose more chemically reactive
- Produces glucose-6-phosphate; since the plasma membrane is relatively impermeable to ions, addition of an electrically charged phosphate group traps the sugar in the cell.

Step 2: An *isomerase* catalyzes the rearrangement of glucose-6-phosphate to its isomer, fructose-6-phosphate.

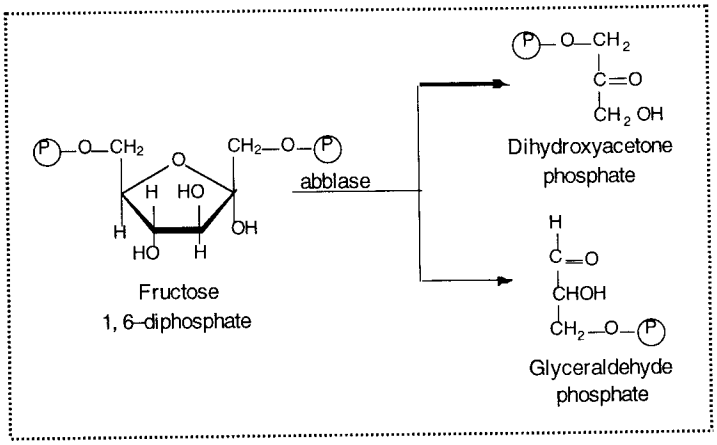


Step 3: Carbon one of fructose-6-phosphate is phosphorylated. This reaction:



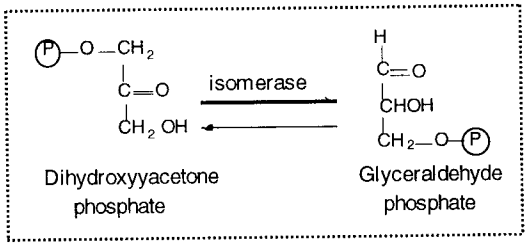
- Requires an investment of another ATP.
- Is catalyzed by *phosphofructokinase*, an allosteric enzyme that controls the rate of glycolysis. This step commits the carbon skeleton to glycolysis, a catabolic process, as opposed to being used to synthesize glycogen, an anabolic process.

Step 4: *Aldolase* cleaves the six-carbon sugar into two isomeric three-carbon sugars.



- This is the reaction for which glycolysis is named.
- For each glucose molecule that begins glycolysis, there are *two* product molecules for this and each succeeding step.

Step 5: An isomerase catalyzes the reversible conversion between the two three-carbon sugars. This reaction:

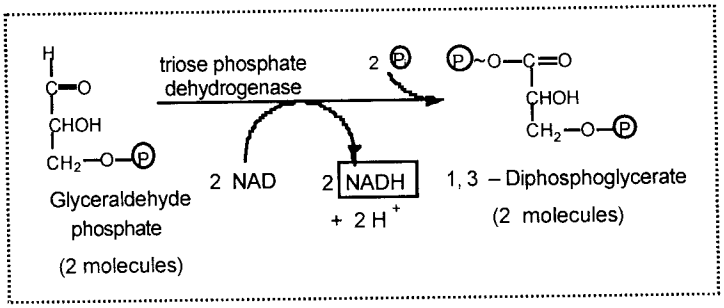


- Never reaches equilibrium because only one isomer, *glyceraldehyde phosphate*, is used in the next step of glycolysis.
- Is thus pulled towards the direction of glyceraldehyde phosphate, which is removed as fast as it forms.
- Results in the net effect that, for each glucose molecule, *two* molecules of glyceraldehyde phosphate progress through glycolysis.

Energy-yielding phase:

The *energy-yielding phase* occurs after glucose is split into two three-carbon sugars. During these reactions, sugar is oxidized, and ATP and NADH are produced.

Step 6: An enzyme catalyzes two sequential reactions:

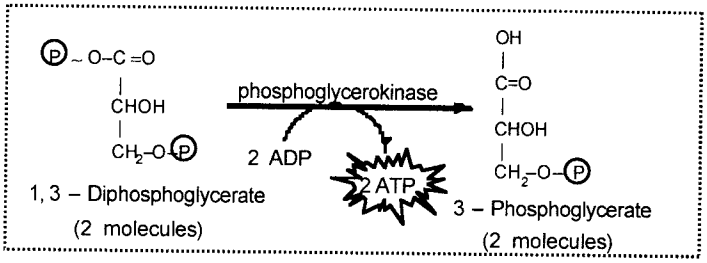


1. Glyceraldehyde phosphate is oxidized and NAD^+ is reduced to $NADH + H^+$.
   - This reaction is very exergonic and is coupled to the endergonic phosphorylation phase ($\Delta G = -10.3$ kcal/ mol).
   - For every glucose molecule, 2 NADH are produced.

2. Glyceraldehyde phosphate is phosphorylated on carbon one.
   - The phosphate source is inorganic phosphate, which is always present in the cytosol.
   - The new phosphate bond is a high energy bond with even more potential to transfer a phosphate group than ATP.

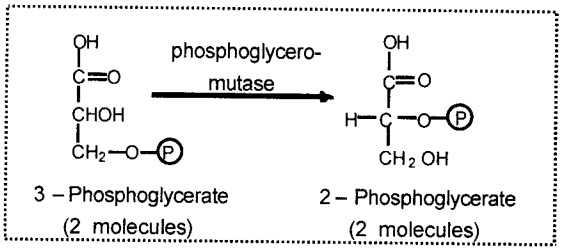
Step 7: ATP is produced by substrate-level phosphorylation.

- In a very exergonic reaction, the phosphate group with the high energy bond is transferred from 1,3-diphosphoglycerate to ADP.



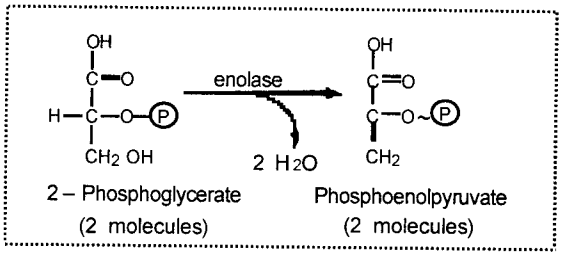
- For each glucose molecule, two ATP molecules are produced. The ATP ledger now stands at zero as the initial debt of two ATP from steps one and three is repaid.

Step 8: In preparation for the next reaction, a phosphate group on carbon three is enzymatically transferred to carbon two.



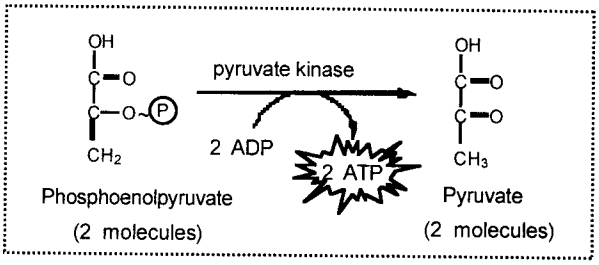
Step 9: Enzymatic removal of a water molecule:

- Creates a double bond between carbons one and two of the substrate.
- Rearranges the substrate's electrons, which transforms the remaining phosphate bond into an unstable bond.



Step 10: ATP is produced by substrate-level phosphorylation.

- In a highly exergonic reaction, a phosphate group is transferred from PEP to ADP.
- For each glucose molecule, this step produces two ATP.



Summary equation for glycolysis:

$$\begin{aligned} & C_6H_{12}O_6 \\ + \; & 2\ NAD^+ \\ + \; & 2\ ADP + 2\text{Ⓟ} \end{aligned} \longrightarrow \begin{aligned} & 2\ C_3H_4O_3\ \text{(Pyruvate)} \\ + \; & 2\ NADH + 2\ H^+ \\ + \; & 2\ ATP \\ + \; & 2\ H_2O \end{aligned}$$

- Glucose has been oxidized into two pyruvate molecules.

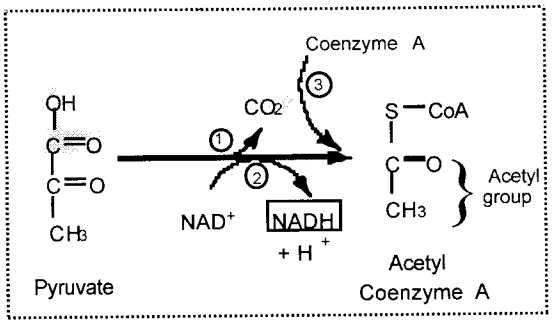
- The process is exergonic ($\Delta G = -140$ kcal/mol or -586 kJ/mol); most of the energy harnessed is conserved in the high-energy electrons of NADH and in the phosphate bonds of ATP.

C. The Krebs cycle completes the energy-yielding oxidation of organic molecules: *a closer look*

Most of the chemical energy originally stored in glucose still resides in the two pyruvate molecules produced by glycolysis. The fate of pyruvate depends upon the presence or absence of oxygen. If oxygen is present, pyruvate *enters the mitochondrion* where it is *completely oxidized* by a series of enzyme-controlled reactions.

1. Formation of acetyl CoA

The junction between glycolysis and the Krebs cycle is the oxidation of pyruvate to acetyl CoA (see Campbell, Figure 9.10):



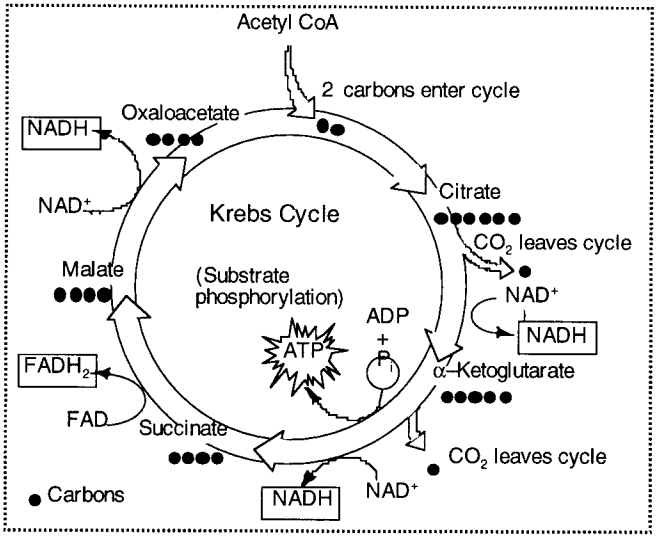
- Pyruvate molecules are translocated from the cytosol into the mitochondrion by a carrier protein in the mitochondrial membrane.
- This step is catalyzed by a *multienzyme complex* which:
  1. Removes CO_2 from the carboxyl group of pyruvate, changing it from a three-carbon to a two-carbon compound. This is the first step where CO_2 is released.
  2. Oxidizes the two-carbon fragment to acetate, while reducing NAD^+ to NADH. Since glycolysis produces two pyruvate molecules per glucose, there are *two* NADH molecules produced.
  3. Attaches coenzyme A to the acetyl group, forming acetyl CoA. This bond is unstable, making the acetyl group very reactive.

2. Krebs cycle

The Krebs cycle reactions oxidize the remaining acetyl fragments of acetyl CoA to CO_2. Energy released from this exergonic process is used to reduce coenzyme (NAD^+ and FAD) and to phosphorylate ATP (substrate-level phosphorylation).

NOTE: The FAD dinucleotide upon reduction accepts two electrons and two protons)

- A German-British scientist, Hans Krebs, elucidated this catabolic pathway in the 1930s.
- The Krebs cycle, which is also known as the *citric acid cycle* or *TCA cycle*, has eight enzyme-controlled steps that occur in the *mitochondrial matrix* (see Campbell, Figure 9.11).



For every turn of Krebs cycle:

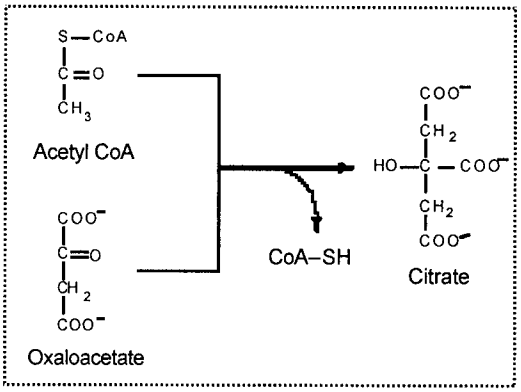
- Two carbons enter in the acetyl fragment of acetyl CoA.
- Two different carbons are oxidized and leave as CO_2.
- Coenzymes are reduced; three NADH and one $FADH_2$ are produced.
- One ATP molecule is produced by substrate-level phosphorylation.
- Oxaloacetate is regenerated.

For every glucose molecule split during glycolysis:

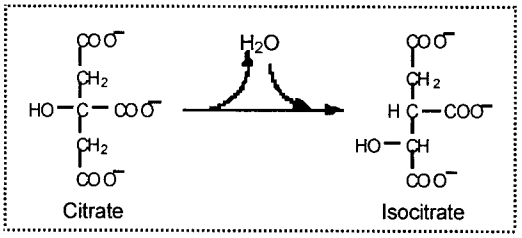
- Two acetyl fragments are produced.
- It takes two turns of Krebs cycle to complete the oxidation of glucose.

Steps of the Krebs cycle (see Campbell, Figure 9.12):

Step 1: The unstable bond of acetyl CoA breaks, and the *two-carbon* acetyl group bonds to the *four-carbon* oxaloacetate to form *six-carbon* citrate.

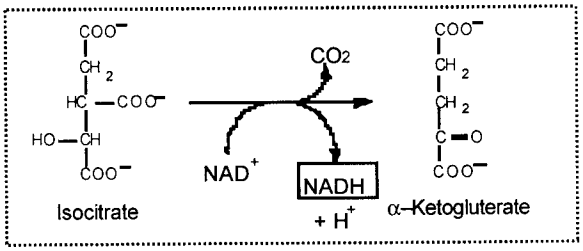


Step 2: Citrate is isomerized to isocitrate.



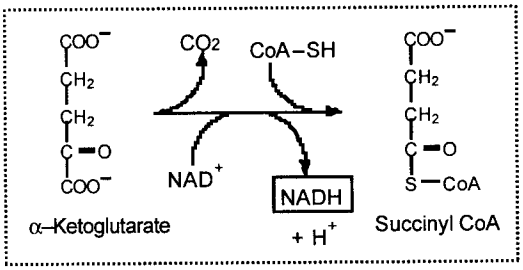
Step 3: Two major events occur during this step:

- Isocitrate loses CO_2 leaving a *five-carbon* molecule.
- The five-carbon compound is oxidized and NAD^+ is reduced.



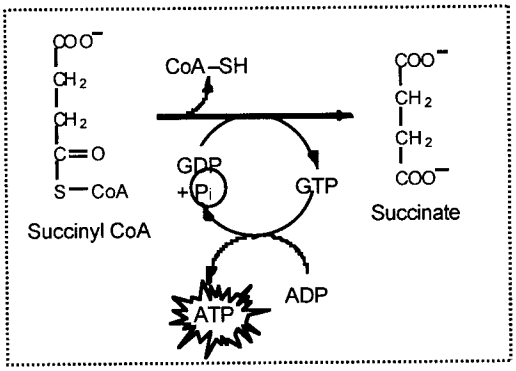
Step 4: A multienzyme complex catalyzes:

- Removal of CO_2
- Oxidation of the remaining *four-carbon* compound and reduction of NAD^+
- Attachment of CoA with a high energy bond to form succinyl CoA



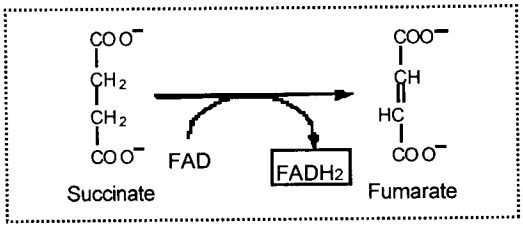
Step 5: Substrate-level phosphorylation occurs in a series of enzyme catalyzed reactions:

- The high energy bond of succinyl CoA breaks, and some energy is conserved as CoA is displaced by a phosphate group.
- The phosphate group is transferred to GDP to form GTP and succinate.
- GTP donates a phosphate group to ADP to form ATP.

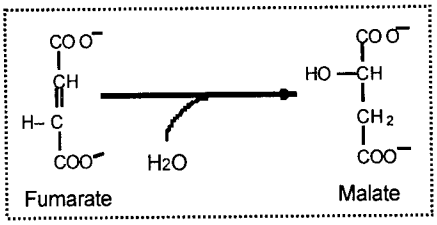


Step 6: Succinate is oxidized to fumarate and FAD is reduced.

- Two hydrogens are transferred to FAD to form $FADH_2$.
- The dehydrogenase that catalyzes this reaction is bound to the inner mitochondrial membrane.

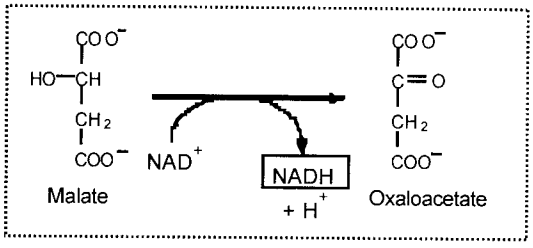


Step 7: Water is added to fumarate which rearranges its chemical bonds to form malate.



Step 8: Malate is oxidized and NAD^+ is reduced.

- A molecule of NADH is produced.
- Oxaloacetate is regenerated to begin the cycle again.



Two turns of the Krebs cycle produce two ATPs by substrate-level phosphorylation. However, *most* ATP output of respiration results from *oxidative phosphorylation*.

- Reduced coenzymes produced by the Krebs cycle (six NADH and two $FADH_2$ per glucose) carry high energy electrons to the electron transport chain.
- The ETC couples electron flow down the chain to ATP synthesis.

D. The inner mitochondrial membrane couples electron transport to ATP synthesis: *a closer look*

Only a few molecules of ATP are produced by substrate-level phosphorylation:

- Two net ATPs per glucose from glycolysis
- Two ATPs per glucose from the Krebs cycle

Most molecules of ATP are produced by oxidative phosphorylation.

- At the end of the Krebs cycle, most of the energy extracted from glucose is in molecules of NADH and $FADH_2$.
- These reduced coenzymes link glycolysis and the Krebs cycle to oxidative phosphorylation by passing their electrons down the electron transport chain to oxygen. (Though the Krebs cycle occurs only under aerobic conditions, it does not use oxygen directly. The ETC and oxidative phosphorylation require oxygen as the final electron acceptor.)
- This exergonic transfer of electrons down the ETC to oxygen is coupled to ATP synthesis.

1. The pathway of electron transport

The *electron transport chain* is made of electron carrier molecules embedded in the inner mitochondrial membrane.

- Each successive carrier in the chain has a higher electronegativity than the carrier before it, so the electrons are pulled downhill towards oxygen, the final electron acceptor and the molecule with the highest electronegativity.
- Except for ubiquinone (Q), most of the carrier molecules are proteins and are tightly bound to *prosthetic groups* (nonprotein cofactors).
- Prosthetic groups alternate between reduced and oxidized states as they accept and donate electrons.

Protein Electron Carriers	Prosthetic Group
flavoproteins	flavin mononucleotide (FMN)
iron-sulfur proteins	iron and sulfur
cytochromes	heme group

Heme group = Prosthetic group composed of four organic rings surrounding a single iron atom

Cytochrome = Type of protein molecule that contains a heme prosthetic group and that functions as an electron carrier in the electron transport chains of mitochondria and chloroplasts

- There are several cytochromes, each a slightly different protein with a heme group.
- It is the iron of cytochromes that transfers electrons.

Sequence of electron transfers along the electron transport chain (see also, Campbell, Figure 9.13):

NADH is oxidized and *flavoprotein* is reduced as high energy electrons from NADH are transferred to FMN.

↓

Flavoprotein is oxidized as it passes electrons to an *iron-sulfur protein*, Fe•S.

↓

Iron-sulfur protein is oxidized as it passes electrons to *ubiquinone* (Q).

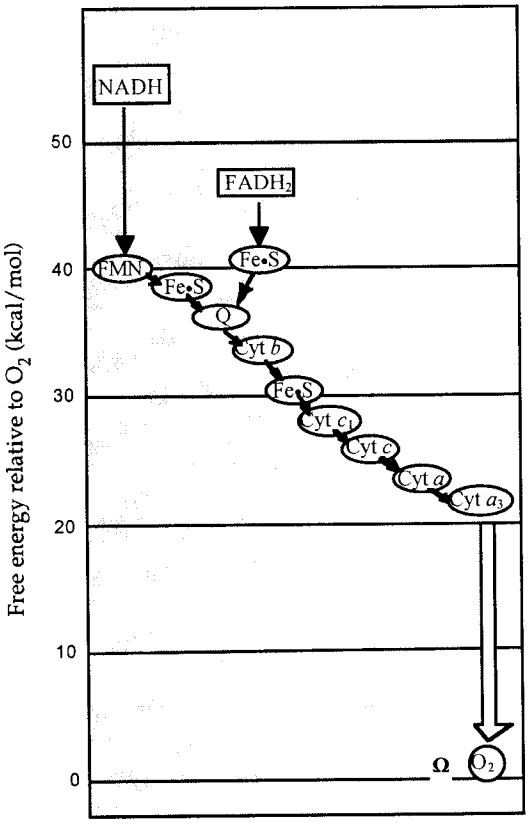
↓

Ubiquinone passes electrons on to a succession of electron carriers, most of which are cytochromes.

↓

Cyt a_3, the last cytochrome passes electrons to molecular oxygen, O_2.

↓



As molecular oxygen is reduced it also picks up two protons from the medium to form water. For every two NADHs, one O_2 is reduced to two H_2O molecules.

- $FADH_2$ also donates electrons to the electron transport chain, but those electrons are added at a lower energy level than NADH.
- The electron transport chain does not make ATP directly. It *generates a proton gradient across the inner mitochondrial membrane*, which stores potential energy that can be used to phosphorylate ADP.

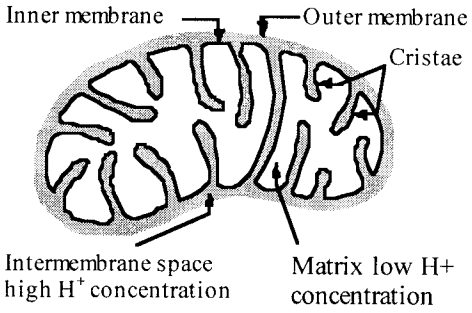
2. Chemiosmosis: the energy-coupling mechanism

The mechanism for coupling exergonic electron flow from the oxidation of food to the endergonic process of oxidative phosphorylation is *chemiosmosis*.

Chemiosmosis = The coupling of exergonic electron flow down an electron transport chain to endergonic ATP production by the creation of a proton gradient across a membrane. The proton gradient drives ATP synthesis as protons diffuse back across the membrane.

- Proposed by British biochemist, Peter Mitchell (1961)

- The term *chemiosmosis* emphasizes a coupling between (1) chemical reactions (phosphorylation) and (2) transport processes (proton transport).
- Process involved in oxidative phosphorylation and photophosphorylation.



The site of oxidative phosphorylation is the inner mitochondrial membrane, which has many copies of a protein complex, *ATP synthase*. This complex:

- Is an enzyme that makes ATP
- Uses an existing *proton gradient* across the inner mitochondrial membrane to power ATP synthesis

Cristae, or infoldings of the inner mitochondrial membrane, increase the surface area available for chemiosmosis to occur.

Membrane structure correlates with the prominent functional role membranes play in chemiosmosis:

- Using energy from exergonic electron flow, the *electron transport chain* creates the proton gradient by pumping H^+s from the *mitochondrial matrix*, across the *inner membrane* to the *intermembrane space*.
- This proton gradient is maintained, because the membrane's phospholipid bilayer is impermeable to H^+s and prevents them from leaking back across the membrane by diffusion.
- *ATP synthases* use the potential energy stored in a proton gradient to make ATP by allowing H^+ to diffuse down the gradient, back across the membrane. Protons diffuse through the ATP synthase complex, which causes the phosphorylation of ADP (see Figure 9.15).

How does the electron transport chain pump hydrogen ions from the matrix to the intermembrane space? The process is based on spatial organization of the electron transport chain in the membrane. Note that:

- Some electron carriers of the transport chain transport only electrons.
- Some electron carriers accept and release protons along with electrons. These carriers are spatially arranged so that protons are picked up from the matrix and are released into the intermembrane space.

Most of the electron carriers are organized into three complexes: 1) NADH dehydrogenase complex; 2) cytochrome b-c_1 complex; and 3) cytochrome oxidase complex (see Campbell, Figure 9.14).

- Each complex is an asymmetric particle that has a specific orientation in the membrane.
- As complexes transport electrons, they also harness energy from this exergonic process to pump protons across the inner mitochondrial membrane.

Mobile carriers transfer electrons between complexes. These mobile carriers are:

1. Ubiquinone (Q). Near the matrix, Q accepts electrons from the NADH dehydrogenase complex, diffuses across the lipid bilayer, and passes electrons to the cytochrome b-c_1 complex.
2. Cytochrome c (Cyt c). Cyt c accepts electrons from the cytochrome b-c_1 complex and conveys them to the cytochrome oxidase complex.

When the transport chain is operating:

- The pH in the intermembrane space is one or two pH units lower than in the matrix.
- The pH in the intermembrane space is the same as the pH of the cytosol because the outer mitochondrial membrane is permeable to protons.

The H^+ gradient that results is called a *proton-motive force* to emphasize that the gradient represents potential energy.

Proton motive force = Potential energy stored in the proton gradient created across biological membranes that are involved in chemiosmosis

- This force is an *electrochemical gradient* with two components:
  1. Concentration gradient of protons (chemical gradient)
  2. Voltage across the membrane because of a higher concentration of positively charged protons on one side (electrical gradient)
- It tends to drive protons across the membrane back into the matrix.

Chemiosmosis couples exergonic chemical reactions to endergonic H^+ transport, which creates the proton-motive force used to drive cellular work, such as:

- ATP synthesis in mitochondria (*oxidative phosphorylation*). The energy to create the proton gradient comes from the oxidation of glucose and the ETC.
- ATP synthesis in chloroplasts (*photophosphorylation*). The energy to create the proton gradient comes from light trapped during the energy-capturing reactions of photosynthesis.
- ATP synthesis, transport processes, and rotation of flagella in bacteria. The proton gradient is created across the plasma membrane. Peter Mitchell first postulated chemiosmosis as an energy-coupling mechanism based on experiments with bacteria.

3. Biological themes and oxidative phosphorylation

The working model of how mitochondria harvest the energy of food illustrates many of the text's integrative themes in the study of life:

- Energy conversion and utilization
- Emergent properties - Oxidative phosphorylation is an emergent property of the intact mitochondrion that uses a precise interaction of molecules.
- Correlation of structure and function - The chemiosmotic model is based upon the spatial arrangement of membrane proteins.
- Evolution - In an effort to reconstruct the origin of oxidative phosphorylation and the evolution of cells, biologists compare similarities in the chemiosmotic machinery of mitochondria to that of chloroplasts and bacteria.

E. Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes: *a review*

During cellular respiration, *most* energy flows in this sequence:

Glucose $\Rightarrow$ NADH $\Rightarrow$ electron transport chain $\Rightarrow$ proton motive force $\Rightarrow$ ATP

The *net* ATP yield from the oxidation of one glucose molecule to six carbon dioxide molecules can be estimated by adding:

1. ATP produced directly by substrate-level phosphorylation during glycolysis and the Krebs cycle.
   - A net of two ATPs is produced during glycolysis. The debit of two ATPs used during the investment phase is subtracted from the four ATPs produced during the energy-yielding phase.

- Two ATPs are produced during the Krebs cycle.

2. ATP produced when chemiosmosis couples electron transport to oxidative phosphorylation.
   - The electron transport chain creates enough proton-motive force to produce a maximum of *three ATPs* for each electron pair that travels from NADH to oxygen. The average yield is actually between two and three ATPs per NADH (2.7).
   - $FADH_2$ produced during the Krebs cycle is worth a maximum of only *two ATPs*, since it donates electrons at a lower energy level to the electron transport chain.
   - In most eukaryotic cells, the ATP yield is lower due to a NADH produced during glycolysis. The mitochondrial membrane is impermeable to NADH, so its electrons must be carried across the membrane in by one of several "shuttle" reactions. Depending on which shuttle is operating, electrons can be transferred to either NAD^+ or FAD^+ . A pair of electrons passed to FAD^+ yields about two ATP, whereas a pair of electrons passed to NAD^+ yields about 13 ATP.
   - Maximum ATP yield for each glucose oxidized during cellular respiration:

Process	ATP Produced Directly by Substrate-level Phosphorylation	Reduced Coenzyme	ATP Produced by Oxidative Phosphorylation	Total
Glycolysis	*Net* 2 ATP	2 NADH	4 to 6 ATP	6-8
Oxidation of Pyruvate	———	2 NADH	6 ATP	6
Krebs cycle	2 ATP	6 NADH 2 $FADH_2$	18 ATP 4 ATP	24
			Total	36-38

   - This tally only *estimates* the ATP yield from respiration (see Campbell, Figure 9.15). Some variables that affect ATP yield include:
     - The proton-motive force may be used to drive other kinds of cellular work such as active transport.
     - The total ATP yield is inflated (~10%) by rounding off the number of ATPs produced per NADH to three.

Cellular respiration is remarkably efficient in the transfer of chemical energy from glucose to ATP.

- Estimated efficiency in eukaryotic cells is about 40%.
- Energy lost in the process is released as heat.

$$\text{Calculated by } \frac{7.3 \text{ kcal/mol ATP} \times 38 \text{ mol ATP/mol glucose}}{686 \text{ kcal/mol glucose}} \times 100$$

III. Related Metabolic Processes

A. Fermentation enables some cells to produce ATP without the help of oxygen

Food can be oxidized under *anaerobic* conditions.

Aerobic = (Aer = air; bios = life); existing in the presence of oxygen

Anaerobic = (An = without; aer = air); existing in the absence of free oxygen

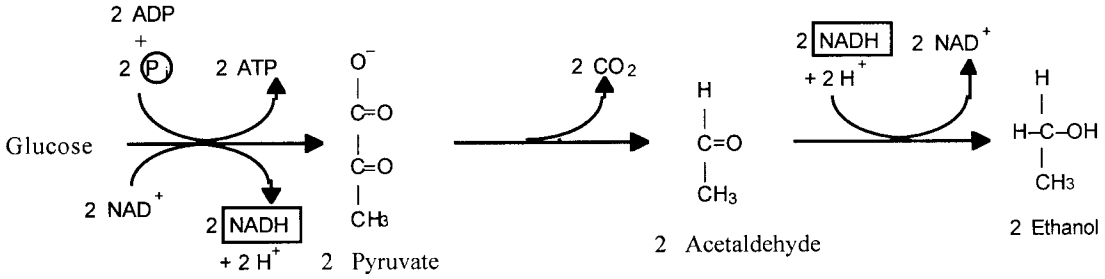
Fermentation = Anaerobic catabolism of organic nutrients

Glycolysis oxidizes glucose to two pyruvate molecules, and the oxidizing agent for this process is NAD^+, *not* oxygen.

- Some energy released from the exergonic process of glycolysis drives the production of two net ATPs by substrate-level phosphorylation.
- Glycolysis produces a net of two ATPs whether conditions are aerobic or anaerobic.
  - *Aerobic conditions*: Pyruvate is *oxidized* further by substrate-level phosphorylation and by oxidative phosphorylation and more ATP is made as NADH passes electrons to the electron transport chain. NAD^+ is regenerated in the process.
  - *Anaerobic conditions*: Pyruvate is *reduced*, and NAD^+ is regenerated. This prevents the cell from depleting the pool of NAD^+, which is the oxidizing agent necessary for glycolysis to continue. No additional ATP is produced.

Fermentation recycles NAD^+ from NADH. This process consists of anaerobic glycolysis plus subsequent reactions that regenerate NAD^+ by reducing pyruvate. Two of the most common types of fermentation are (1) alcohol fermentation and (2) lactic acid fermentation (see Campbell, Figure 9.16).

Alcohol fermentation:

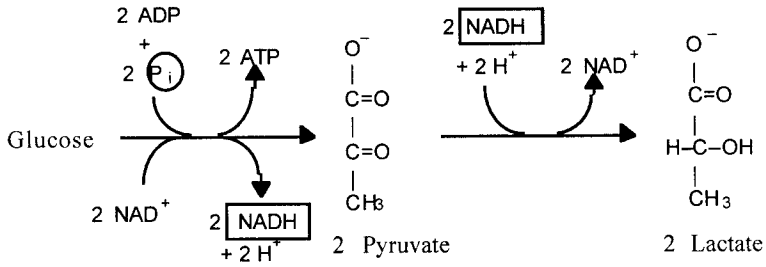


Pyruvate is converted to ethanol in two steps:

a. Pyruvate loses carbon dioxide and is converted to the two-carbon compound acetaldehyde.
b. NADH is oxidized to NAD^+ and acetaldehyde is reduced to ethanol.

Many bacteria and yeast carry out alcohol fermentation under anaerobic conditions.

Lactic acid fermentation:



NADH is oxidized to NAD^+ and pyruvate is reduced to lactate.

- Commercially important products of lactic acid fermentation include cheese and yogurt.
- When oxygen is scarce, human muscle cells switch from aerobic respiration to lactic acid fermentation. Lactate accumulates, but it is gradually carried to the liver where it is converted back to pyruvate when oxygen becomes available.

1. Fermentation and respiration compared

The anaerobic process of fermentation and aerobic process of cellular respiration are similar in that both metabolic pathways:

- Use glycolysis to oxidize glucose and other substrates to pyruvate, producing a net of two ATPs by substrate phosphorylation
- Use NAD^+ as the oxidizing agent that accepts electrons from food during glycolysis

Fermentation and cellular respiration differ in:

- How NADH is oxidized back to NAD^+. Recall that the oxidized form, NAD^+, is necessary for glycolysis to continue.
  - During fermentation, NADH passes electrons to pyruvate or some derivative. As pyruvate is reduced, NADH is oxidized to NAD^+. Electrons transferred from NADH to pyruvate or other substrates are not used to power ATP production.
  - During cellular respiration, the stepwise electron transport from NADH to oxygen not only drives oxidative phosphorylation, but regenerates NAD^+ in the process.
- Final electron acceptor
  - In fermentation, the final electron acceptor is pyruvate (lactic acid fermentation), acetaldehyde (alcohol fermentation), or some other organic molecule.
  - In cellular respiration, the final electron acceptor is oxygen.
- Amount of energy harvested
  - During fermentation, energy stored in pyruvate is unavailable to the cell.
  - Cellular respiration yields 18 times more ATP per glucose molecule than does fermentation. The higher energy yield is a consequence of the Krebs cycle which completes the oxidation of glucose and thus taps the chemical bond energy still stored in pyruvate at the end of glycolysis.
- Requirement for oxygen
  - Fermentation does not require oxygen.
  - Cellular respiration occurs only in the presence of oxygen.

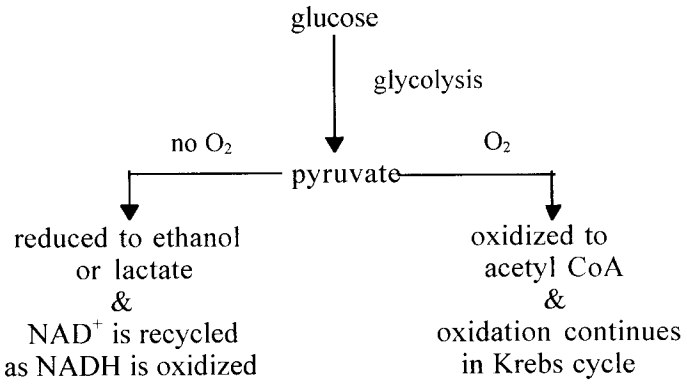
Organisms can be classified based upon the effect oxygen has on growth and metabolism.

Strict (obligate) aerobes = Organisms that require oxygen for growth and as the final electron acceptor for aerobic respiration.

Strict (obligate) anaerobes = Microorganisms that only grow in the absence of oxygen and are, in fact, poisoned by it.

Facultative anaerobes = Organisms capable of growth in either aerobic or anaerobic environments.

- Yeasts, many bacteria, and mammalian muscle cells are facultative anaerobes.
- Can make ATP by fermentation in the absence of oxygen or by respiration in the presence of oxygen.
- Glycolysis is common to both fermentation and respiration, so pyruvate is a key juncture in catabolism (see Campbell, Figure 9.18).



3. The evolutionary significance of glycolysis

The first prokaryotes probably produced ATP by glycolysis. Evidence includes the following:

- Glycolysis does not require oxygen, and the oldest known bacterial fossils date back to 3.5 billion years ago when oxygen was not present in the atmosphere.
- Glycolysis is the most widespread metabolic pathway, so it probably evolved early.
- Glycolysis occurs in the cytosol and does not require membrane-bound organelles. Eukaryotic cells with organelles probably evolved about two billion years after prokaryotic cells.

B. Glycolysis and the Krebs cycle connect to many other metabolic pathways

1. The versatility of catabolism

Respiration can oxidize organic molecules other than glucose to make ATP. Organisms obtain most calories from fats, proteins, disaccharides and polysaccharides. These complex molecules must be enzymatically hydrolyzed into simpler molecules or monomers that can enter an intermediate reaction of glycolysis or the Krebs cycle (see Campbell, Figure 9.19).

Glycolysis can accept a wide range of carbohydrates for catabolism.

- Starch is hydrolyzed to glucose in the digestive tract of animals.
- In between meals, the liver hydrolyzes glycogen to glucose.
- Enzymes in the small intestine break down disaccharides to glucose or other monosaccharides.

Proteins are hydrolyzed to amino acids.

- Organisms synthesize new proteins from some of these amino acids.
- Excess amino acids are enzymatically converted to intermediates of glycolysis and the Krebs cycle. Common intermediates are pyruvate, acetyl CoA, and α-ketoglutarate.
- This conversion process deaminates amino acids, and the resulting nitrogenous wastes are excreted and the carbon skeleton can be oxidized.

Fats are excellent fuels because they are rich in hydrogens with high energy electrons. Oxidation of one gram of fat produces twice as much ATP as a gram of carbohydrate.

- Fat sources may be from the diet or from storage cells in the body.
- Fats are digested into glycerol and fatty acids.
- Glycerol can be converted to glyceraldehyde phosphate, an intermediate of glycolysis.

- Most energy in fats is in fatty acids, which are converted into acetyl CoA by *beta oxidation*. The resulting two-carbon fragments can enter the Krebs cycle.

2. Biosynthesis (anabolic pathways)

Some organic molecules of food provide the carbon skeletons or raw materials for the synthesis of new macromolecules.

- Some organic monomers from digestion can be used *directly* in anabolic pathways.
- Some precursors for biosynthesis do not come directly from digested food, but instead come from glycolysis or Krebs cycle intermediates which are diverted into anabolic pathways.
- These anabolic pathways *require energy* (ATP) produced by catabolic pathways of glycolysis and respiration.
- Glycolysis and the Krebs cycle are metabolic interchanges that can convert one type of macromolecule to another in response to the cell's metabolic demands.

C. Feedback mechanisms control cellular respiration

Cells respond to changing metabolic needs by controlling reaction rates.

- Anabolic pathways are switched off when their products are in ample supply. The most common mechanism of control is *feedback inhibition* (see Campbell, Chapter 6).
- Catabolic pathways, such as glycolysis and Krebs cycle, are controlled by regulating enzyme activity at strategic points.

A key control point of catabolism is the third step of glycolysis, which is catalyzed by an allosteric enzyme, *phosphofructokinase* (see Campbell, Figure 9.20).

- The ratio of ATP to ADP and AMP reflects the energy status of the cell, and phosphofructokinase is sensitive to changes in this ratio.
- Citrate (produced in Krebs cycle) and ATP are *allosteric inhibitors* of phosphofructokinase, so when their concentrations rise, the enzyme slows glycolysis. As the rate of glycolysis slows, Krebs cycle also slows since the supply of acetyl CoA is reduced. This synchronizes the rates of glycolysis and Krebs cycle.
- ADP and AMP are *allosteric activators* for phosphofructokinase, so when their concentrations relative to ATP rise, the enzyme speeds up glycolysis which speeds up the Krebs cycle.
- There are other allosteric enzymes that also control the rates of glycolysis and the Krebs cycle.

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