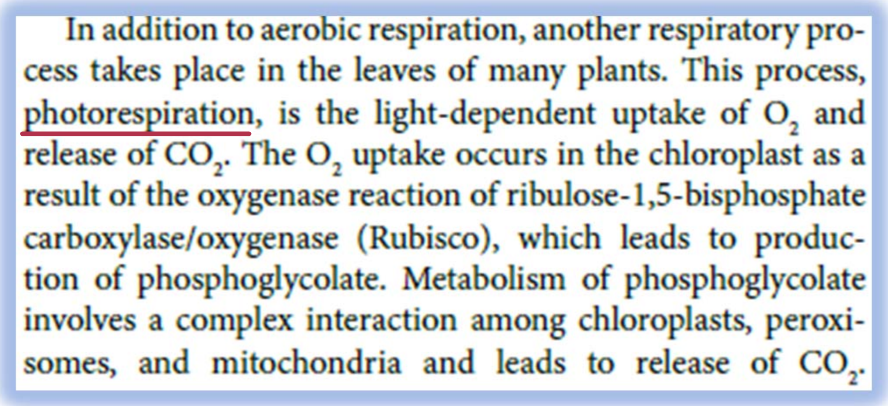
Respiration

INTRODUCTION

- plants use light energy to assimilate carbon into sugars and starch, and these molecules are subsequently broken down and converted into organic acids and other compounds.
- In this chapter, we address aerobic respiration—the further oxidation of these compounds to carbon dioxide (CO2) and water (H2O) in the mitochondrion—and review the mechanisms by which the energy released during respiration is conserved as ATP, a process called oxidative phosphorylation.



In addition to aerobic respiration, another respiratory process takes place in the leaves of many plants. This process, photorespiration, is the light-dependent uptake of O_2 and release of CO_2. The O_2 uptake occurs in the chloroplast as a result of the oxygenase reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which leads to production of phosphoglycolate. Metabolism of phosphoglycolate involves a complex interaction among chloroplasts, peroxisomes, and mitochondria and leads to release of CO_2.

Type of Respiration

Dark respiration

Photo respiration

OVERVIEW OF RESPIRATION

- a process common to almost all eukaryotic organisms.
- controlled oxidation of reduced organic substrates to CO2 and H2O.
- Numerous compounds can serve as substrates for respiration, including carbohydrates, lipids, proteins, amino acids, and organic acids.
- Respiration releases a large amount of free energy, which is conserved in ATP molecules.
- This chemical bond energy can be used to drive metabolic reactions involved in the growth, development, and maintenance of the plant.
- The primary pathways of respiration provide metabolic intermediates that serve as substrates for the synthesis of nucleic acids, amino acids, fatty acids, and many secondary metabolites.
- Although the general process of respiration in plants is the same as in other eukaryotes, several features are unique to plants.
- These modifications apparently evolved to cope with the environmental and metabolic circumstances commonly faced by plants.

PLANT MITOCHONDRIA

- The **mitochondrion** is the principal organelle of eukaryotic respiration.
- The number of mitochondria per plant cell varies and is related primarily to the metabolic activity of the tissue. however, usually remains similar during different developmental stages of the same cell type. For example, the small cells of the root cap of maize seedlings contain approximately 200 mitochondria each, whereas the much larger mature root tip cells may have 2,000 or so mitochondria per cell. In very active cells such as phloem companion cells, secretory cells, and transfer cells, up to 20% of the volume of the cytoplasm may be occupied by mitochondria.

Mitochondria contain two sets of membranes that divide the organelle into four compartments: the **outer membrane**, the region between the two membranes (**intermembrane space**), a highly invaginated **inner membrane**, and the aqueous phase contained within the inner membrane (mitochondrial **matrix**) (Fig.). Invaginations of the inner membrane give rise to **cristae**, which appear as sac-like structures under the electron microscope

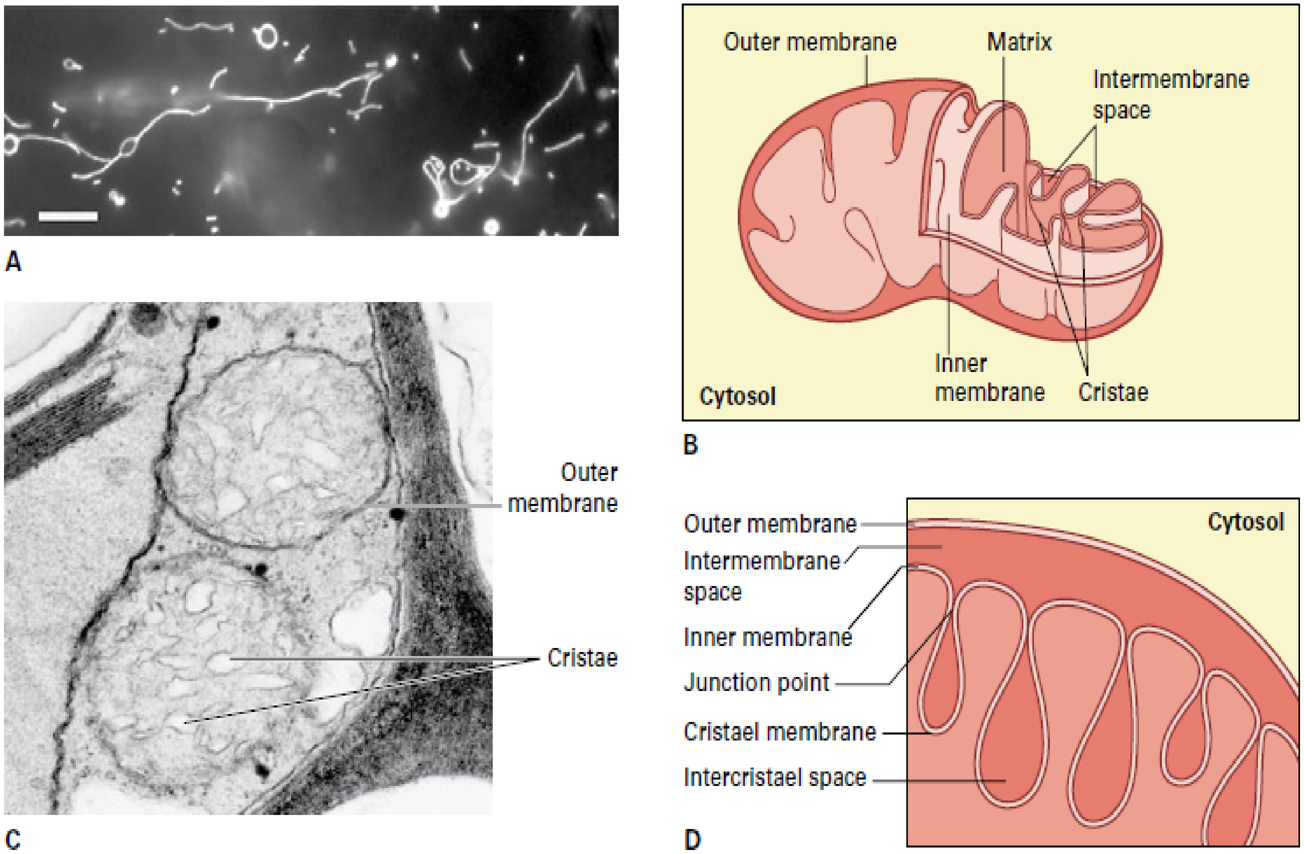
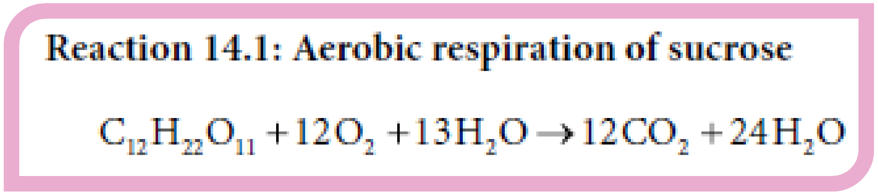


FIGURE 14.1 *Structural organization of the mitochondrion. (A) Fluorescence image of GFP-tagged mitochondrial structures in* Arabidopsis *leaf epidermis. (B) Diagram of the membrane organization of a plant mitochondrion. The outer membrane is permeable to molecules of 10 kDa or less because it contains a pore-forming protein called porin. The highly invaginated inner membrane, which contains the components of the mitochondrial electron transport chain and ATP synthase, serves as the primary permeability barrier for the organelle. The space between the two membranes is called the intermembrane space. The mitochondrial matrix, the region bounded by the inner membrane, contains the mitochondria genome, the enzymes associated with the citric acid cycle, and the machinery required for mitochondrial protein synthesis. (C) Thin-section electron micrograph of a plant mitochondrion, showing the highly folded inner membrane structures (cristae) and the surrounding outer membrane. (D) Details of the mitochondrial outer and inner membranes.*

THE PRINCIPAL PRODUCTS OF RESPIRATION ARE CO2, H2O, AND FREE ENERGY CONSERVED AS ATP

➥process of oxidizing food to release energy inside cells

- Although respiration can oxidize many compounds, the principal substrates provided by photosynthesis for plant respiration are sucrose and starch.
- Using sucrose as an example of a respiratory substrate, one can represent the overall process of aerobic respiration as follows:

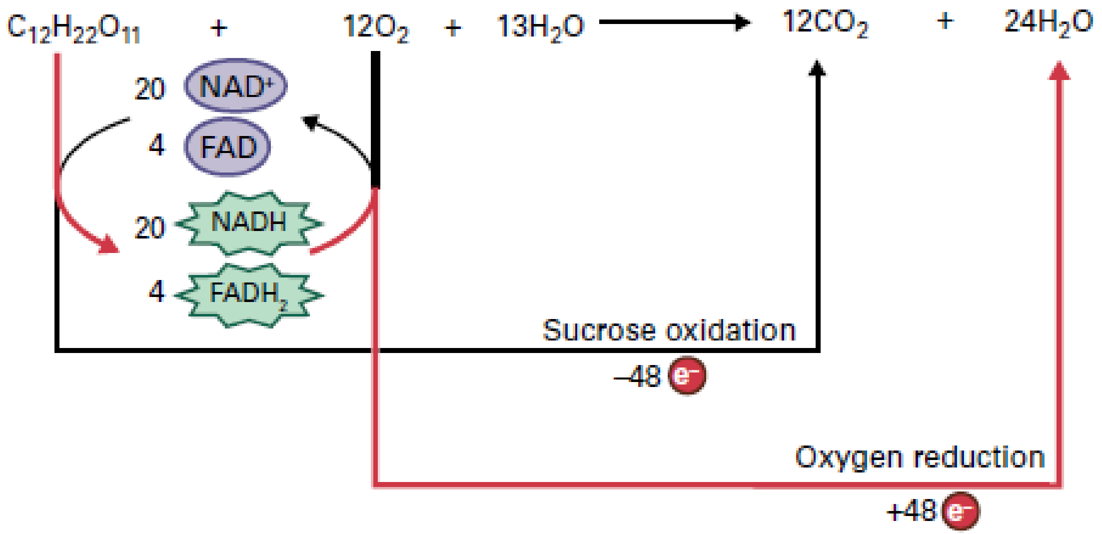


Reaction 14.1: Aerobic respiration of sucrose

$$C_{12}H_{22}O_{11} + 12O_2 + 13H_2O \rightarrow 12CO_2 + 24H_2O$$

- This reaction scheme is the reverse of photosynthesis, and it represents a coupled pair of redox reactions in which sucrose is completely oxidized to CO2, while O2, the terminal electron acceptor, is reduced to H2O (Fig. 14.2). The standard free energy change ($\Delta G°'$) for this exergonic reaction, −5764 kJ mol−1, provides the thermodynamic driving force for the production of ATP.

FIGURE 14.2 *Redox reactions of respiration. The oxidation of carbohydrate to carbon dioxide (CO_2) is coupled to the reduction of oxygen (O_2) to water (H_2O). The free energy released during this process is linked to the synthesis of ATP.*



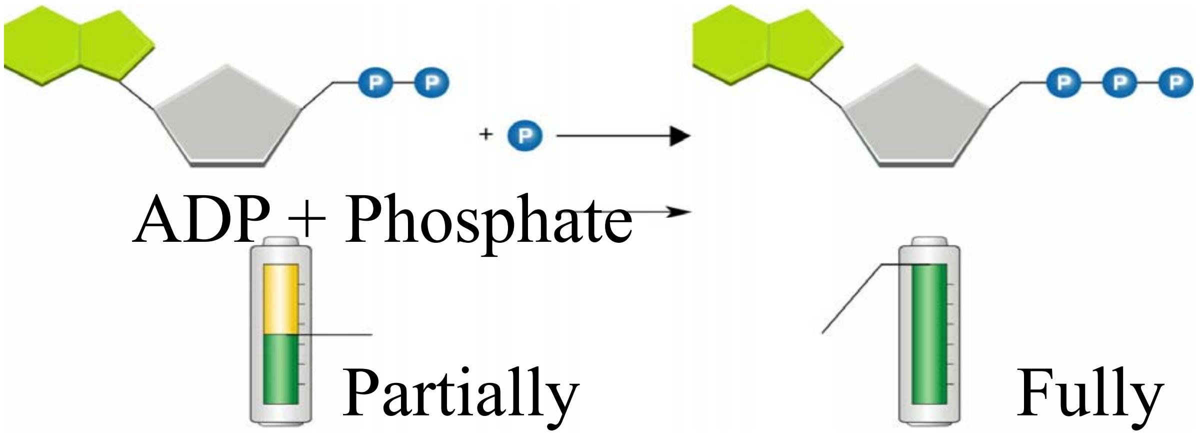
ATP

- energy is mainly released as heat and ATP during respiration in cells
- however, during respiration, some ATP should be consumed first before other ATP can be formed
- the ATP consumed is used to form other ATP from ADP and phosphate groups

ADP TO ATP!

Energy is released when ATP is changed into ADP.

Energy is stored when ADP is converted to ATP.

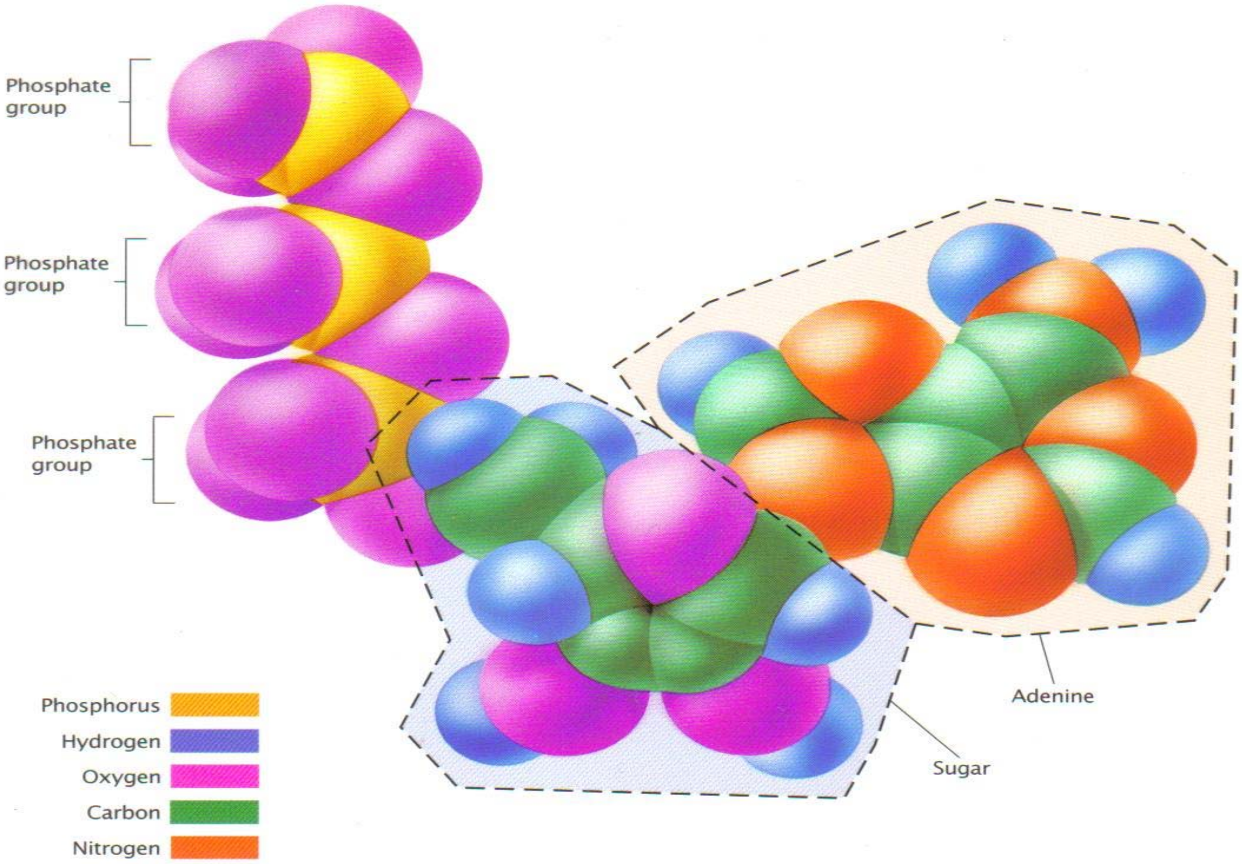


ADP + Phosphate →

Partially
charged
battery

Fully
charged
battery

ATP = ADENOSINE TRIPHOSPHATE

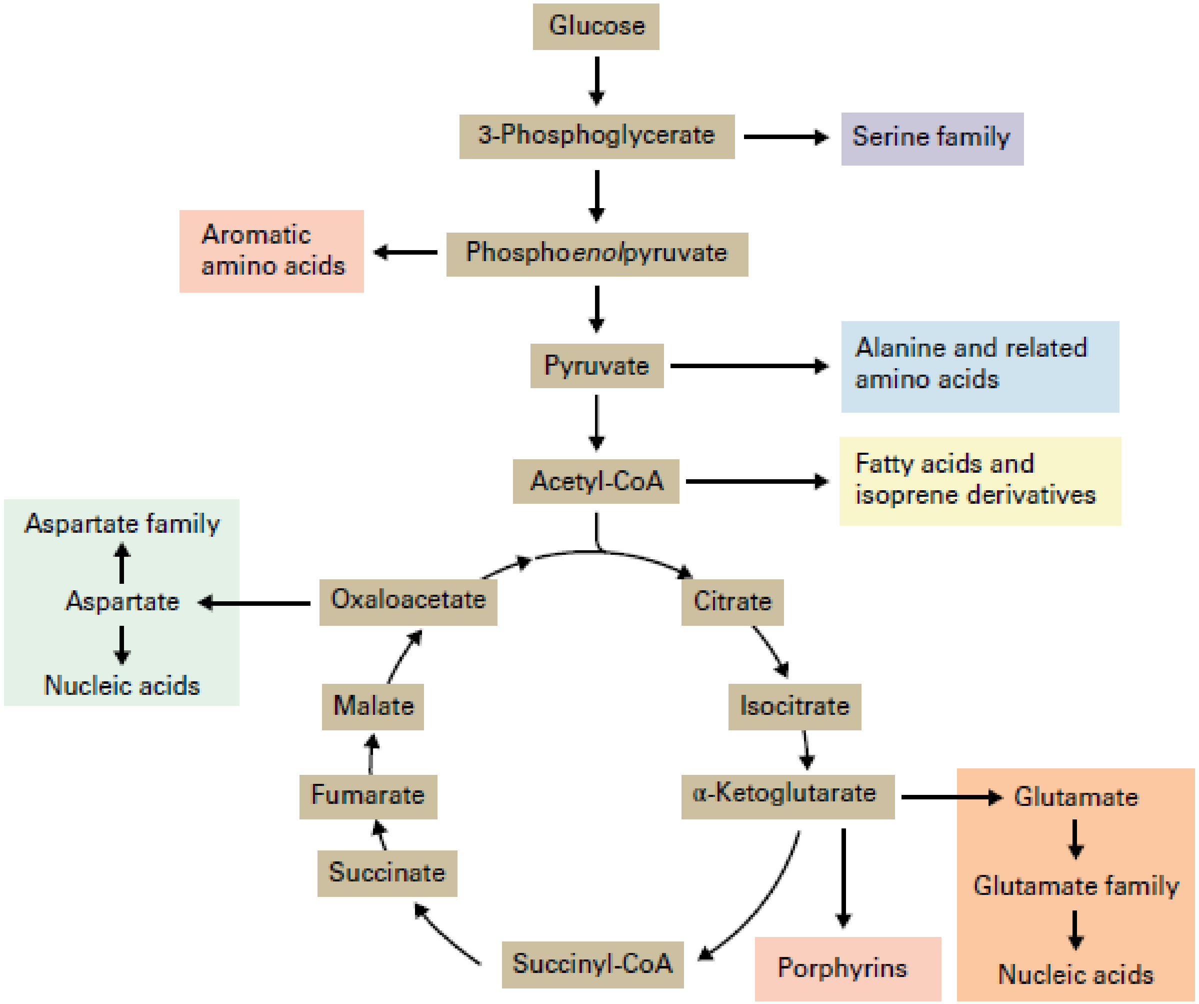


ADP + phosphate + energy → ATP

- ATP is a high-energy compound while ADP is a low-energy one
- ATP can only store energy for a short period
- ATP is made inside organelles, mitochondria, which is scattered in the cytoplasm of a cell

- The three stages of respiration—**glycolysis**, the **citric acid cycle**, and **electron transfer/oxidative phosphorylation**— take place in different subcellular locations.

- **Glycolysis** involves a series of soluble cytosolic enzymes that oxidize sugars to organic acids. In the reaction above, one sucrose molecule is broken into two hexoses, which are then converted by glycolysis to four molecules of the three-carbon (C3) compound pyruvate.
- The reactions of the **citric acid** cycle occur within the mitochondrial matrix and can completely oxidize pyruvate to CO_2; The electrons are transferred to NAD^+ and another cofactor, FAD, yielding NADH and $FADH_2$. The citric acid cycle also phosphorylates ADP directly.
- Finally, in the inner mitochondrial membrane, the reduced cofactors generated during glycolysis and the citric acid cycle are oxidized by a set of **electron transfer proteins** that ultimately donate the electrons to O_2, producing H_2O. The energy stored in this electrochemical gradient subsequently drives the conversion of ADP and Pi to ATP by a process known as *oxidative phosphorylation*.
- Not every molecule of sucrose or C6 unit of starch metabolized via glycolysis and the citric acid cycle, however, is completely oxidized in this manner; many intermediates from these two pathways are diverted along the way to provide the primary carbon building blocks for other important cellular compounds (Fig.).



Glucose

3-Phosphoglycerate

Serine family

Aromatic amino acids

Phospho*enol*pyruvate

Pyruvate

Alanine and related amino acids

Acetyl-CoA

Fatty acids and isoprene derivatives

Aspartate family

Aspartate

Nucleic acids

Oxaloacetate

Citrate

Malate

Isocitrate

Fumarate

α-Ketoglutarate

Glutamate

Glutamate family

Nucleic acids

Succinate

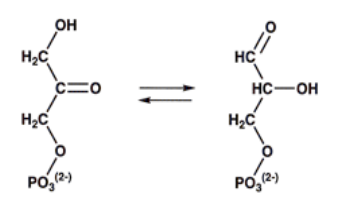
Succinyl-CoA

Porphyrins

FIGURE 14.6 *Intermediates produced during the reactions of glycolysis and the citric acid cycle serve as substrates for numerous plant biosynthetic pathways. The primary intermediates for the production of amino acids, lipids, nucleic acids, cell wall sugars, and many other essential components of the plant cell are derived from compounds that originate from glycolysis or the citric acid cycle.*

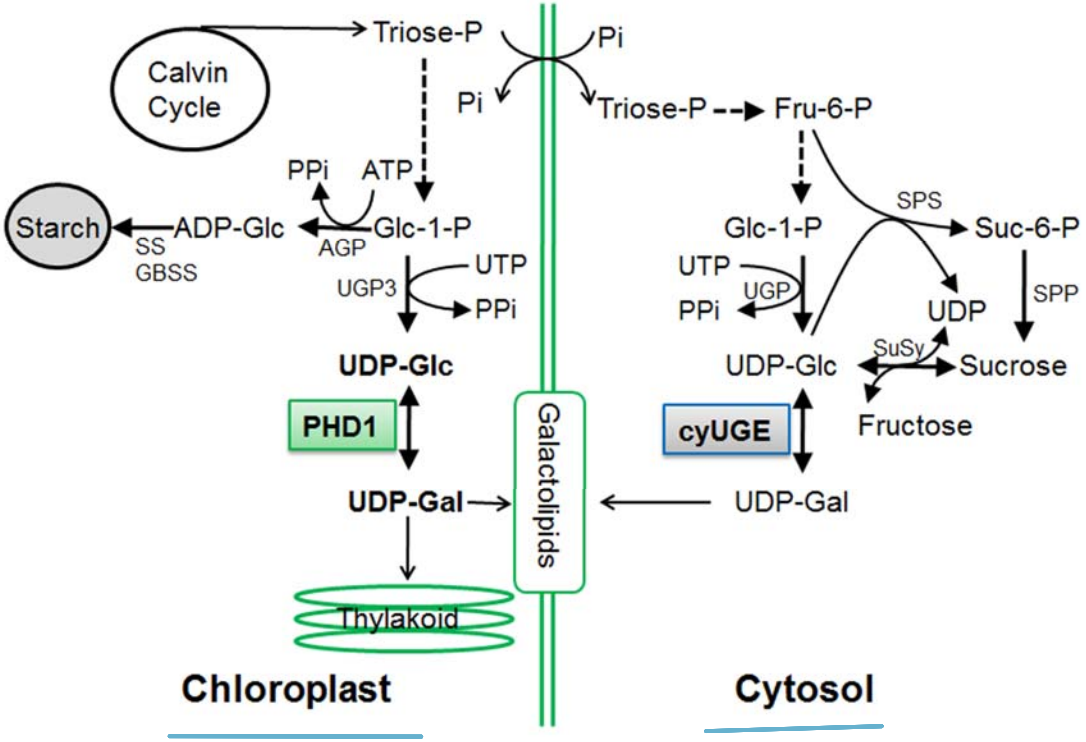
GLYCOLYSIS

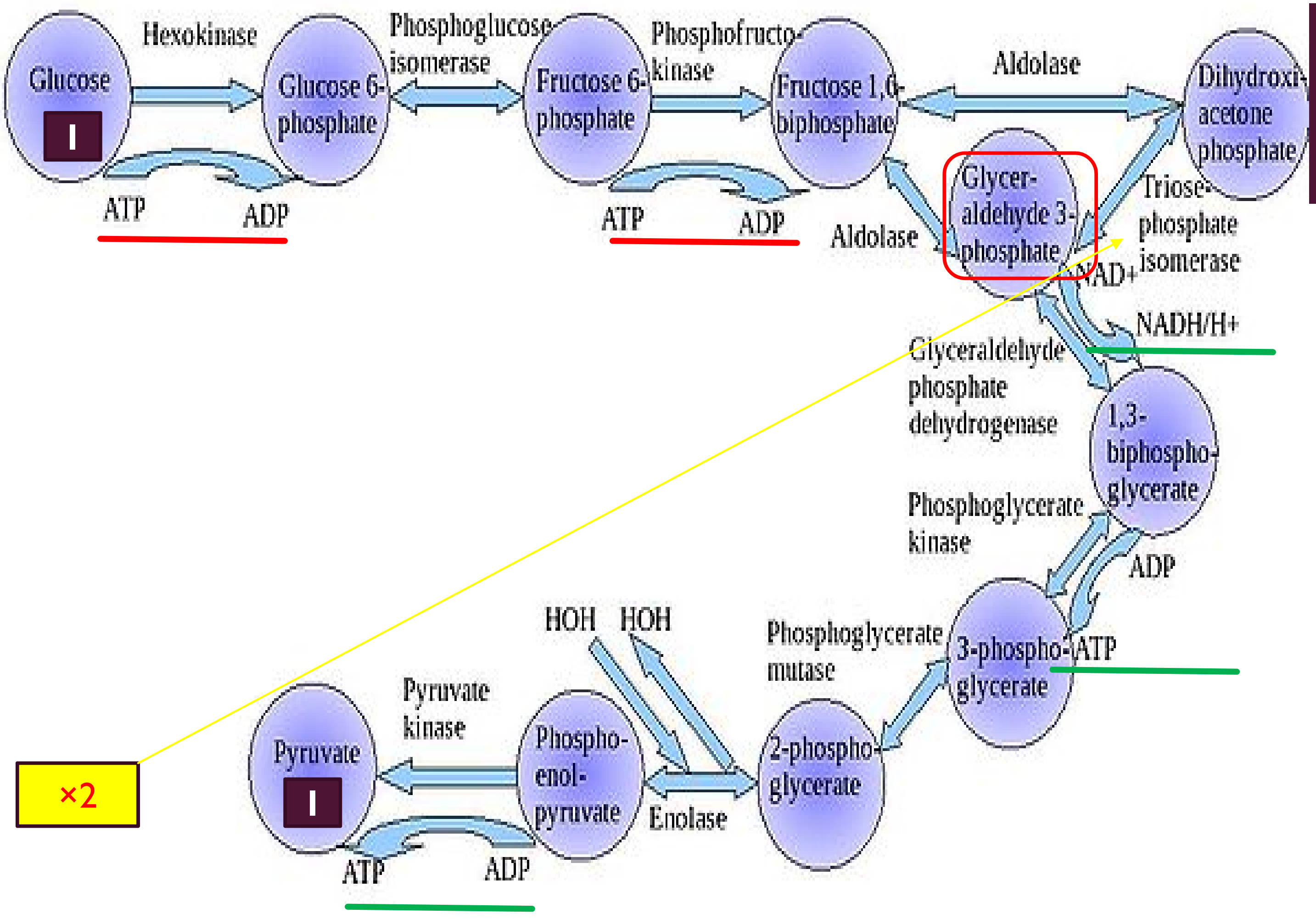
- The reactions of the lower half of glycolysis, triose phosphate molecules are oxidized to form pyruvate and energy is conserved in high-energy phosphate compounds that can be used to synthesize ATP from ADP.
- These high-energy compounds are generated by two enzymes, **glyceraldehyde-3- phosphate dehydrogenase**, which forms the mixed acid anhydride **1,3-bisphosphoglycerate** (Reaction 13.32), and **enolase**, which generates a phosphate ester linked to the enol form of pyruvic acid, phospho*enol*pyruvate (Fig. 13.34; see also Section 13.9.3).
- Because the instability of the enol form of pyruvate gives the phosphate ester a high negative free energy of hydrolysis, the phosphate group can be transferred to ADP, yielding ATP.



dihydroxyacetone phoshate (DHAP)

D-glyceraldehyde-3-phosphate (GAP)




Glucose
Hexokinase
ATP
ADP
Glucose 6-phosphate
Phosphoglucose isomerase
Fructose 6-phosphate
Phosphofructokinase
ATP
ADP
Fructose 1,6-biphosphate
Aldolase
Dihydroxiacetone phosphate
Aldolase
Glyceraldehyde 3-phosphate
Triose-phosphate isomerase
NAD+
NADH/H+
Glyceraldehyde phosphate dehydrogenase
1,3-biphosphoglycerate
Phosphoglycerate kinase
ADP
ATP
3-phosphoglycerate
Phosphoglycerate mutase
2-phosphoglycerate
HOH
HOH
Enolase
Phosphoenolpyruvate
Pyruvate kinase
ATP
ADP
Pyruvate
×2

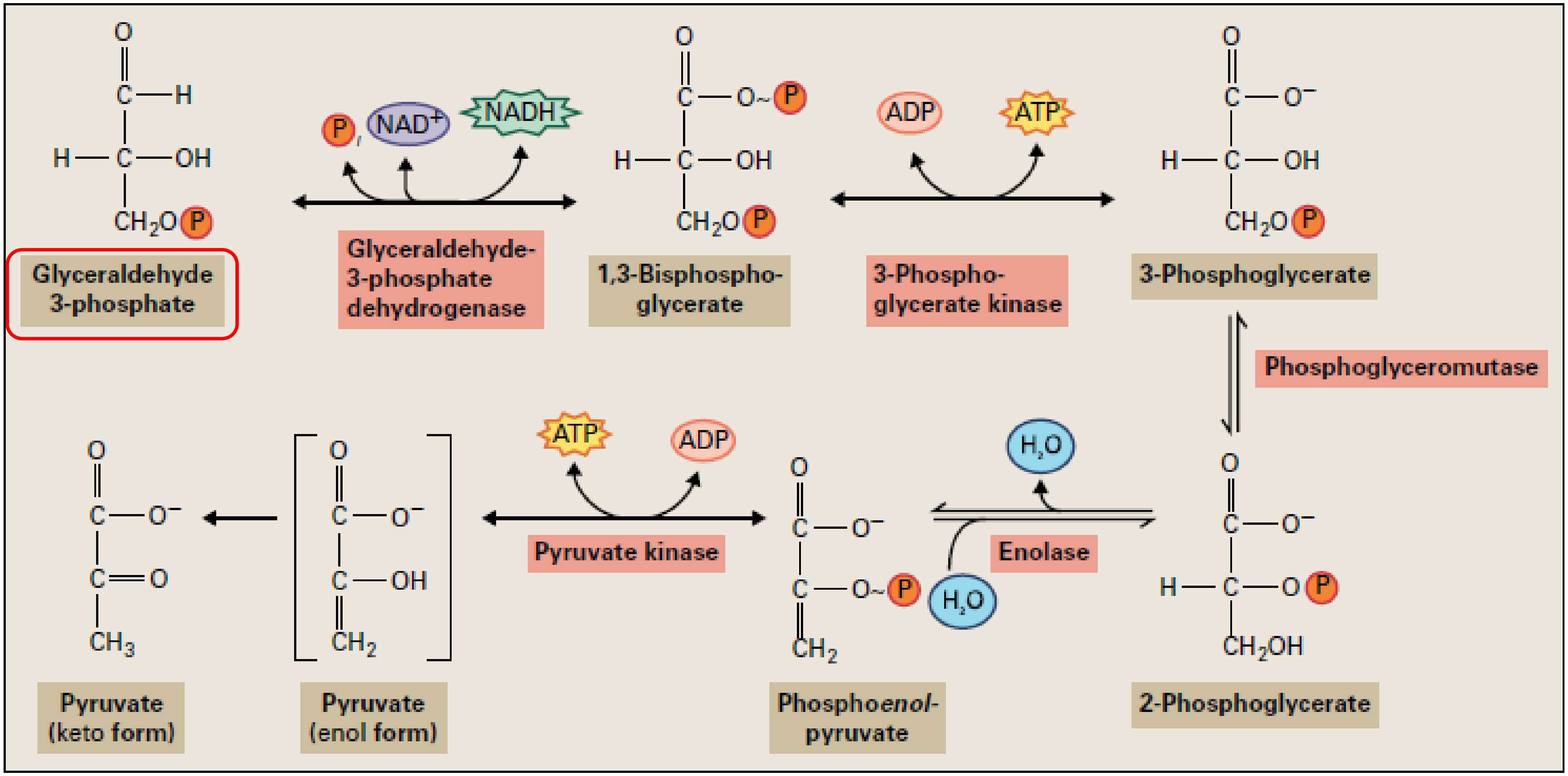
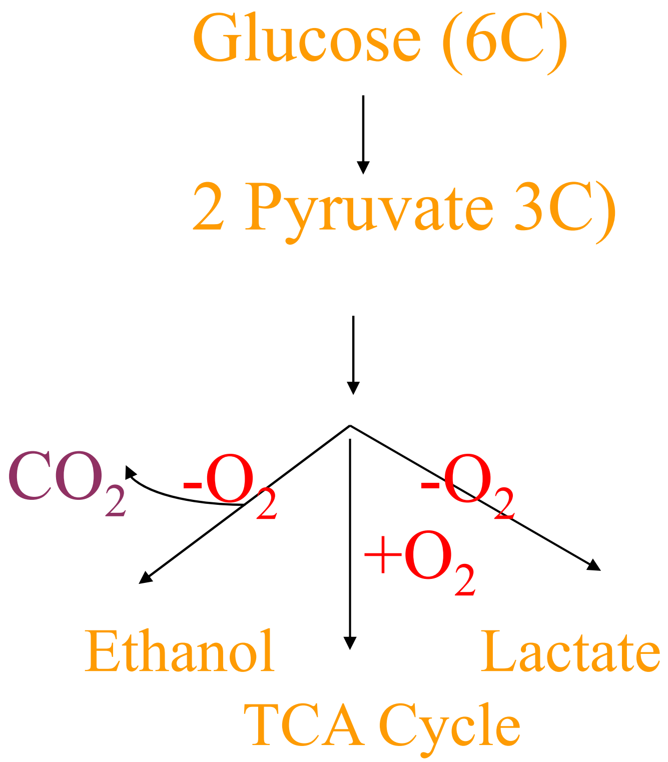


FIGURE 13.34 *Oxidative and ATP-forming reactions of the glycolytic pathway, showing the relationship between the oxidation of the three-carbon molecules of the lower half of glycolysis and the creation of phosphate compounds with high negative free energies of hydrolysis (~).*

GLYCOLYSIS

- Occurs in all living organisms
- Only stage which can occur without oxygen
- Oldest stage of respiration
  - operated for billions of years in anaerobic organisms
- Converts glucose to 2 pyruvates in cytosol
  - with O_2 goes on to TCA cycle
  - without O_2 pyruvate is converted to lactate or ethanol (fermentation)

GLYCOLYSIS



Glucose (6C)

2 Pyruvate 3C)

CO_2 $-O_2$ $-O_2$

$+O_2$

Ethanol

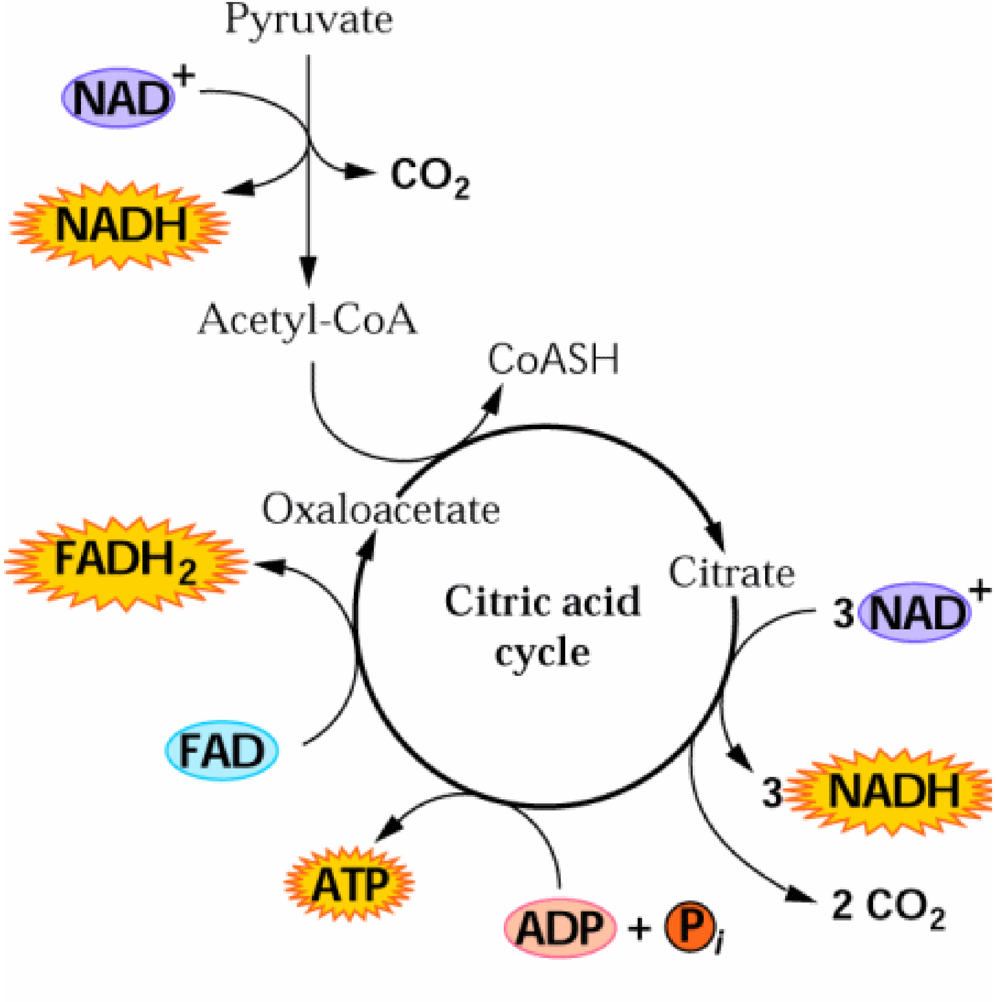
Lactate

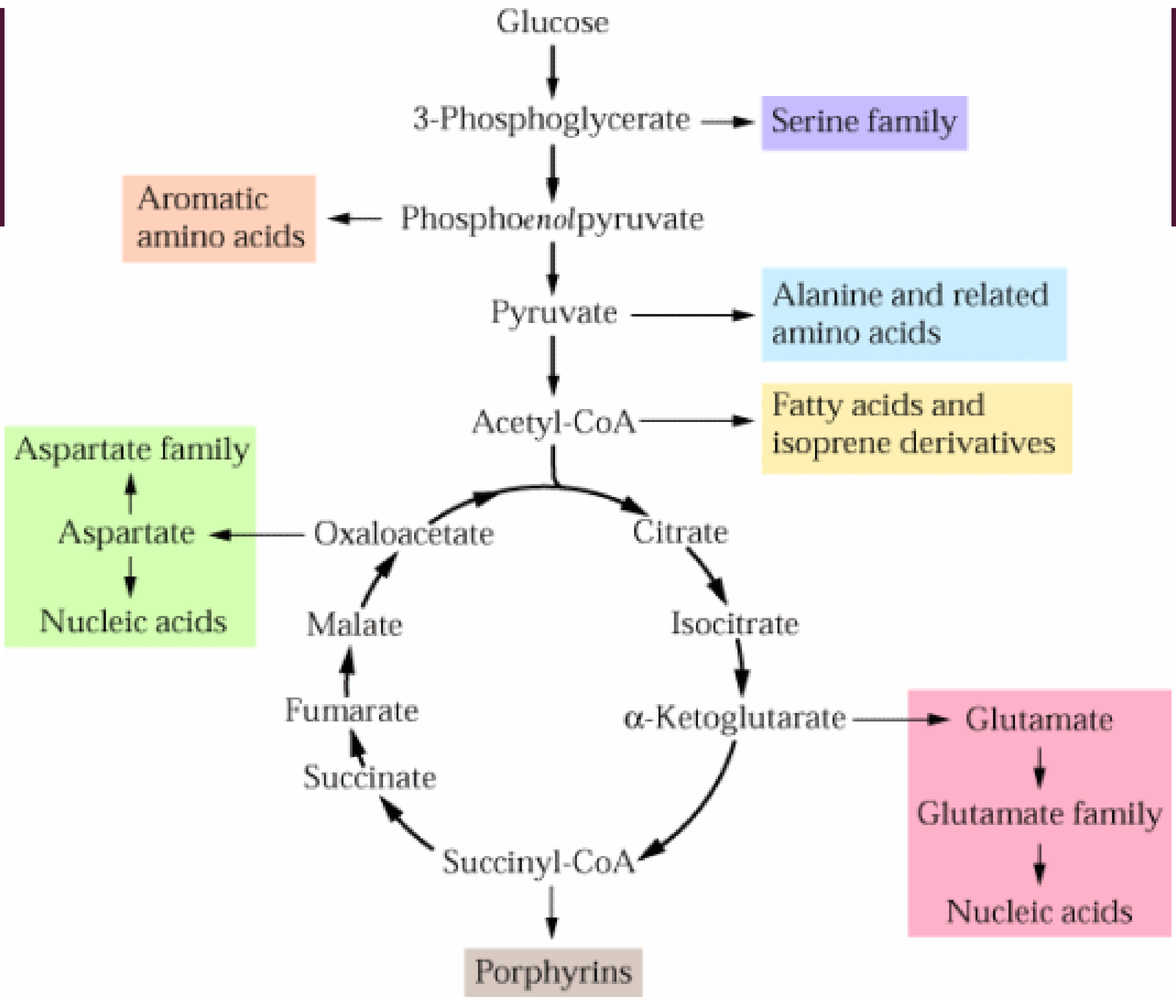
TCA Cycle

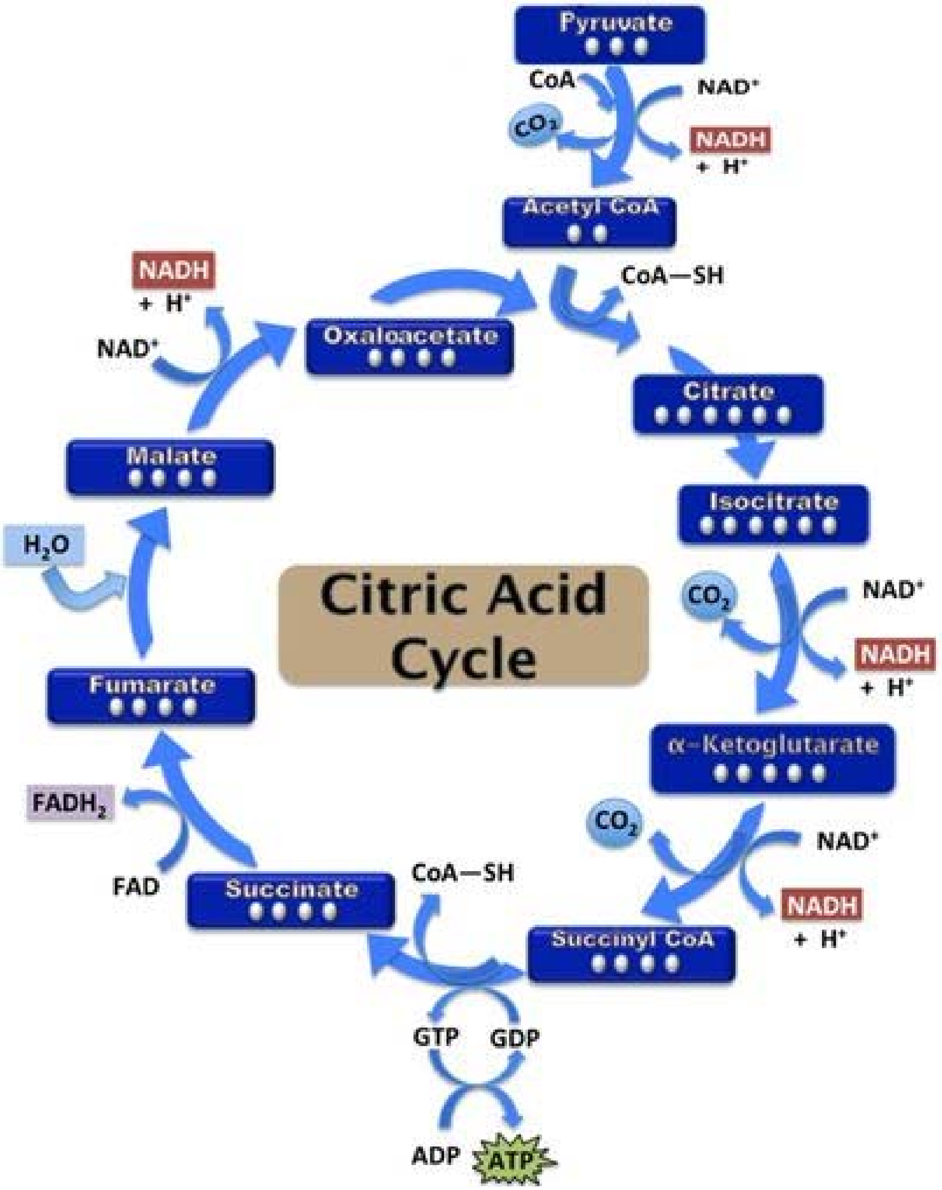
CITRIC ACID CYCLE

- Known by several names, including the tricarboxylic acid cycle (TCA) and Krebs cycle (after its discoverer, Hans Krebs), the citric acid cycle oxidizes organic acids to produce CO2 and transfers the resulting electrons to reduce the redox cofactors NAD+ and FAD, forming NADH and FADH2.
- Cytosolic reactions generate products that are transported into the mitochondria to feed the citric acid cycle
- Pyruvate is transported directly into the mitochondrion.
- OAA is either transported directly into the mitochondrion or first reduced to malate by cytosolic malate dehydrogenase.
- The mitochondrial inner membrane contains separate carriers by which malate and pyruvate are taken into the mitochondrial matrix.

TCA cycle


Pyruvate
NAD^+
CO$_2$
NADH
Acetyl-CoA
CoASH
Oxaloacetate
Citrate
Citric acid cycle
$FADH_2$
3 NAD^+
FAD
3 NADH
ATP
ADP + P_i
2 CO$_2$


Glucose
3-Phosphoglycerate
Serine family
Aromatic amino acids
Phospho*enol*pyruvate
Pyruvate
Alanine and related amino acids
Acetyl-CoA
Fatty acids and isoprene derivatives
Aspartate family
Aspartate
Nucleic acids
Oxaloacetate
Citrate
Malate
Isocitrate
Fumarate
α-Ketoglutarate
Glutamate
Succinate
Glutamate family
Succinyl-CoA
Nucleic acids
Porphyrins


Citric Acid Cycle
Pyruvate
CoA
CO₂
NAD⁺
NADH + H⁺
Acetyl CoA
CoA—SH
Citrate
Isocitrate
CO₂
NAD⁺
NADH + H⁺
α-Ketoglutarate
CO₂
NAD⁺
NADH + H⁺
Succinyl CoA
CoA—SH
GTP
GDP
ADP
ATP
Succinate
FAD
FADH₂
Fumarate
H₂O
Malate
NAD⁺
NADH + H⁺
Oxaloacetate

PLANT MITOCHONDRIAL ELECTRON TRANSPORT

- The mitochondrial electron transport chain conserved among eukaryotes consists of four membrane-bound, multiple subunit protein complexes, commonly referred to as complexes I–IV (Fig. 14.12).
- These protein complexes catalyze the multistep transfer of electrons from NADH and FADH2 to O2, forming H2O and translocating protons from the matrix to the intermembrane space.
- In this way, an **endergonic** (energy-consuming) reaction—proton pumping—is driven by the **exergonic** (energy-releasing) transfer of electrons from strong reducing agents to a strong oxidant.
- The reduction of ½ O2 by two electrons from NADH involves a reduction potential difference ($\Delta E^{\circ\prime}$) of 1.14 V (Fig.).
- This translates to 219.2 kJ of free energy released for every mole of NADH oxidized.
- If NADH or FADH2 reduced O2 directly, the heat released would not be biologically useful.
- The electron transport chain facilitates several modestly exergonic redox reactions, rather than a single explosive one, and conserves energy through proton translocation.

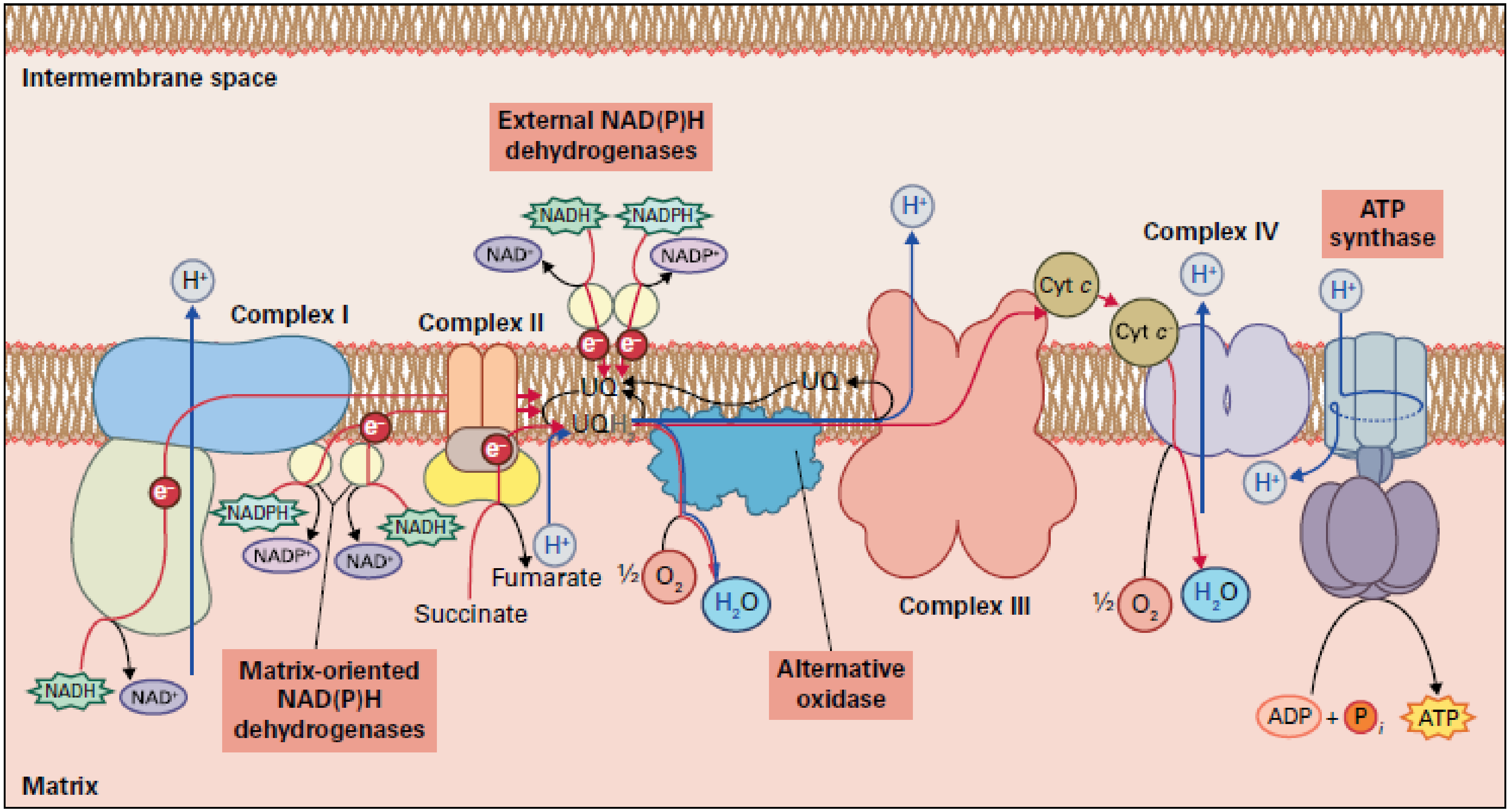
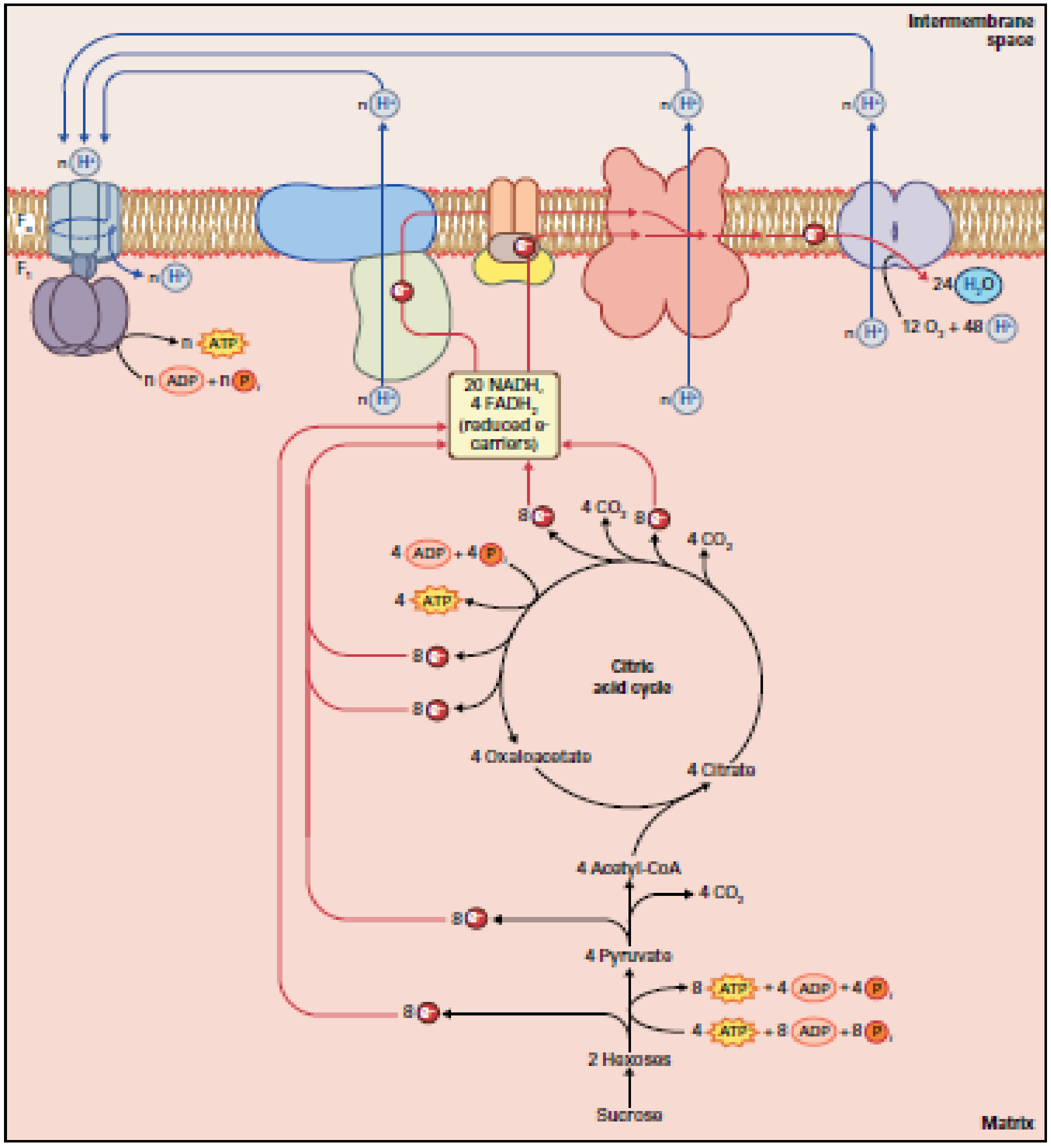
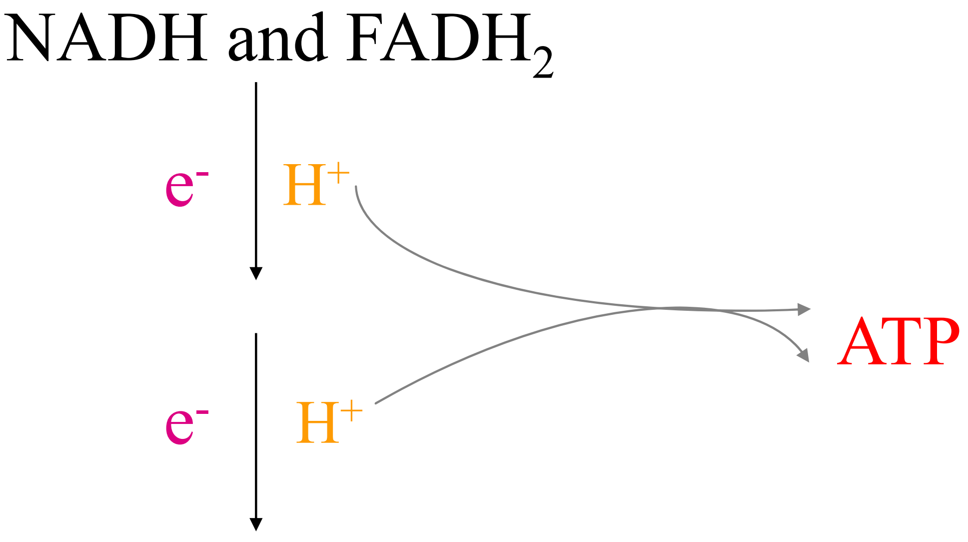


FIGURE 14.12 *Organization of the plant mitochondrial electron transport chain and ATP synthase in the inner mitochondrial membrane. Electron transfer complexes I–IV, ATP synthase, alternative NAD(P)H dehydrogenases, and the alternative oxidase (AOX) are diagrammed, incorporating what is currently known about their topological orientation in the inner membrane. Red lines indicate electron transfer pathways. A large pool of oxidized ubiquinone (UQ) and reduced ubiquinol (UQH_2) freely diffuses within the inner membrane and transfers electrons derived from the dehydrogenases to either complex III or the AOX. Blue arrows designate the direction of proton translocation at complexes I, III, and IV. Transmembrane proton movement generates a proton electrochemical gradient that drives the synthesis of ATP from ADP and P_i via the ATP synthase.*



General mechanism of oxidative phosphorylation in mitochondria. Electrons released during the oxidative steps of glycolysis and the citric acid cycle produce 20 molecules of NADH and 4 molecules of FADH2. These reduced coenzymes are subsequently oxidized bythe mitochondrial electron transport chain. The free energy released during mitochondrial electron transfer is coupled to the translocation of protons across the inner mitochondrial membrane (from the matrix into the intermembrane space), which generates a proton electrochemical gradient (ΔµH+) across the inner membrane. As protons move back across the inner membrane through the Fo proton channel of the ATP synthase complex, the free energy released is used by the complex's F1 component to catalyze conversion of ADP and Pi to ATP in the mitochondrial matrix.The efficiency of net H+ transfer and ATP synthesis can vary (n),

ELECTRON TRANSPORT SYSTEM



NADH and $FADH_2$

e^- H^+

ATP

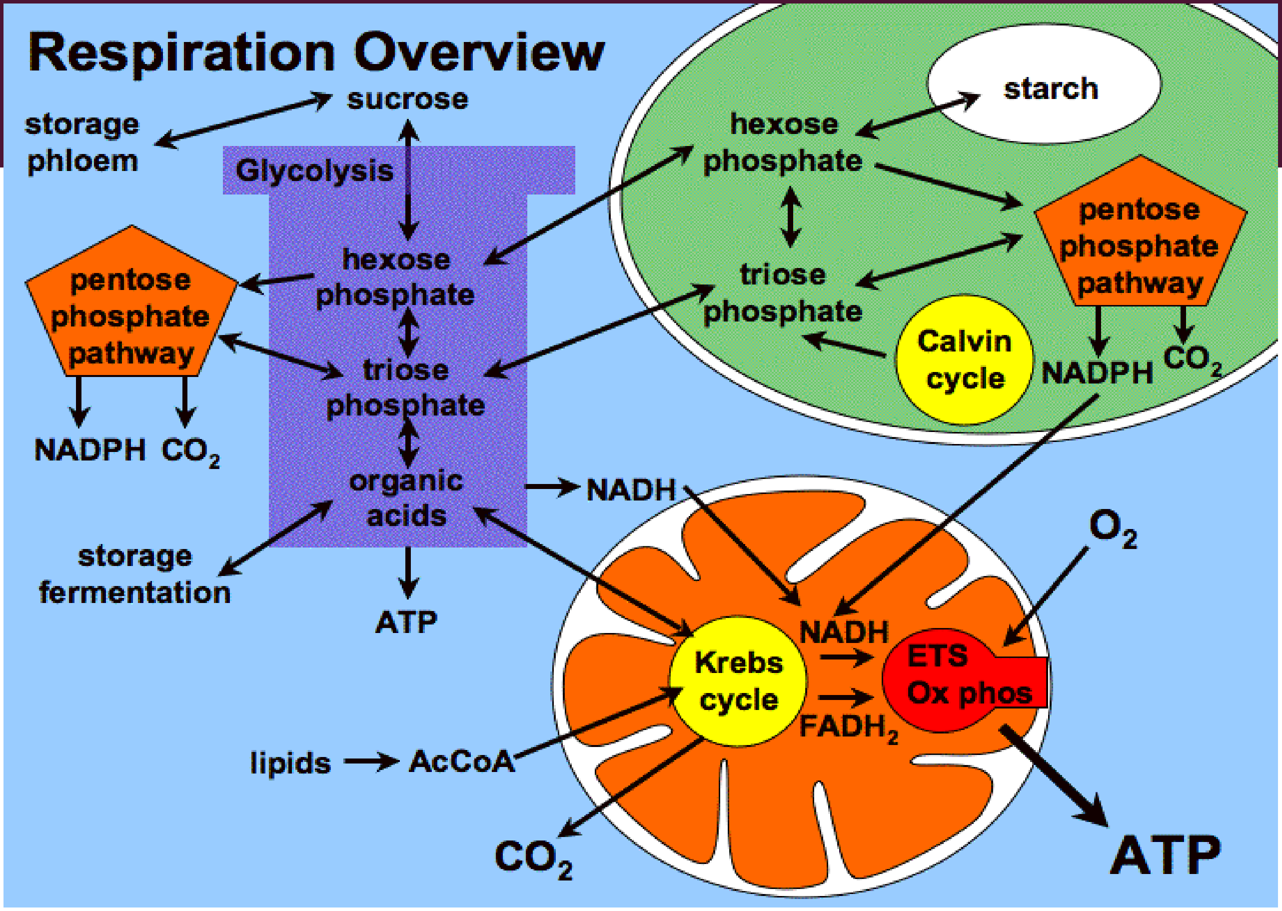
e^- H^+

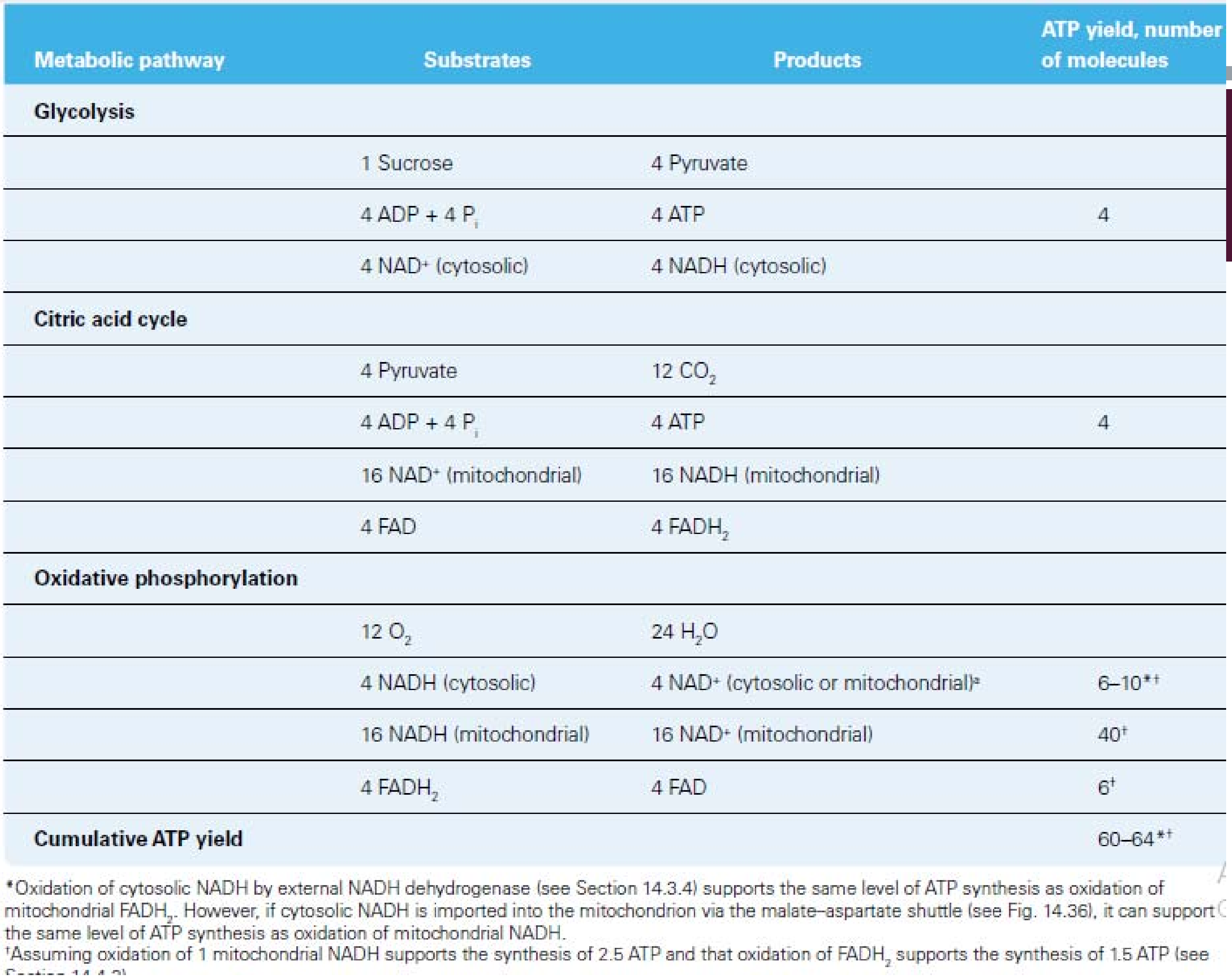
$$4e^- + 4H^+ + O_2 \longrightarrow 2H_2O$$

$$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$$

3 STAGES OF RESPIRATION

- Glycolysis
  - cytoplasm
  - with or without oxygen present
  - breaks glucose (6C) into 2 pyruvates (3C)
- TCA Cycle
  - mitochondrial matrix
  - only if oxygen present
  - converts pyruvate via acetyl CoA into CO_2; generates NADH and $FADH_2$
- Electron Transport Chain
  - mitochondrial membranes = cristae
  - transfers electrons from NADH and $FADH_2$ to reduce O_2 to H_2O and generate ATP


Respiration Overview
storage phloem
sucrose
Glycolysis
hexose phosphate
triose phosphate
organic acids
pentose phosphate pathway
NADPH
CO2
storage fermentation
ATP
NADH
starch
hexose phosphate
triose phosphate
pentose phosphate pathway
Calvin cycle
NADPH
CO2
O2
NADH
Krebs cycle
ETS Ox phos
FADH2
lipids
AcCoA
CO2
ATP



Metabolic pathway	Substrates	Products	ATP yield, number of molecules
Glycolysis			
	1 Sucrose	4 Pyruvate	
	4 ADP + 4 P_i	4 ATP	4
	4 NAD^+ (cytosolic)	4 NADH (cytosolic)	
Citric acid cycle			
	4 Pyruvate	12 CO_2	
	4 ADP + 4 P_i	4 ATP	4
	16 NAD^+ (mitochondrial)	16 NADH (mitochondrial)	
	4 FAD	4 $FADH_2$	
Oxidative phosphorylation			
	12 O_2	24 H_2O	
	4 NADH (cytosolic)	4 NAD^+ (cytosolic or mitochondrial)[a]	6–10[*†]
	16 NADH (mitochondrial)	16 NAD^+ (mitochondrial)	40[†]
	4 $FADH_2$	4 FAD	6[†]
Cumulative ATP yield			60–64[*†]

[*] Oxidation of cytosolic NADH by external NADH dehydrogenase (see Section 14.3.4) supports the same level of ATP synthesis as oxidation of mitochondrial $FADH_2$. However, if cytosolic NADH is imported into the mitochondrion via the malate–aspartate shuttle (see Fig. 14.36), it can support the same level of ATP synthesis as oxidation of mitochondrial NADH.

[†] Assuming oxidation of 1 mitochondrial NADH supports the synthesis of 2.5 ATP and that oxidation of $FADH_2$ supports the synthesis of 1.5 ATP (see

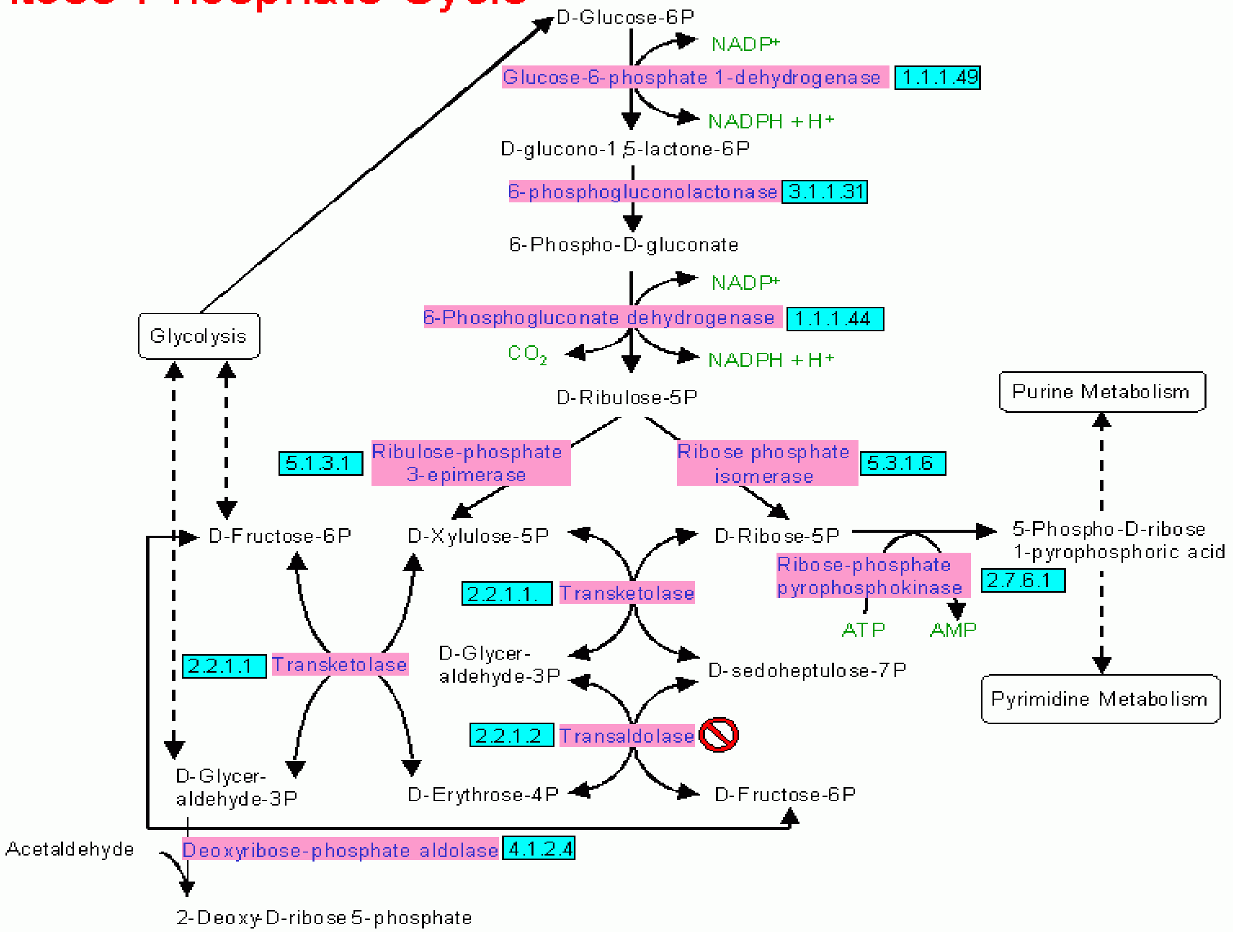
SUMMARY

- Aerobic respiration involves the controlled oxidation of reduced organic compounds to CO_2 and H_2O.
- Much of the free energy released during respiration is conserved in the formation of ATP.
- In glycolysis, the first stage of respiration, carbohydrates are oxidized to organic acids in the cytosol.
- The organic acids produced during glycolysis are completely oxidized to CO_2 in the mitochondrial matrix via the citric acid cycle.
- The electrons released during the operation of the citric acid cycle are transferred through a series of multiprotein complexes located in the inner mitochondrial membrane, ultimately reducing O_2 to H_2O.

SUMMARY

- The free energy released during mitochondrial electron transfer is used to generate a proton electrochemical gradient across the inner membrane.
- The energy available in the proton gradient is subsequently used by another protein complex, ATP synthase, to synthesize ATP from ADP and Pi.
- Mitochondrial respiration is regulated by the availability of ADP and Pi and by the presence of additional electron transfer complexes that allow respiration to proceed without forming a proton gradient.
- Plant mitochondria participate in several metabolic processes besides respiration, including providing reducing equivalents to other cellular compartments and carbon skeletons for amino acid biosynthesis. Plant mitochondria also participate in the biosynthesis of sugars from lipids in some germinating seeds

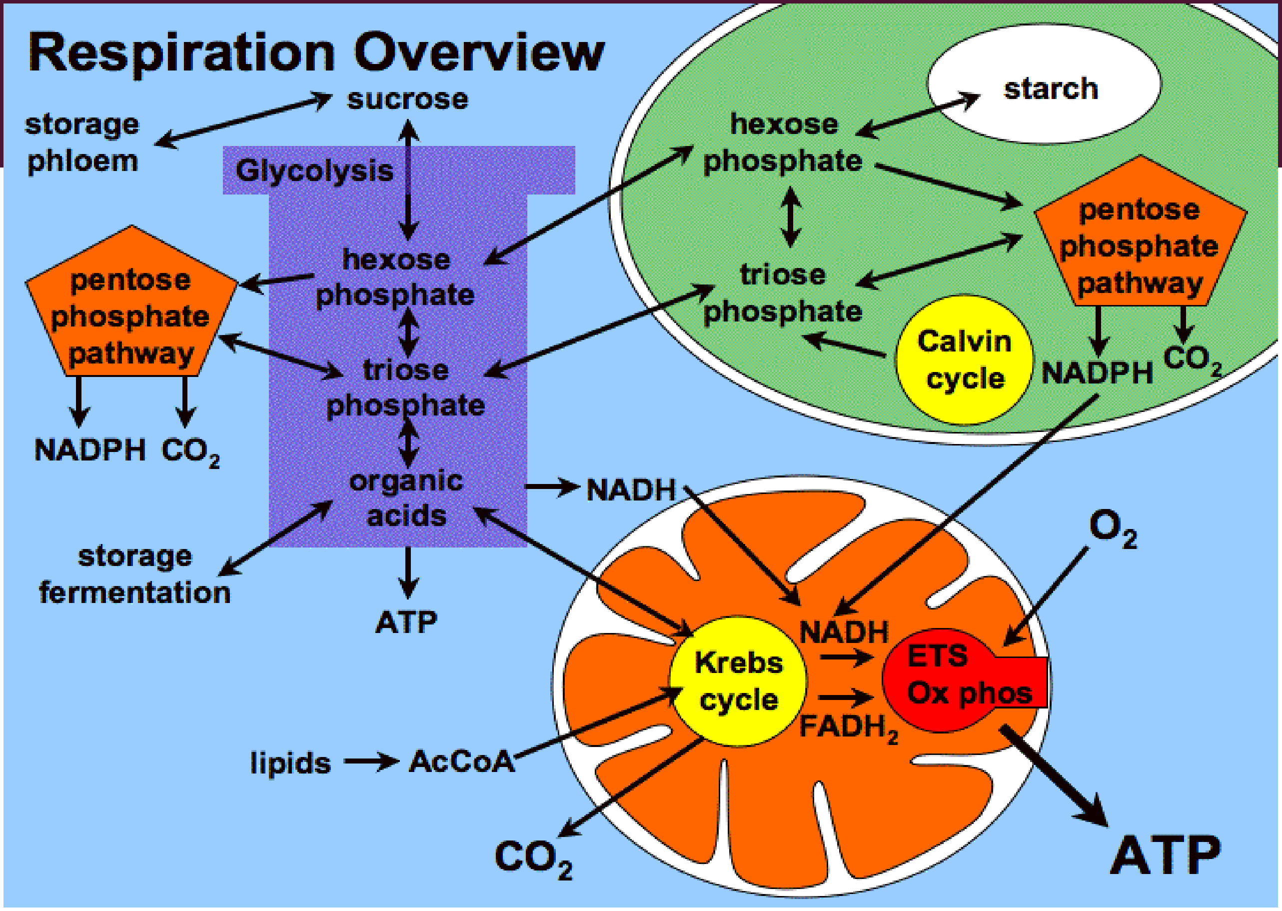
Pentose Phosphate Cycle



Sugar ⟶ CO2

Does not produce ATP in ETS

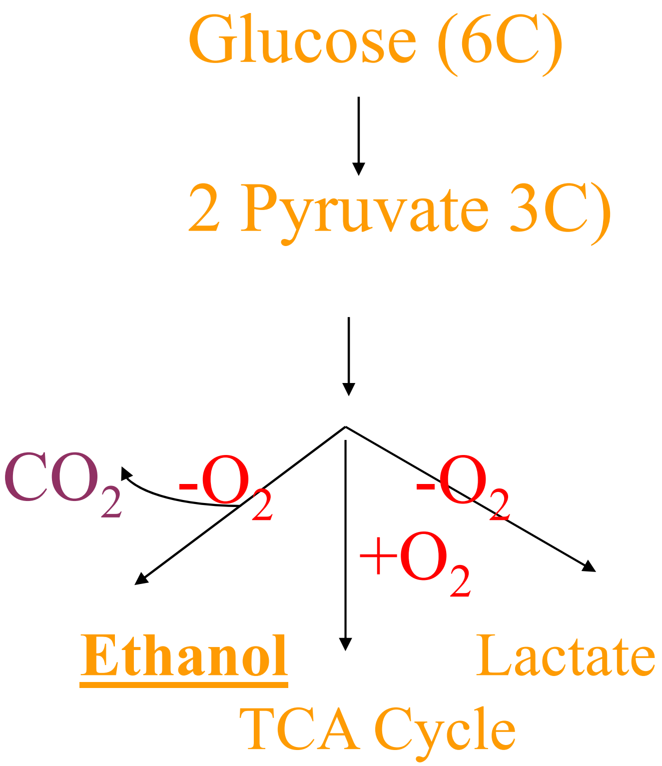
Produce ribose-5-phosphate (nucleic acids), NADP (light reaction, fatty acid production), 3, 4, 5, 6, 7 carbon skeleton for biosynthesis reactions


Respiration Overview
storage phloem
sucrose
Glycolysis
hexose phosphate
pentose phosphate pathway
triose phosphate
NADPH
CO2
organic acids
NADH
storage fermentation
ATP
starch
hexose phosphate
pentose phosphate pathway
triose phosphate
Calvin cycle
NADPH
CO2
O2
NADH
Krebs cycle
ETS Ox phos
FADH2
lipids
AcCoA
CO2
ATP

FERMENTATION

- The process by which a glucose is broken down in the absence of oxygen.
  - Fermentation allows a cell to continue the supply of ATP, in anerobic (without oxygen) conditions.
  - Lactic Acid Fermentation: Pyruvates can be converted into lactic acid, NAD+ is recycled by lactic acid when glucose runs out.

GLYCOLYSIS



Glucose (6C)

2 Pyruvate 3C)

CO_2 $-O_2$

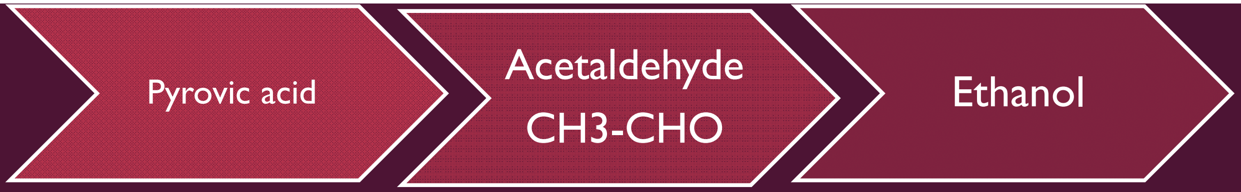
$-O_2$

$+O_2$

Ethanol

Lactate

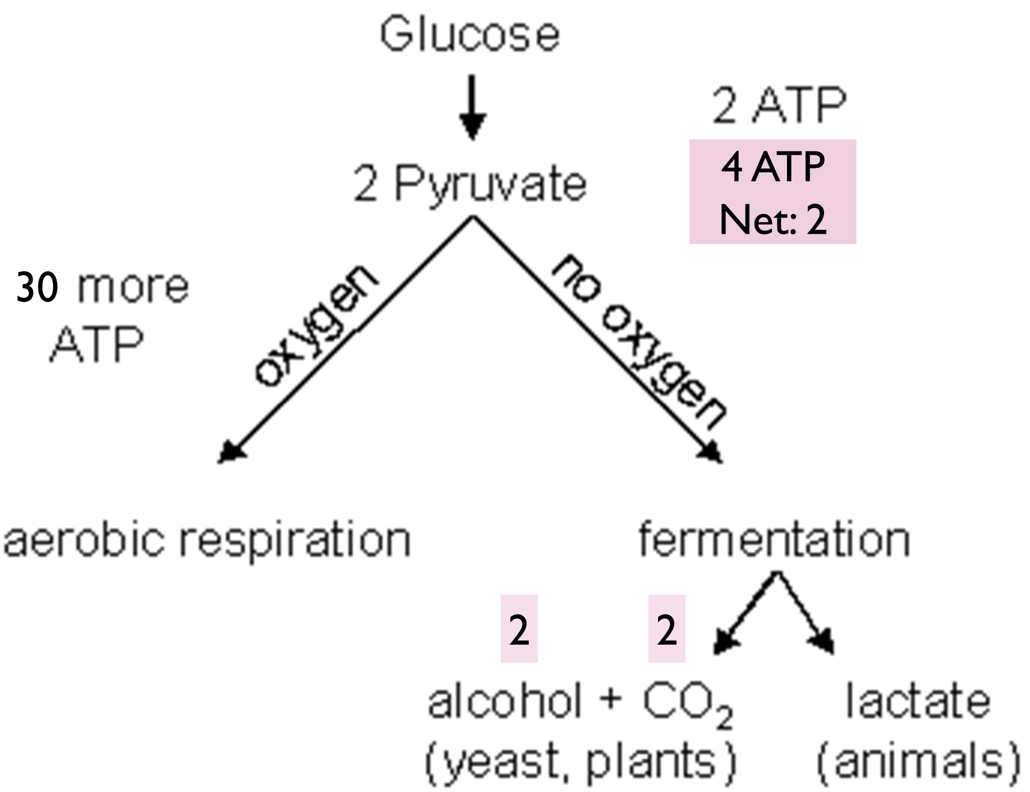
TCA Cycle



Pyrovic acid

Acetaldehyde
CH3-CHO

Ethanol

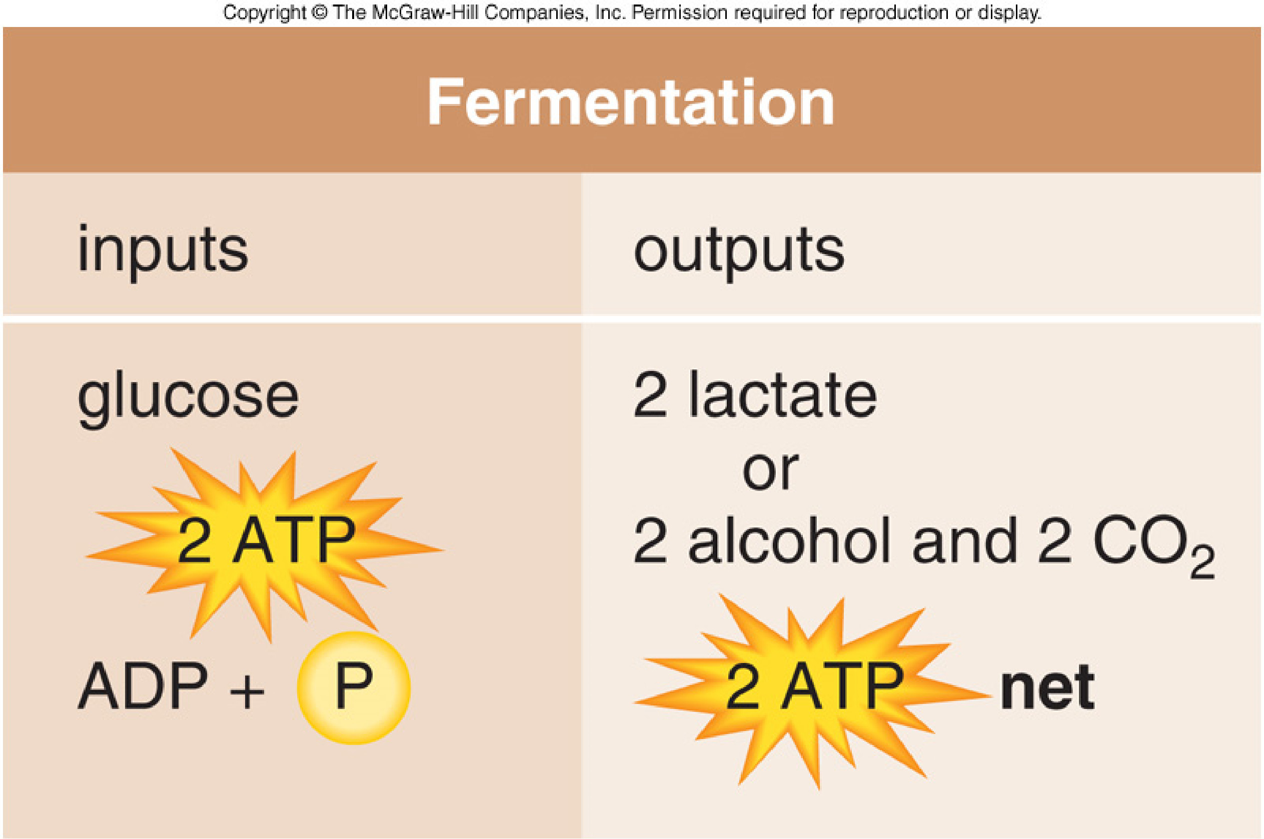


4 ATP
Net: 2

30

2

2



Fermentation	
inputs	outputs
glucose 2 ATP ADP + P	2 lactate or 2 alcohol and 2 CO_2 2 ATP **net**

Comparison of Aerobic and Anaerobic Respiration

- Similarity
  - Sugar is broken down to release energy
  - ATP is made
  - Both are controlled by enzymes

ॐ Differences

	aerobic respiration	anaerobic respiration
oxygen requirement	essential	nil
oxidation of sugar	complete oxidation	incomplete oxidation
energy released	large amount	small amount

	aerobic respiration	anaerobic respiration
end products	inorganic: CO_2 and H_2O	organic: ethanol or lactic acid
occurrence	in most living cells	in lower organisms (e.g. bacteria and yeast) and vertebrate muscles

ALTERNATE FATES OF GLUCOSE C

- Not all C respired to CO_2
- Intermediates of respiration branch off:
  - amino acids
  - pentoses for cell wall structure
  - nucleotides
  - porphyrin biosynthesis
  - fatty acid synthesis
  - lignin precursors
  - precursors for carotenoid synthesis, hormones

USES OF ENERGY

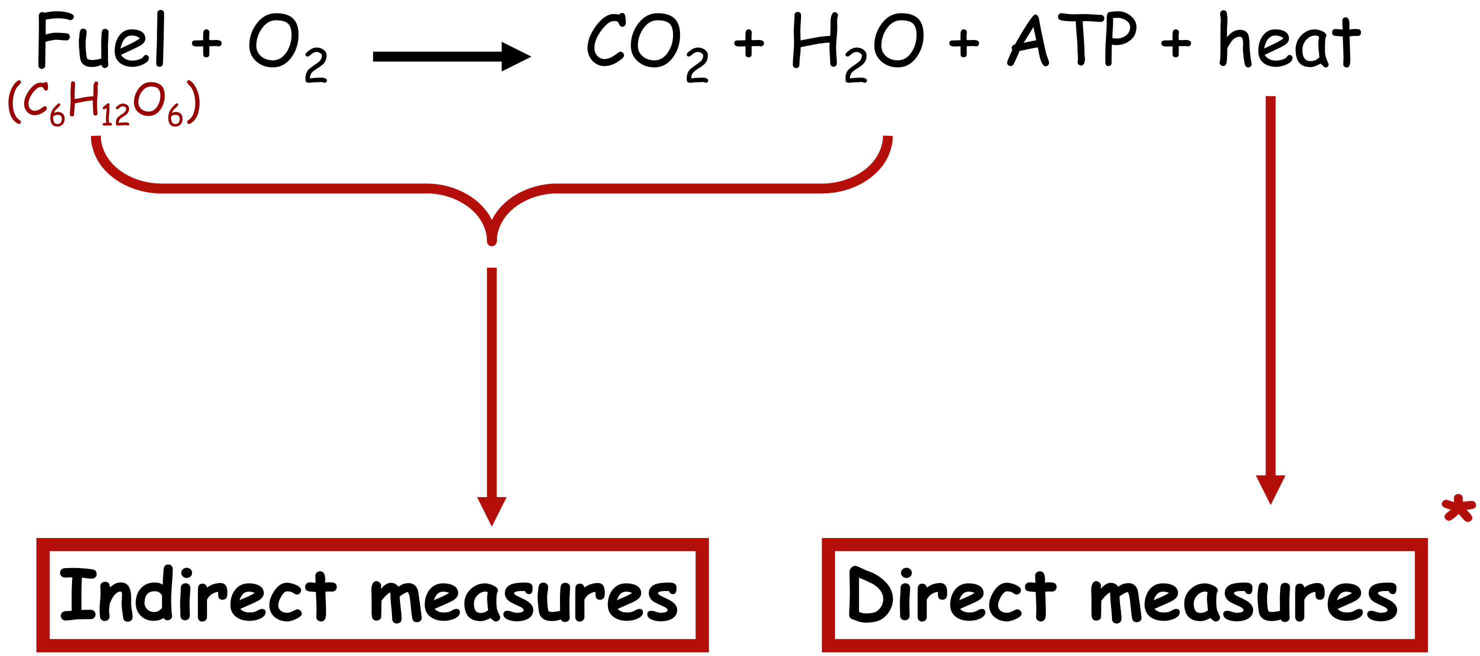
- Biosynthesis
  - Replacing body structures
  - Growth
  - Reproduction
  - Storage (Fat, Glycogen)
  - Exported Materials
- Maintenance (homeostasis)
- External Work (e.g, movement)

WHAT AFFECTS METABOLIC RATE?

- Chemical Activity
- Environmental Temperature
- Digestive Processing (Specific Dynamic Action)
- Age
- Gender
- Circadian Rhythms
- Aquatic Salinity (Osmoregulation)

How can we measure MR?

Aerobic metabolism:



$$\text{Fuel} + O_2 \longrightarrow CO_2 + H_2O + ATP + \text{heat}$$

$(C_6H_{12}O_6)$

Indirect measures

Direct measures *

- Amount of CO_2 formed does not always equal amount of O_2 consumed
- *Respiratory Quotient* (RQ)
  - Amt. CO_2 produced/O_2 consumed
  - Varies for different energy sources

BUT, heat production varies with foodstuff being oxidized…

HEAT PRODUCTION (KJ)

	Per gram of food	Per liter of O_2 consumed	Per liter of CO_2 produced	RQ
carbohydrates	17.1	21.1	21.1	1.0
lipids	38.9	27.9	19.8	0.7
Proteins	17.6	23.3	18.7	0.8

What if you aren’t sure what animal ate?

$$\text{“Respiratory Quotient”} = \frac{\text{Rate of } CO_2 \text{ production}}{\text{Rate of } O_2 \text{ production}}$$

Differences between Respiration and Photosynthesis

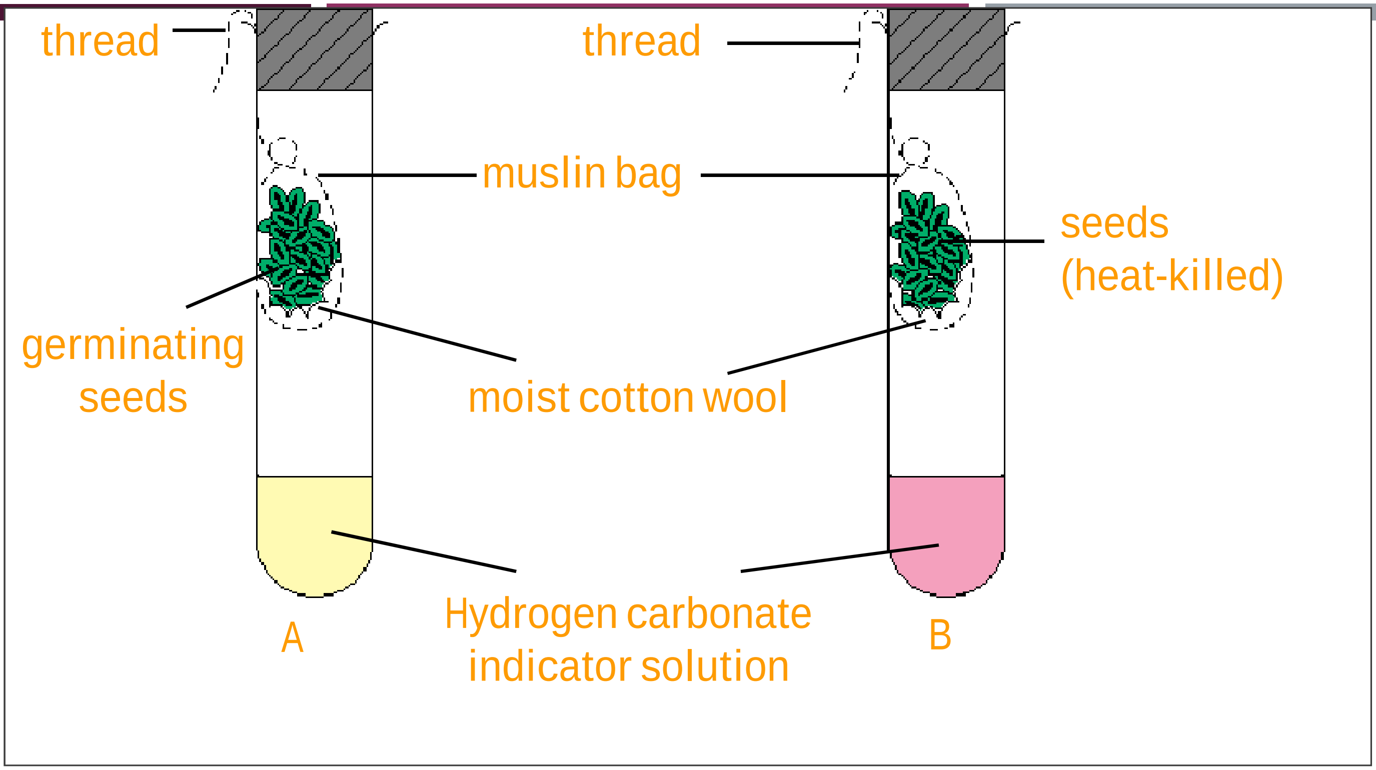
aerobic respiration	photosynthesis
produces carbon dioxide and water	requires carbon dioxide and water
energy is released	energy (light) is absorbed
an oxidative process	a reductive process

aerobic respiration	photosynthesis
a breaking down process	a synthetic process
occurs in all living cells at all times	occurs in green plants only when light is available
occurs in mitochondria	occurs in chloroplasts

~ END ~

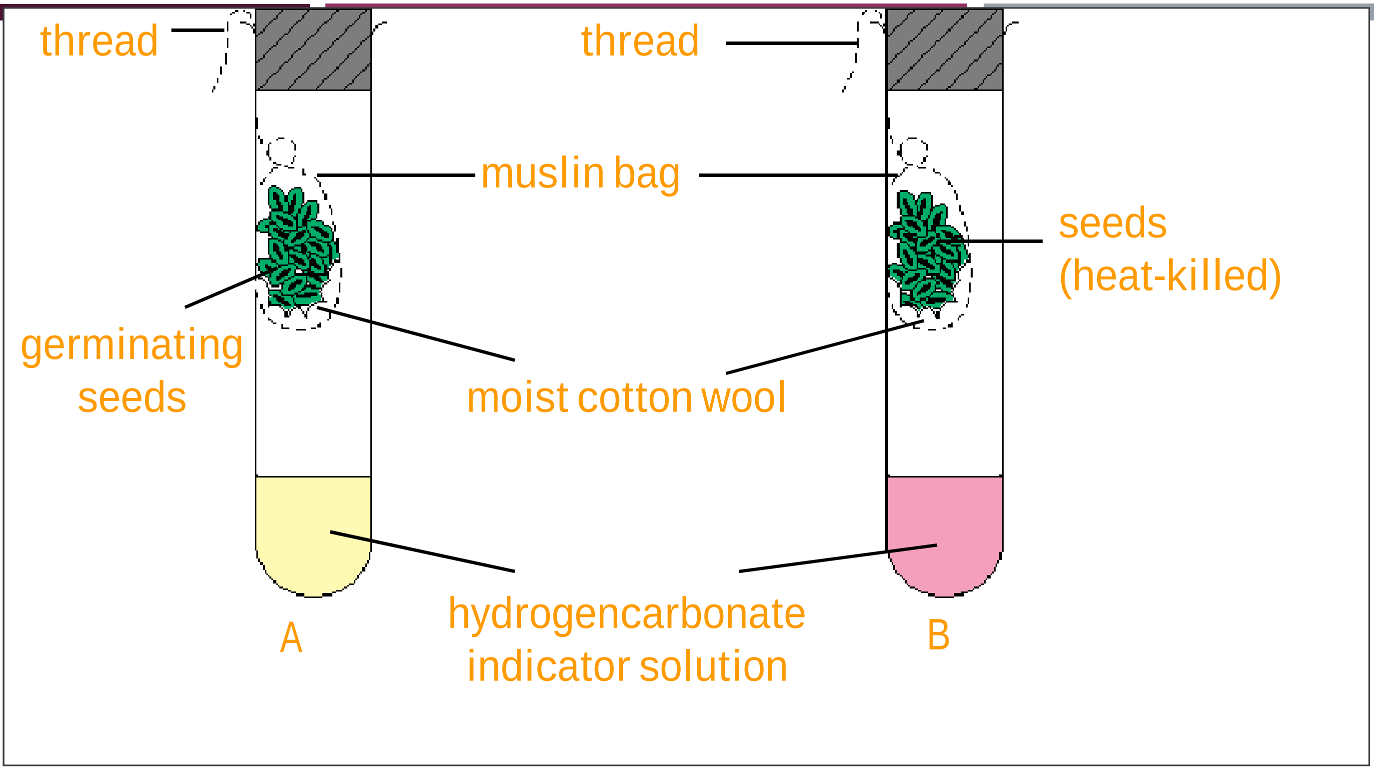
Investigation 1

To Show the Release of Carbon Dioxide by Germinating Seeds



What is the function of the moist cotton wool inside the muslin bag ?

Ans: It prevents the seeds from drying out.

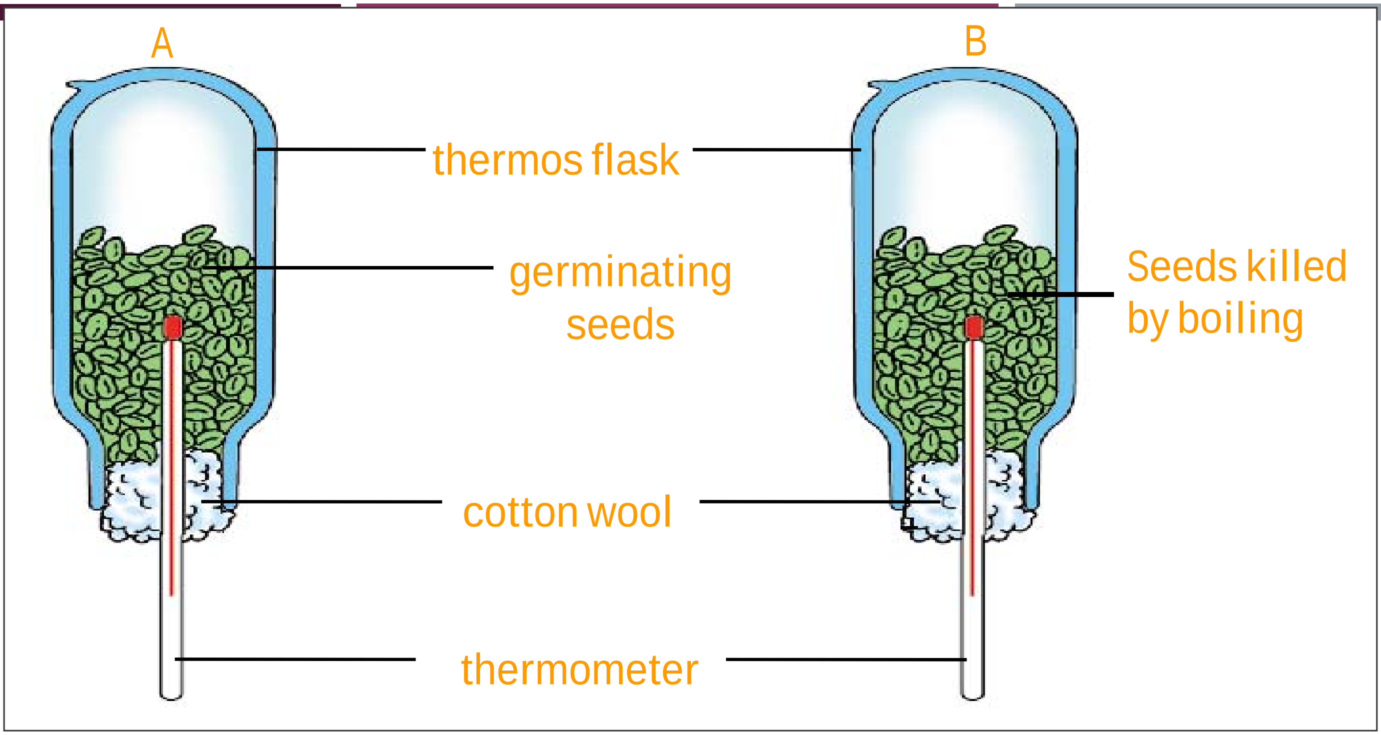


What has happened to the hydrogen carbonate indicator solution in the two tubes ?

Ans: Indicator solution in tube A turns yellow while the indicator solution in tube B remains unchanged.

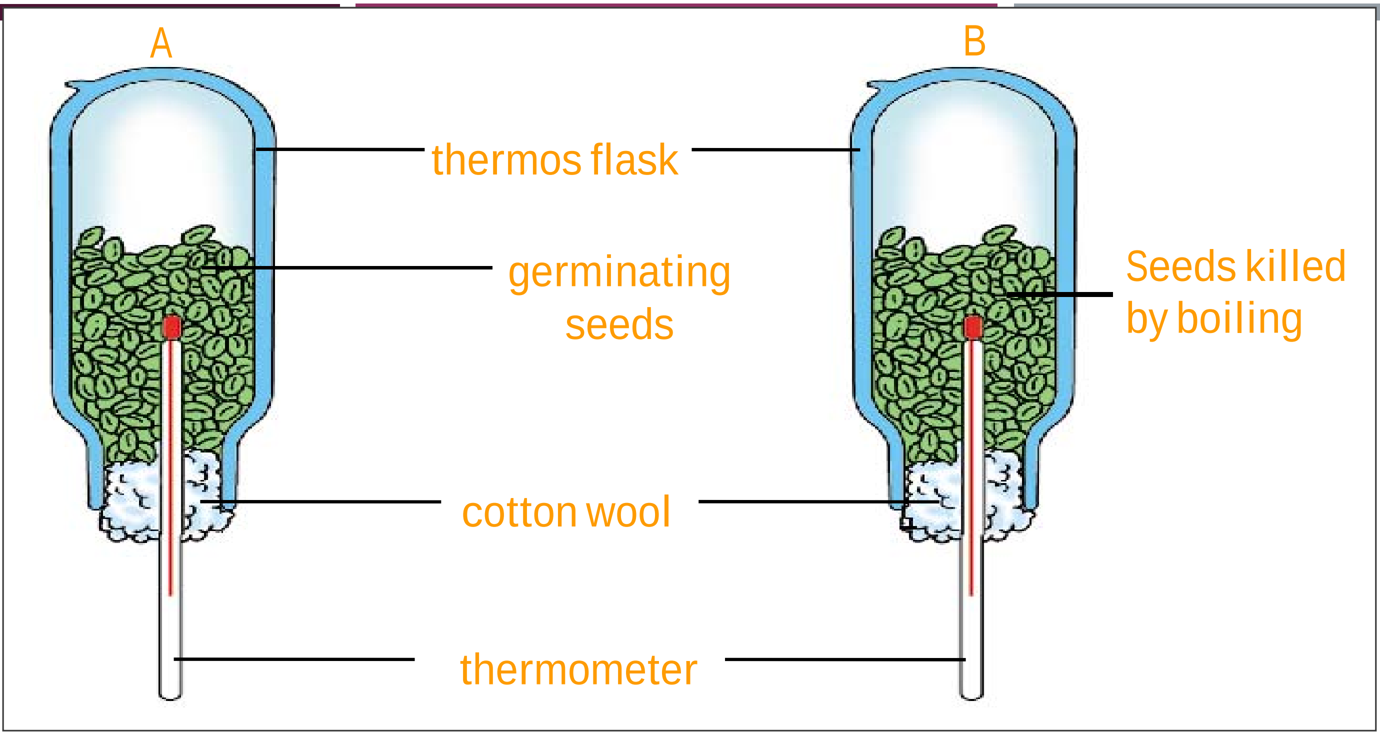
Investigation 2

To Demonstrate Heat Production by Germinating Seeds using Thermos Flasks



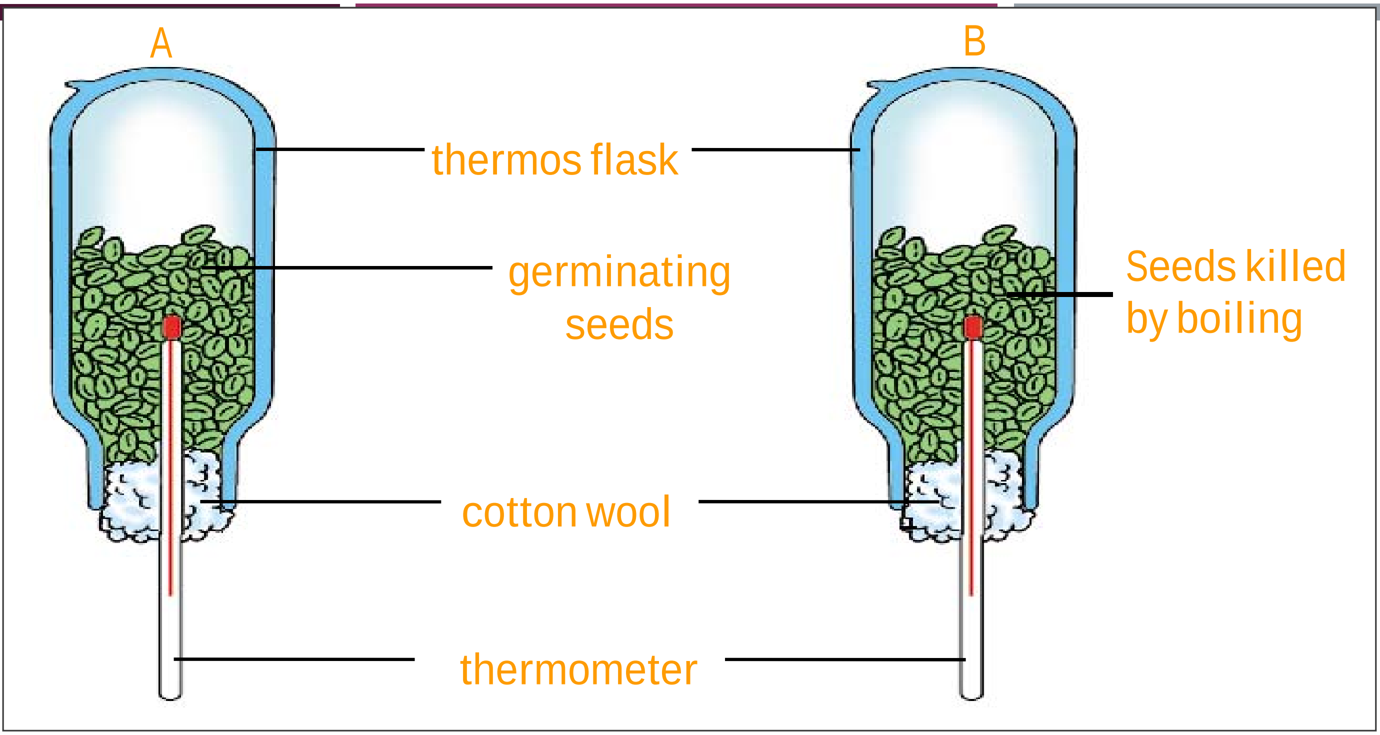
Which thermos flask shows a higher temperature at the end of the experiment ?

Ans: Flask A showed a higher temperature at the end of the experiment.



It is advisable not to fill the flasks completely with seeds. Why ?

Ans: To provide air for the seeds to respire.



Why are the flasks supported in the upside-down position ?

Ans: The flasks are supported in the upside-down position because

- carbon dioxide, produced during respiration of the living seeds, is a heavier gas and may escape through the cotton wool.
- hot air rises up, and inverting it will ensure that heat will not escape through the cotton wool.
- it is easier to read the thermometer as the thermos flasks are not transparent.

SUMMARY

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