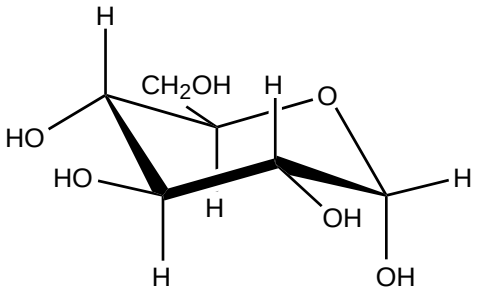
BIOCHEMISTRY AND MOLECULAR BIOLOGY

Problem Unit Four 1999/2000



Carbohydrate Metabolism

Module 1: Glycolysis, Glycogenesis, Glycogenolysis and Gluconeogenesis

Module 2: The Mitochondria, the Tricarboxylic Cycle, the Respiratory Chain, and Oxidative Phosphorylation

Module 3: Other Pathways of Carbohydrate Metabolism and Abnormalities

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Learning Resources:

ESTIMATED WORK TIME: 32 hours.

A. This study guide is provided in two forms: printed and electronic. ***It is best viewed in electronic form as a pdf file which can be read on your computer using Adobe Acrobat Reader.*** See **Appendix I** for an introduction on how to view a pdf file. The pdf file can be downloaded from the biochemistry server (**http://www.siu.edu/departments/biochem**) and Acrobat Reader can be downloaded free from Adobe's web page (**http://www.adobe.com/acrobat**). They should also be installed on the student computers. There are a number of advantages to using the electronic version including color, a hypertext index, and hypertext links within the text. Hypertext links in the text body are in blue underlined characters (**such as this)**. Clicking on these will lead to a jump to the linked material for further details. The destination material is indicated by red underlined characters (**such as this)**.

This and other study guides are provided to help you focus on the topics that are important in the biochemistry curriculum. These are designed to guide your studying and provide information that may not be readily available in other resources. They are not designed to replace textbooks, and are not intended to be complete. They are guides for starting your reading and reviewing the material at a later date.

B. Textbooks:

1. Devlin, Textbook of Biochemistry with Clinical Correlations, 4th ed. ('97), Wiley-Liss. Core text for Biochemistry & Molecular Biology.

2. Champ & Harvey, Lippincotts Illustrated Reviews: Biochemistry, 2nd ed. (‘94), Lippincott. Efficient presentation of basic principles.

3. Murray et al., Harper's Biochemistry, (24th ed.) ('96), Appleton & Lange. A review text for examinations.

4. Marks, Marks, and Smith, Basic Medical Biochemistry: A Clinical Approach, (‘96), Williams & Wilkins. Good basic presentation with clinical relevance.

5. Cohn and Roth, Biochemistry and Disease, ('96), Williams & Wilkins. A good bridge between the basic sciences and clinical medicine.

6. Garrett and Grishham, Biochemistry, 2nd ed., ('99), Saunders College Publishing.

7. Salway, Metabolism at a Glance, 2nd ed., ('99), Blackwell Science. An excellent presentation of metabolic pathways.

Most texts of biochemistry have sections on metabolism. It is a mature subject in biochemistry, and consequently, the content of the subject is much the same from text to text; the differences are basically in style and rigor. The Study Guide in this Problem Unit will set the level of rigor expected of you. Read the sections on metabolism in Devlin and Murray. What differences there will be between these texts and the Study Guide will be helpful to you in gaining perspective on the subject.

Additional material can be found on the web at the National Institutes of Health (**http://www.nih.gov**), the National Library of Medicine (**http://www.nlm.nih.gov**), and the free MEDLINE PubMED Search system at the National Library of Medicine (**http://www3.ncbi.nlm.nih.gov/PubMed/**).

C. Lecture/Discussions

The lectures will provide an overview of the material and concentrate on key points.

D. **Practice Exam Questions** are contained at the end of this Problem Unit along with **answers**. An **on-line quiz** (containing the Practice Exam Questions) is also available from the PU4 web page.

Evaluation Criteria:

A pacing quiz will be given during Neuro Block 1. A written examination and one recycle covering the objectives will be scheduled during CVR Block 2. Answers to questions and the solving of problems will be judged against the learning resources of which the Study Guide, the biochemistry text by Devlin, other texts and lectures are the primary resources. You will need to receive 70% or better on the final examination in order to pass PU04.

Module I: Glycolysis, Gluconeogenesis, Glycogenolysis and Glycogenesis,

Introduction to Metabolism:

A living organism is a highly complex and unstable system. Its existence is precarious because of its complexity. **Metabolism** is the sum total of all biochemical reactions designed to maintain and replicate the structure of the organism and to counteract a continuous drive towards an increase in the disorder, or entropy, of the system.

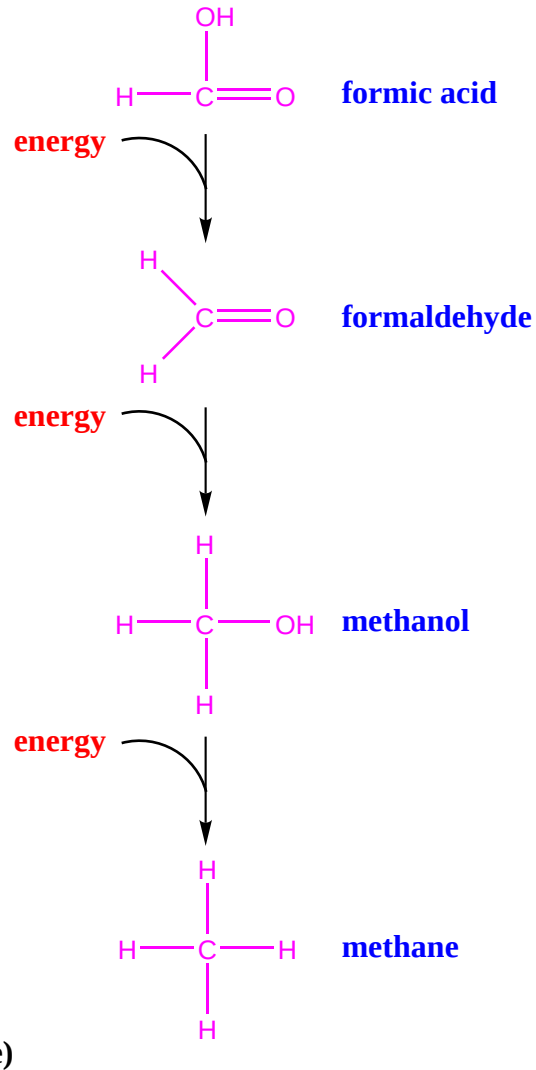
To build complex structures requires energy, i.e., a system needs energy for synthesis and maintenance. Human metabolism derives energy from degradation of organized structures (food) from plants, and, therefore, indirectly from the sun. In more affluent societies of the world, a significant amount of energy is obtained from animals which themselves have survived by utilizing plants and other animals. For the most part, **food** represents complex organic molecules which require energy to be synthesized and therefore can yield energy by being destroyed. (Food also contains some constituents which are not utilized for energy production, but have important roles in maintaining life, e.g., minerals and vitamins.)

Some General Principles:

Organic molecules are composed primarily of carbon, oxygen, nitrogen, and hydrogen. The most stable form of carbon in our atmosphere is CO_2. The energy of CO_2 may be increased by reduction to yield organic molecules.

Organic **reduction** of a molecule results in an increase in energy resulting in the addition of hydrogen atoms (a proton and an electron) and the removal of oxygen. Organic **oxidation** is the reverse, i.e., the removal of hydrogen with the evolution of energy. Thus, complex organic molecules may be degraded by oxidation to liberate energy. We oxidize (burn) fossil fuels to obtain energy to run engines and heat our society. Similarly, a living organism oxidizes organic compounds in a highly controlled manner to capture the energy to allow maintenance, synthesis, movement, and replication. Some heat is produced intentionally to maintain body temperature in warm-blooded animals.

(Most stable)



(Least stable)

The tendency for a compound to undergo a reaction (e.g., to be degraded) is indicated by the **free energy change** (ΔG) of the reaction. A decrease in free energy means that the free energy of the products is less than the free energy of the reactants so that

$$\Delta G = G \text{ (products)} - G \text{ (reactants)} \leq 0.$$

A reaction will proceed if $\Delta G < 0$. Such a reaction is exergonic. The energy could be captured for utilization if the systems are available for trapping it. The energy that is not used will be lost as **heat**.

Even though the ΔG for a reaction may be quite large, the reaction may still not proceed quickly. The sign for ΔG only indicates whether or not a reaction will occur, not how fast it will occur.

Biochemical reactions occur with the aid of **enzymes** which are examples of **catalysts**. Catalysts are substances which speed up chemical reactions without themselves being altered. Most enzymes are proteins. Enzymes are important in biological systems not only because they accelerate reactions, but also because they can be regulated, i.e., they can be turned on or off, thus allowing for the reaction rate to be controlled.

Enzymes also control the type of reaction which is occurring so that one reaction may not be allowed to occur without another simultaneously occurring, i.e., the two are tightly **coupled**. This aspect of enzyme catalysis is often overlooked It is this characteristic of enzymes which allows for the capture of energy resulting from the degradation of organic foods and the prevention of its being lost as heat. Specific examples will be presented many times below so that it will not be necessary to give details here. Try to remember that enzymes are more than mere catalysts. In many instances they direct the flow of energy to/from specific molecules by defining **metabolic pathways**. It is this property of enzymes which prevent chaos and which is responsible for the order in biological systems. In the normal physiological state, enzymes permit the unstable organic molecules we ingest as food to be degraded only if the energy is simultaneously captured. The constant ingestion of food and its degradation results in a maintenance of order (life). The "driving force" for life is therefore the dissipation of the energy in the environment. Hence the maintenance of life requires the constant loss of energy by the universe, consistent with the laws of thermodynamics.

Objectives:

1. Be able to classify metabolic intermediates in the metabolism of carbohydrates as alcohols, aldehydes, inorganic and organic esters, acids, ketones, phosphates and nucleotides.

2. Discuss the role of enzymes in living systems as catalysts, regulatory gates, and coupling devices.

3. Distinguish between spontaneity and rate of a reaction. Which is controlled by ΔG?

4. Summarize glycolysis by giving a diagram showing the metabolic intermediates and the coenzymes in the pathway. Given such a diagram, identify the missing components. What are the products of anaerobic and aerobic glycolysis?

5. Given the name of any enzyme of the glycolytic pathway, write the complete reaction catalyzed by that enzyme including the coenzymes required, their reaction (if any), and the structures of

the substrate(s) and product(s).

6. List the three thermodynamically irreversible steps in glycolysis. How are these steps bypassed in gluconeogenesis? Identify the metabolic and hormonal regulators that control the activity of these enzymes. Are these reactions truly irreversible?

7. Give the major regulatory step in glycolysis and describe how the activity of this enzyme is controlled. Explain how hormones such as glucagon, epinephrine and insulin regulate the glycolytic pathway.

8. List the reactions that generate NADH, and list the reactions that regenerate NAD^+ during both anaerobic and aerobic glycolysis. Show how and explain why the reactions that generate NADH and those that use NADH are coupled.

9. Answer questions concerning the general structure of glycogen, including the types of glycosidic linkages and the number of reducing sugar residues present per glycogen molecule. Explain the biological role of glycogen, and tell why it is "fit" for this role.

10. Glycogen phosphorylase and a debranching enzyme (4-α-D-glucanotransferase/amylo-α-1,6-glucosidase) are necessary for the complete degradation of glycogen. Identify products of the reactions catalyzed by each of these enzymes and show how they lead to the complete degradation of glycogen.

11. Diagram the pathway and name the enzymes that are required for glycogenesis from glucose-6-phosphate. When given such a diagram, identify missing components.

12. Two of the enzymes that are required for the synthesis of glycogen are glycogen synthase and a branching enzyme. Name and identify the substrates and products of glycogen synthase. Explain why branching is important to the biological function of glycogen.

13. Explain why there are separate pathways for the synthesis and degradation of glycogen.

14. Give the substrates and products of the reaction catalyzed by UDP-glucose pyrophosphorylase. Describe the significance of the formation of pyrophosphate and the metabolic fate of pyrophosphate in the cell.

15. The degradation and synthesis of glycogen is under hormonal control. Describe the cascade of reactions in liver and muscle that are initiated by epinephrine and glucagon and lead to the activation of phosphorylase and the inhibition of glycogen synthase. Include the following details: a) the binding sites for epinephrine and glucagon, b) the effect of epinephrine and glucagon on adenylate cyclase, c) the substrates and products of adenylate cyclase, d) the effect of increased intracellular levels of cAMP on protein kinase A, e) the effect of protein kinase A on phosphorylase kinase and glycogen synthase, f) the activation of glycogen phosphorylase, g) the role of phosphoprotein phosphatase, and phosphodiesterase, h) the role of inhibitor protein. Show how the response of heart tissue differs from that of liver during stimulation by epinephrine. Describe the action of insulin on this cascade.

16. Describe the significance of the cascade and predict the effect of an absence of any one of these enzymes on the levels of glycogen and cAMP in muscle tissue.

17. List the factors that regulate intracellular cAMP concentration. Describe cAMP synthesis and degradation. Write the structure of cAMP.

18. Summarize gluconeogenesis by giving a diagram showing the metabolic intermediates and the coenzymes in the pathway. Given such a diagram, identify the missing components.

19. Given the name of any enzyme of the gluconeogenic pathway, write the complete reaction catalyzed by that enzyme including the coenzymes required, their reaction (if any), and the structures of the substrate(s) and product(s).

20. Use the above objectives and previous objectives to solve new problems.

21. Define and use correctly the terms in the **Nomenclature and Vocabulary** list.

Nomenclature and Vocabulary:

2-phosphoglycerate
3-phosphoglycerate
1,3-bisphosphoglycerate
2,3-bisphosphoglycerate
α-1,6 glucosidase
adenylate cyclase
adenosine cyclic monophosphate
aerobic glycolysis
aldose
aldolase
AMP
amylase

amylose
anabolism
anaerobic glycolysis
anomer
ATP
branching enzyme
calmodulin
cAMP
carbohydrates
catabolism
catalyst
cellulose
citric acid cycle
debranching enzyme
dihydroxyacetone phosphate
dinucleotide
disaccharide
enolase
enzymes
exergonic
fructose
fructose-1,6-bisphosphate
fructose-2,6-bisphosphate
fructose-6-phosphate
fructose bisphosphatase
glucokinase
glucose
gluconeogenesis
glucose-1-phosphate
glucose-6-phosphate
glucose-6-phosphatase
glyceraldehyde-3-phosphate dehydrogenase
glyceraldehyde-3-phosphate
glycogen synthase
glycogen
glycogenin
glycogenesis
glycogenolysis
glycogenosis
glycolysis
hexokinase
hypoglycemia
inhibitor protein (IP)
ketose
Kreb's cycle
lactate
lactic acid
lactate dehydrogenase
metabolic pathway
metabolism
monosaccharide
nicotinamide adenine dinucleotide (NAD)
oxaloacetic acid
oxidation
pentose
PEP
phosphodiesterase
phosphoenol pyruvate
phosphoenol pyruvate carboxykinase
phosphofructokinase II
phosphofructokinase I
phosphoglucomutase
phosphoglycerate kinase
phosphoglyceromutase
phosphohexoisomerase
phosphorylase a
phosphorylase b
phosphorylase kinase
phosphorylase
Pi

protein kinase A
pyruvate kinase
pyruvate carboxylase
reducing sugar
saccharide
starch
TCA cycle
UDP-glc pyrophosphorylase
UDPG
pyrophosphate
pyruvate
reducing equivalents
reduction
second messenger
sucrose
triosephosphate isomerase
UDP-glc
ΔG

STUDY GUIDE - I

What is metabolism?

Metabolism is the total of anabolism and catabolism. **Anabolism** refers to the synthetic activities of a cell; it uses energy and forms more complex molecules from simpler ones. **Catabolism** refers to the degradation reactions in a cell; it produces energy which is most commonly stored as ATP. Anabolism requires catabolism because of its need for energy.

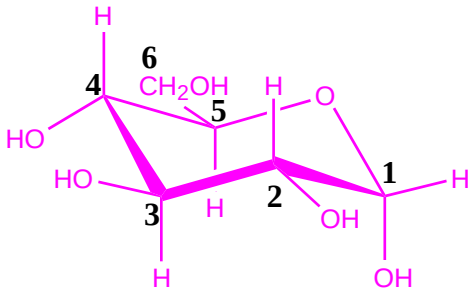
Life is characterized by growth, movement, and reproduction. Each of these properties depends on controlled chemical reactions for the biosynthesis of organic compounds (anabolism) and the production of usable energy (catabolism). Control is attained by enzyme catalysis of the reactions of metabolic pathways.

Each of the activities of a cell requires energy. The cell constantly uses energy for the biosynthesis of chemical substances, mechanical work (e.g. muscle contraction), maintenance of cell turgor (osmotic pressure), etc. Chemical energy is also transferred into the electrical energy of nerve impulses.

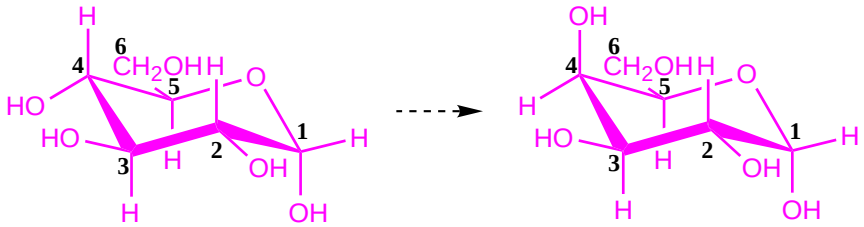
A living cell is inherently an unstable organization. It maintains the complexity and orderliness of its fragile structure only by constantly rebuilding itself. Most cell components are in a dynamic state, i.e., are constantly broken down and replaced.

Basic Sugar Chemistry

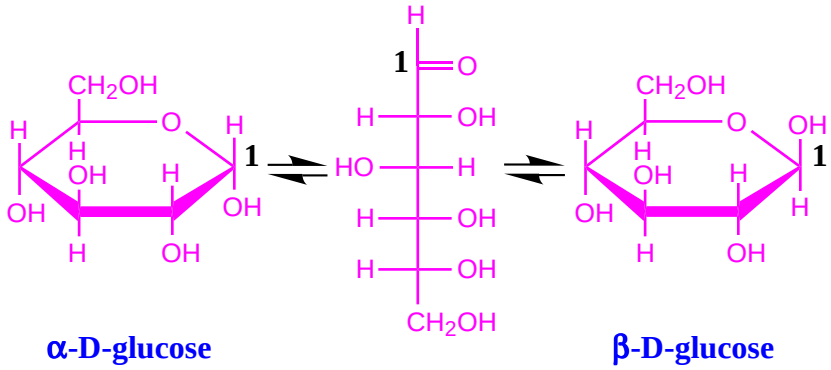
Carbohydrates are **saccharides** or sugars, either single monomeric units (**monosacchardes**) such as glucose or fructose, or polymeric chains (**disaccharides** or polysaccharides) of sugar units such as glycogen or amylopectin. Most of the monosaccharides involved in human biochemistry are either six carbon (**hexoses**) or five carbon (**pentoses**) sugars. The most important sugar in carbohydrate metabolism is glucose. **Glucose** is shown here in its six membered ring form with each carbon atom numbered.



The configuration of the hydroxyls on carbons 2, 3, and 4 are important and define this molecule as glucose. Changing one of these creates a different sugar. For example, moving the equatorial hydroxyl on carbon 4 to the axial position creates galactose.

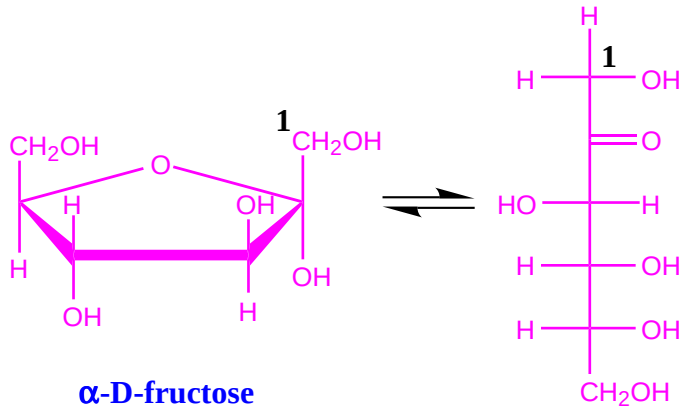


In aqueous solution the glucose ring is constantly opening and closing as shown below. When the ring closes, the configuration of the hydroxyl on the C1 carbon (the so-called **anomeric carbon**) can be in either of two positions. The two forms are different isomers of glucose and are referred to as α and β **anomers**.



The open chain form (shown above in the Fisher projection style) is always present in solution to a small extent for all of the monosaccharides. In the case of glucose the C1 carbon is an aldehyde carbon, thus glucose is referred to as an **aldose**. It is a **reducing sugar** since the aldehyde can reduce other species and simultaneously be oxidized to an acid.

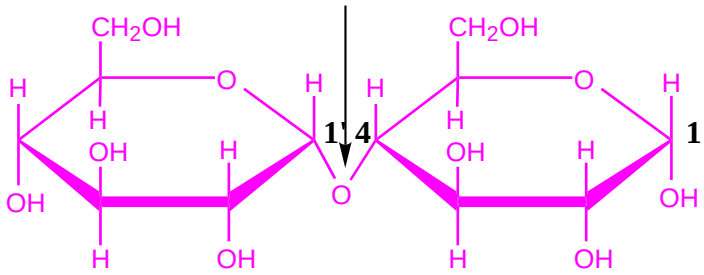
Fructose is another hexose. In this case, the anomeric carbon is not C1 but C2. When the ring opens in solution (as shown below) the sugar is a ketone rather than an aldehyde, thus fructose is referred to as a **ketose**. Closing of the ring can lead to either α or β forms (only the α is shown here).



α-D-fructose

Sugar units can be linked together in various ways to make polymers or polysaccharides. An example of a disaccharide is **maltose**, shown here.

α-(1→4) linkage

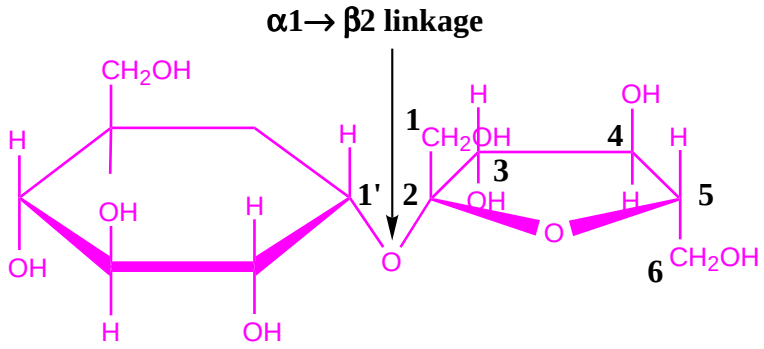


α-maltose

Note that the linkage of one unit to the other is via the anomeric carbon of one to the C4 carbon of the other. The configuration of the C1' carbon is α, thus this is an α-(1→4) linkage. Linkage of numerous glucose units in this way creates **amylose**. Branching can be accomplished by linking to the C6 carbon, e.g. α-(1→6) linkages which are essential in **starch** and **glycogen**. Note that maltose and other polysaccharides similar to it have a free anomeric carbon, thus these are still reducing sugars.

Cellulose is formed from β-(1→4) linkages between glucose units. The human digestive system cannot break the β-(1→4) linkages, thus cellulose is indigestible and is a component of dietary fiber.

Finally, another common disaccharide is **sucrose**.



sucrose

Sucrose is α-glucose-(1→2)-β-fructose. Note that the fructose ring is flipped 180° relative to the view given previously, and the anomeric C2 carbon is involved in the linkage to the glucose unit, i.e. both anomeric carbons are involved in the disaccharide linkage and thus the rings cannot open to expose either the aldehyde or ketone. This is therefore a **non-reducing sugar**.

Sugar Absorption and Fate

Carbohydrate is ingested primarily as starch and glycogen, although considerable amounts of sucrose are also eaten. Starch is digested primarily in the small intestine by the action of amylases. Limited hydrolysis takes place by the action of salivary **amylase** (ptyalin) in the mouth and hydrochloric acid in the stomach. In the lumen of the small intestine, pancreatic amylase converts starch into oligosaccharides. α-(1→6) linkages are hydrolyzed by **α-(1→6)-glucosidase**s. Disaccharidases in the brush border complete the hydrolysis to D-glucose which is absorbed and goes into the portal vein.

Following digestion, most of the glucose and other monosaccharides pass into the portal blood and are carried directly to the liver, through which they must pass before entering the systemic circulation. The functional state of the liver has a profound influence on the carbohydrate metabolism of the entire body. It is here that part is stored as glycogen. Part is also oxidized to produce energy (ATP) and part is oxidized via the HMP pathway (pentose phosphate pathway) to provide reducing power (NADPH) which, together with glucose itself, is used in synthesis of such molecules as amino acids, nucleotides, fats and cholesterol. The following scheme summarizes the typical demand for glucose and the metabolic pathways available to various tissues.

Brain uses ~120 g glucose per day for its ATP needs (glycolysis plus citric acid cycle)

Tissues that depend primarily on glycolysis for ATP production use ~40 g glucose per day

Kidney, medulla, testis, leukocytes and white muscle fibers have few mitochondria

Cornea, lens and regions of retina have limited blood supply and lack mitochondria

Red blood cells lack mitochondria (glycolysis plus pentose phosphate pathway)

In the liver, glucose is also converted into other sugars, for example, galactose for the synthesis of glycolipids, glucosamine for the synthesis of glycoproteins and glycosaminoglycans, neuraminic acid for the synthesis of glycoproteins, and glucuronic acid for detoxification. The liver, kidneys, and intestinal epithelial cells are the only organs that contain **glucose-6-phosphatase** activity, located within the membranes of the endoplasmic reticulum (see below). Thus, these are the only tissues that can release glucose into the bloodstream. In other tissues, glucose is trapped once it enters the cell and becomes phosphorylated by hexokinase.

Once glucose is in the systemic circulation, it becomes available for utilization by extrahepatic tissues. There glucose can be oxidized to produce ATP, oxidized to produce NADPH, converted to glycogen for storage, converted to fat for storage (adipose tissue) and used for synthesis of other carbohydrates, e.g., lactose (mammary glands) and glycosaminoglycans (mucopolysaccharides).

Glucose is transported into cells by the action of a family of protein carriers, denoted as GLUT. There are six characterized members (GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, and GLUT7), which are expressed in specific tissues.

Table 1: Characteristics of Glucose Transporters

Transporter	Characteristics
GLUT1	In many tissues including erythrocytes, brain, muscle and fat
GLUT2	Primarily in liver and pancreatic β cells, high K_m (~60 mM)

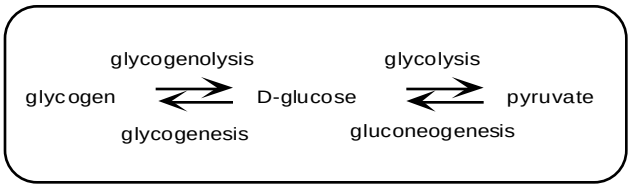
Table 1: Characteristics of Glucose Transporters

Transporter	Characteristics
GLUT3	In brain and nerve tissues, may work with GLUT1
GLUT4	Insulin-sensitive (dependent) occuring only in muscle and adipose tissue, relatively low K_m (~2-5 mM)
GLUT5	Primarily in small intestine, unrelated to Na^+-glucose cotransporter system, may be important for fructose transport
GLUT7	In endoplasmic reticulum of liver cells

NOTE: Under normal dietary conditions in a healthy individual, the brain, the retina, mature erythrocytes, the renal medulla, and germinal epithelium will only use glucose as an energy source.

Carbohydrate metabolism overview

Sugars can also be synthesized. The anabolic aspects of carbohydrate metabolism consist of the formation of glucose from noncarbodrate precursors (**gluconeogenesis**) and the synthesis of glycogen (**glycogenesis**), the storage form of glucose that is found in muscle and liver. The catabolic aspects of carbohydrate metabolism consist of **glycogenolysis** and **glycolysis**.

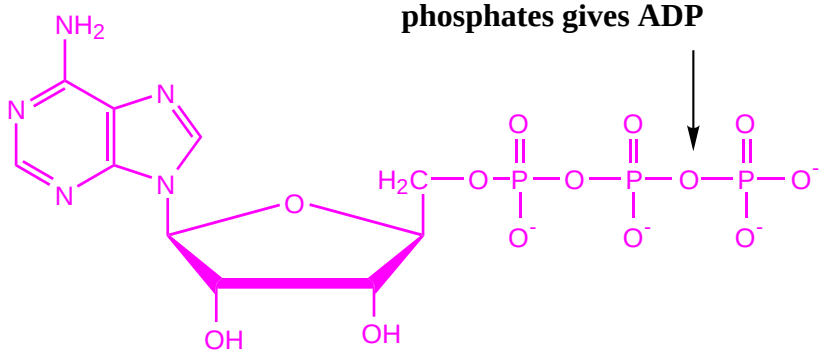


The catabolism of glucose involves its oxidation to carbon dioxide and water.

$$C_6H_{12}O_6 + 6\ O_2 \Leftrightarrow 6\ H_2O + 6\ CO_2 + 686\ \text{kcal/mole}$$

Much of the released energy can be viewed as being stored temporarily as **adenosine triphosphate (ATP)**.

hydrolysis of the phosphoester linkage between the β and γ phosphates gives ADP



Adenosine Triphosphate (ATP)

The triphosphate chain in ATP is energetically unstable. Energy must be put into the synthesis of ATP by extending the diphosphate chain in adenosine diphosphate. ATP is therefore a high energy compound that serves as a reservoir of energy that can be released when required by breaking (hydrolyzing) the triphosphate to give diphosphate (ADP) and phosphate (orthophosphate sometimes called inorganic phosphate, or **P_i**).

Even though cells are better than most nonliving systems in energy transformation, they cannot achieve 100% efficiency, i.e., not all the energy released by oxidation of glucose is captured in the form of ATP. Part of the energy is lost as heat. Indeed, some maintenance of the creation of heat through metabolism is essential to regulate the body temperature.

The total oxidation of glucose is accomplished in two stages. The first, the pathway of **glycolysis** or the Embden-Myerhof pathway, takes place in the cytosol of most cells in either the presence or absence of oxygen (i.e. it *does not require oxygen*), and *produces little energy.* The second, the **citric acid, tricarboxylic acid (TCA) or Kreb's cycle**, takes place in mitochondria, *requires oxygen* indirectly, and when coupled with electron transport and oxidative phosphorylation, produces most of the usable energy in the form of ATP.

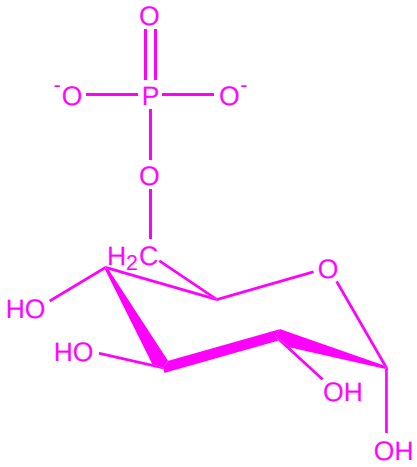
Glycolysis, literally the splitting of carbohydrate, is the conversion of a glucose molecule or a glucopyranosyl unit of glycogen into pyruvate. If oxygen is present (**aerobic glycolysis**), the pyruvate is oxidized further via the **TCA cycle** and the electron transport chain to produce CO_2 and H_2O. In the absence of oxygen (**anaerobic glyco-**

lysis), pyruvate is converted to lactate.

Glycolysis

Glycolysis represents the initial degradation of glucose. It is an anaerobic process, i.e. oxygen is not utilized. It is useful in emergencies when oxygen is not available since a minor amount of ATP is produced.

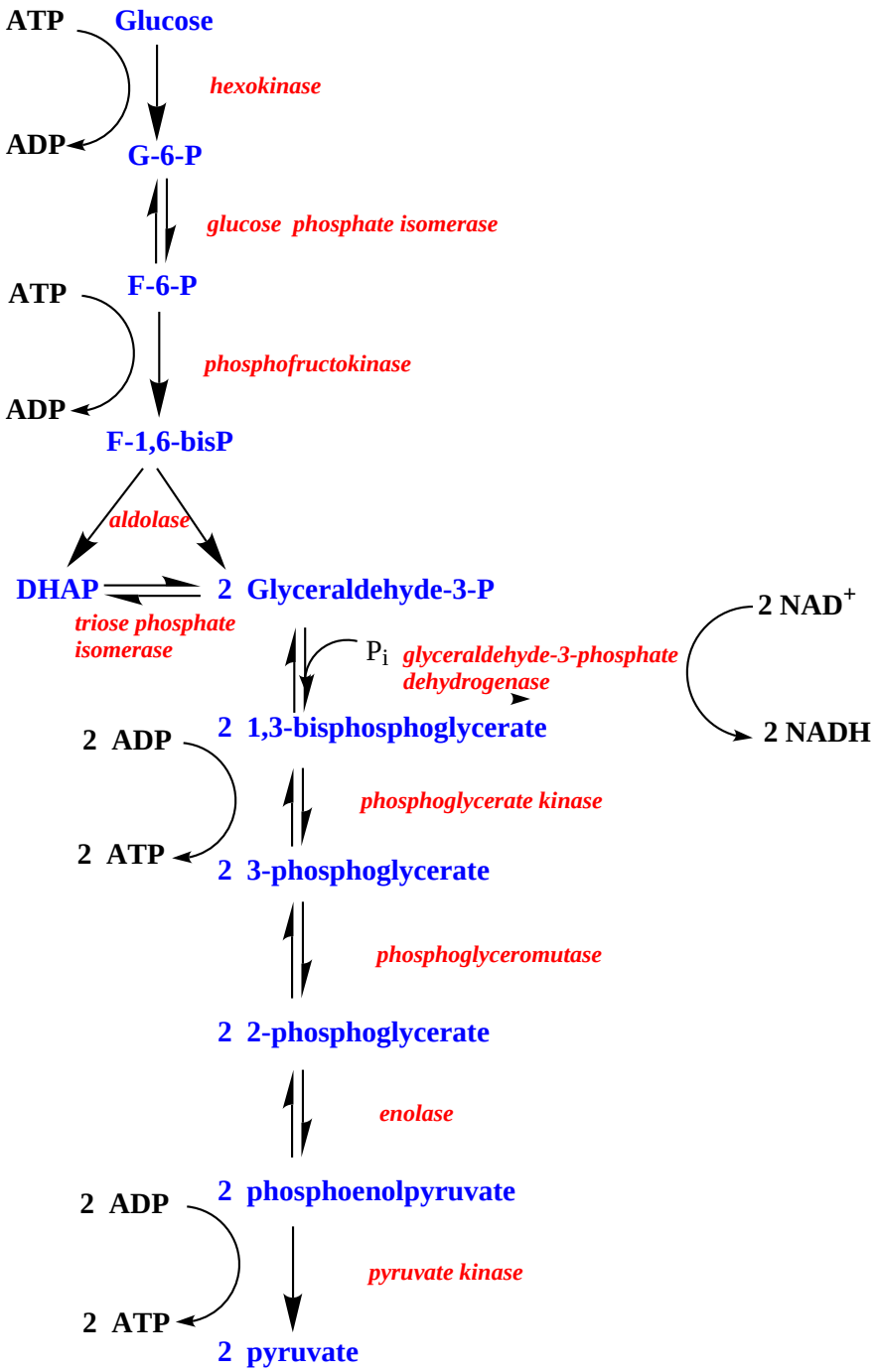
The first step involves the phosphorylation of glucose on C6 to give **glucose-6-phosphate** (G6P). The phosphorylation is catalyzed by



either **hexokinase** or **glucokinase** (depending on the cell) and requires ATP. This reaction is commonly referred to as being essentially irreversible. Of course all reactions are reversible. The intention here is to draw attention to the fact that there is so little ADP in the cell, that the reaction of ADP and G6P to give back ATP and glucose rarely occurs. Phosphorylation of glucose gives it a negative charge (all intermediates in glycolysis are charged) and thus membrane impermeable. The glucose is committed to the cell once phosphorylated. It can be either broken down via glycolysis or stored in glycogen. Only liver, kidneys, and intestinal epithelia contain a **glucose-6-phosphatase** which remove the phosphate and free glucose. Clearly, this is essential in these tissues to permit movement of glucose to other parts of the body.

Hexokinase, found in muscle and other tissues, is allosterically regulated (inhibited) by G6P. It displays normal Michaelis-Menton kinetics. The isozyme glucokinase is unique to the liver; it has a high K_m for glucose and is thus active only when glucose levels are high. This isozyme displays sigmoidal (non-Michaelis-Menton) kinetcs, which is unusual for a monomeric enzyme. Its role is to provide G6P for glycogen synthesis. The high K_m of glucokinase in the liver gives the brain and muscle first call on glucose when its supply is limited.

GLYCOLYSIS



Following phosphorylation, G6P is converted to **fructose-6-phosphate** (F6P) by **phosphohexoisomerase**. This reaction involves essentially no energy change and is freely reversible. F6P is then phosphorylated at the C1 position by **phosphofructokinase I** to give

fructose-1,6-diphosphate. This reaction is allosterically controlled by ATP (-), AMP (+), citrate (-) and fructose-2,6-bisphosphate (+) (the signs indicate positive or negative regulation). **AMP** is used as an allosteric effector since its concentration changes significantly under anaerobic conditions (see Table below). This is the first reaction which commits sugars to catabolism rather than storage. It is irreversible in that ATP is utilized.

Table 2: Changes in ATP, ADP, AMP, and P_i in going to anaerobic conditions in perfused rat heart

	Aerobic	Anaerobic
ATP	4.2 mM	3.4 mM
ADP	0.8	1.2
AMP	0.06	0.2
P_i	1.5	5.2

F-1,6-bisP is then broken by **aldolase** into two 3 carbon fragments: **dihydroxyacetone phosphate** and **glyceraldehyde-3-phosphate**. This reaction is freely reversible. **Triose phosphate isomerase** converts DHAP to Gly3P, which then proceeds on to be oxidized by **glyceraldehyde-3-phosphate dehydrogenase** to **1,3-bisphosphoglycerate**. This is an unusual enzyme in that it utilizes inorganic phosphate to make a high energy compound - i.e. substrate phosphorylation. In addition, the oxidation of the glyceraldehyde releases reducing equivalents (hydride, H^-) which are deposited in NAD^+ to give NADH (hydrogen carrier).

Arsenate is poisonous at least in part because it can substitute for phosphate in the glyceraldehyde-3-dehydrogenase catalyzed reaction. Arsenate is then spontaneously released, leading to no incorporation of phosphate.

1,3-bisphosphoglycerate can be converted to **2,3-bisphosphoglycerate** by bisphosphoglycerate mutase. This is especially important in red blood cells since 2,3-bisphosphoglycerate is important in regulating the oxygen affinity of hemoglobin.

The high energy **1,3-bisphosphoglycerate** can now release its energy to make ATP in a reaction catalyzed by **phosphoglycerate kinase**. Thus the incorporation of inorganic phosphate by glyceraldehyde dehydrogenase ultimately goes to ATP.

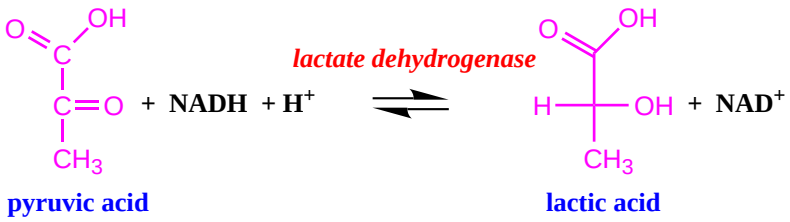
The resulting **3-phosphoglycerate** is converted to **2-phosphoglycer-**

ate by **phosphoglyceromutase**. **Enolase** then converts this to **phosphoenolpyruvate (PEP)**. All of these reactions are freely reversible.

Finally, **pyruvate kinase** converts PEP to **pyruvate** with the production of ATP. This enzyme is allosterically controlled by F-1,6-bisP (+), PEP (+), ATP (-), citrate (-), and acetyl CoA (-). In muscle it is not activated by F-1,6-bisP. Note that in liver this is a secondary forward regulation site. Increased flux through glycolysis will increase F-1,6-bisP which will accelerate the efflux through the pyruvate kinase reaction. The forward control therefore ensures increased flow.

Anaerobic glycolysis

Under anaerobic conditions, the NADH produced by oxidation of Gly3P will consume all of the NAD^+ and therefore prevent continued flux through glycolysis. Therefore, the supply of NAD^+ is replenished by **lactate dehydrogenase** which converts pyruvate to **lactic acid (lactate)**. A good indication of the utilization of this pathway is shown by erythrocytes which lack mitochondria where the lactate concentration is typically on the order of 3 mM and pyruvate is 0.5 mM. Increased production of lactic acid in muscle during heavy exercise leads to cramping and decreased pH. This is the only reaction in the body that uses lactate.



Lactate dehydrogenase is a tetramer. There are actually two different subunits that can be used to assemble LDH - the H form predominates in heart tissue, and the M form in muscle. Isozymes with composition H_4, H_3M, H_2M_2, HM_3 and M_4 may be found in specific cell types. An increase in the H_4 isoform in the blood can be used as a clear indication of cardiac infarct. The H_4 isoform has a higher affinity for substrates than the M_4 isoform and is allosterically inhibited by high concentrations of pyruvate. This presumably prevents the buildup of lactate in continuously exercising heart muscle. It is thought that the M_4 isoform is optimize for anaerobic metabolism that occurs in muscle when oxygen levels are low.

Irreversible Control Steps in Glycolysis

There are three thermodynamically irreversible steps of glycolysis: those catalyzed by hexokinase, phosphofructokinase and pyruvate kinase. All of them are allosterically controlled. In fact, this is why they are considered irreversible. In a very real sense, they represent positions on a river that is dammed. Reverse flow is not possible (via these pathways).

The conversion of fructose-6-phosphate (F6P) into fructose-1,6-bis-phosphate (F-1,6-bisP) is the rate limiting step in liver because the concentration of phosphofructokinase is low. The addition of FBP increases glycolysis.

The Role of NAD as a coenzyme with glycer-aldehyde-3-phosphate dehydrogenase

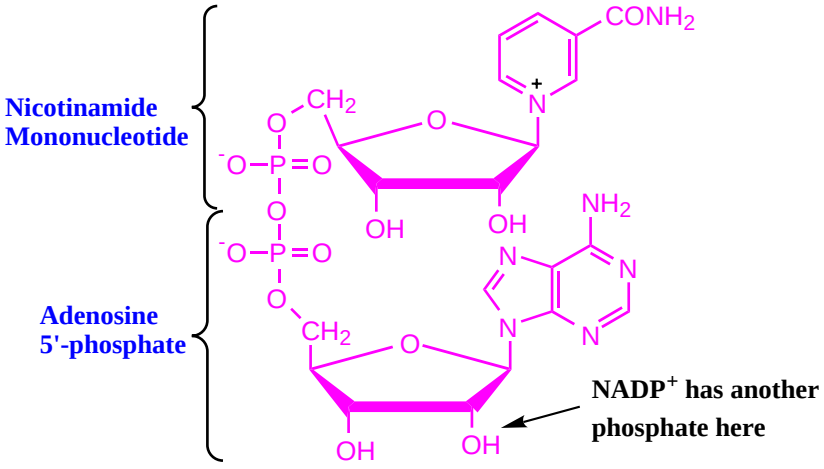
NAD stands for **nicotinamide adenine dinucleotide**. A nucleotide is a compound of the following structure:

nitrogen base-(ribose or deoxyribose)-phosphate

NAD is a **dinucleotide** in which the two parts are joined at their phosphates:

nitrogen base-sugar-phosphate-phosphate-sugar-nitrogen base

In NAD, the nitrogen bases are adenine and nicotinamide, the amide of the **B vitamin nicotinic acid or niacin**. NADP possesses an additional phosphate group and is primary involved in anabolic path-ways.



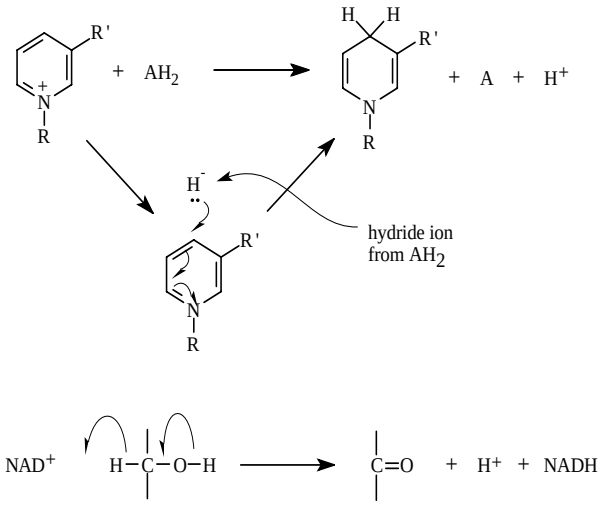
NAD^+ (Nicotinamide Adenine Dinucleotide)

Both NAD and NADP are coenzymes in oxidation-reduction reac-tions, i.e. “redox” reactions, of the type

$$SH_2 + NAD^+ \quad \Leftrightarrow \quad P + NADH + H^+$$

(S = substrate, P = product)

Biological oxidations frequently are of this type, i.e., the removal of hydrogen. Hence, the enzymes involved are called **dehydrogenases**. However, it is important to remember that **oxidation** generally represents the removal of electrons. In the above example, two electrons are transferred from the substrate to NAD^+. This movement of electrons is not apparent from the equation because protons are removed at the same time. The net result is the removal of 2 hydrogen atoms (**reducing equivalents**) from the substrate. A more detailed view of the reduction of NAD^+ is shown in the figure below. (Note that it is customary to not explicitly show the hydrogen atoms on an aromatic ring. Thus each carbon on the niacin ring prior to reduction has one proton attached, except for the carbon with the R' attached. After reduction, the pre-existing hydrogen along with the newly attached hydrogen are both shown at the top of the reduced niacin ring below - only one has been added as a result of the reduction.)



It is important to note that oxidation and **reduction** always occur simultaneously. If a substrate is oxidized, something else must be reduced; and *vice versa*. There must be an acceptor for the electrons removed. The acceptor in this case is NAD^+; and it is reduced to NADH. Molecules that can reversibly accept and donate electrons serve as coenzymes in "redox" reactions.

The term nicotinamide adenine dinucleotide (NAD) may mean either the oxidized or reduced form, so it can be described simply as a coenzyme for redox reactions. However, when a specific reaction is

being considered, the oxidized form (NAD^+) and the reduced form (NADH) should be distinguished. NADH can be reoxidized to NAD^+ by transferring two electrons to another molecule.

Summary of Glycolysis

Glycolysis can be summarized as follows:

$$\text{glucosyl unit of glycogen} + 3\ P_i + 3\ ADP + 2\ NAD^+ \Leftrightarrow 2\ \text{pyruvate} + 3\ ATP + NADH$$

or

$$\text{D-glucose} + 2\ P_i + 2\ ADP + 2\ NAD^+ \Leftrightarrow 2\ \text{pyruvate} + 2\ ATP + 2\ NADH$$

Actually the process never stops at pyruvate. Under anaerobic conditions, (i.e., when oxygen concentration is low) NADH is oxidized to NAD^+ constantly with the reduction of pyruvate to lactate by lactate dehydrogenase. Under aerobic conditions, pyruvate is oxidized to CO_2 and water in the tricarboxylic acid cycle and NADH is reoxidized to NAD^+ by the enzymes of the electron transport chain. This is necessary because NAD^+ must be replenished to sustain glycolysis.

How much energy is captured in glycolysis?

Under aerobic conditions, the two moles of NADH produced require two moles of oxygen atoms for reoxidation. These two mole-atoms of oxygen form two moles of water. Therefore, we can write the equation for aerobic glycolysis as:

$$\text{D-glucose}\ (C_6H_{12}O_6) + O_2 \Leftrightarrow 2\ \text{pyruvate} + 2\ H_2O$$

This may be viewed as a highly controlled "burning" of glucose. Note that the equation just represents a summary of the process; oxygen is not directly involved in the oxidation of glucose or pyruvate. As we will learn in the next module, participation of oxygen is confined to the last step in the electron transport chain.

Mitochondrial oxidation of pyruvate (as we will see below) can be summarized as follows:

$$2\ \text{pyruvate} + 5\ O_2 \Leftrightarrow 6CO_2 + 4\ H_2O$$

Therefore, the complete catabolism of D-glucose is:

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

Glycolysis is an **exergonic** process, i.e., it releases energy. If one mole of glucose is burned in a calorimeter, 686 kcal (686,000 cal) of heat are produced.

$$\text{D-glucose} + 6\ O_2 \Leftrightarrow 6\ CO_2 + 6\ H_2O + 686\ \text{kcal}$$

If one mole of L-lactic acid is burned in a calorimeter, 314 kcal of heat are produced.

$$2\ \text{L-lactic acid} + 6\ O_2 \Leftrightarrow 6\ CO_2 + 6\ H_2O + 628\ \text{kcal}$$

The energy available from converting glucose to lactate is the difference between these two processes. Therefore, the amount of energy released if glucose is burned to lactic acid is small: 686-628 = 58 kcal.

From each mole of glucose, anaerobic glycolysis produces a net gain of two moles of ATP (three if starting from glycogen). The free energy of hydrolysis of ATP under typical conditions in a normal cell (hence the amount of energy stored in ATP) is approximately 11 kcal/mole. Thus, the total amount of energy produced in glycolysis is 2 x 11 = 22 kcal/mole. Glycolysis is therefore approximately 38% efficient.

Gluconeogenesis

The synthesis of glucose (the reverse of glycolysis) from pyruvate is called **gluconeogenesis.** Most of the required ATP is derived from β-oxidation of fatty acids. While other metabolic intermediate can supply the carbon atoms for glucose, pyruvate is a convenient starting point for the discussion of this pathway. However, gluconeogenesis is not a complete reversal of glycolysis. Because of the irreversibility of the reactions catalyzed by pyruvate kinase, phosphofructokinase and hexokinase, additional enzymes are required:

1. **pyruvate carboxylase**
2. **phosphoenolpyruvate carboxykinase**
3. **fructose bisphosphatase**
4. **glucose-6-phosphatase**

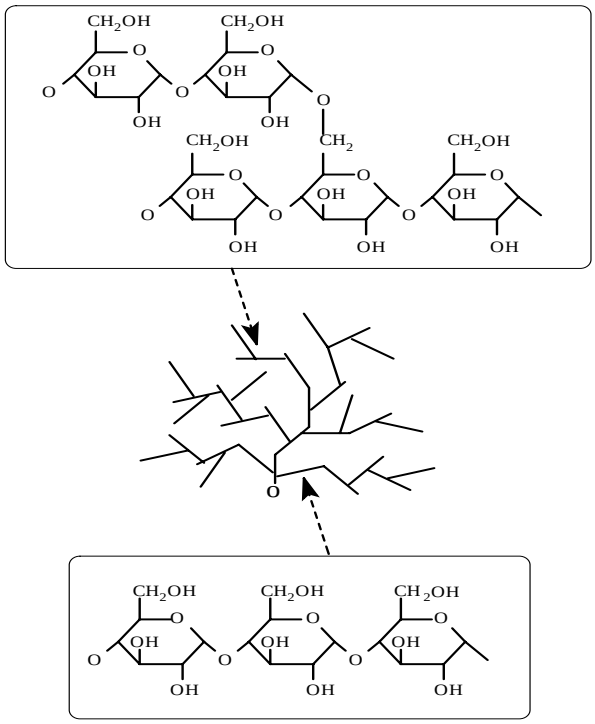
The first converts pyruvate to **oxaloacetate** by direct incorporation of CO_2, and the second converts this to phosphoenolpyruvate with the release of CO_2. The glycolytic pathway reactions are reversible up to fructose-1,6-bisphosphate which is converted to fructose-6-phosphate by the third enzyme above. Glucose-6-phosphatase in the

membranes of the **endoplasmic reticulum** of liver and kidney cells removes the phosphate so that glucose contained within forming vesicles can fuse with the plasma membrane and be released into the circulation. This is thought to involve both GLUT7 and GLUT2.

Glycogen and its function:

Glycogen is the storage form of glucose. When there is an excess of glucose, it is converted into glycogen or fat. Glycogen is a large polymer of glucose units connected through linear α-D-(1→4) links with α-D-(1→6)-linkages creating branches. It is found primarily in the liver and muscle. Because glycogen is a very large, only slightly soluble molecule, it can accomplish the storage of sugar without increasing osmotic pressure. For a typical cellular glycogen concentration of 10 nM, the equivalent glucose concentration would be ~0.4 M. A cell containing an equivalent amount of glucose would burst!

Glycogen exists as a compact, globular structure. In native glycogen, there are approximately 12 tiers instead of what is shown in the abbreviated structure below. The highly branched structure yields numerous nonreducing ends. Why would this be valuable? Consider that glycogen must store a large amount of glucose in a small volume. Glycogen is optimized for the degree of branching and chain length. This ensures that the maximun amount of glucose can be released from a well-designed spherical glycogen particle. The single reducing end is shown on the branched structure below with an O.



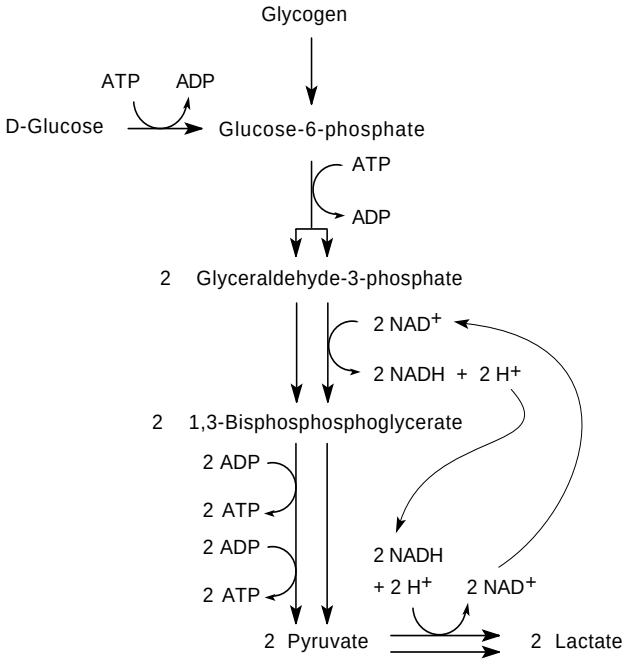
The linkages glycogen are not *ether* linkages but glycosidic linkages, i.e., acetal groups. This is important because ethers are very stable chemically and are not very susceptible to either enzyme-catalyzed or acid-catalyzed hydrolysis. Glycosidic (acetal) bonds undergo hydrolysis, phosphorolysis, and transglycosylation much more readily.

When **hypoglycemia** (i.e. low blood sugar levels) occurs, glycogen stored in the liver is converted into glucose. The glucose is released from the liver into the blood. Thus, liver glycogen is involved in the regulation of blood glucose levels and in maintaining homeostasis. In muscles, glycogen serves as the emergency energy source. Both of these functions are under hormonal control.

Glycogenesis

Glycogenesis is the synthesis of glycogen. Glycogen synthesis requires **glucose-1-phosphate**, so the first requirement is to convert G-6-P to G-1-P, catalyzed by **phosphoglucomutase**. **UDPG pyrophosphorylase** transfers G-1-P to the high energy triphosphate compound UTP, uridine triphosphate (similar to ATP except the adenine is replaced with uracil) to create **UDP-glucose (UDPG)** and pyrophosphate, PP_i. The product UDP-glucose is then used by **glycogen synthase** to transfer a single glucose unit to pre-existing "glycogen primer" at a non-reducing end creating an $\alpha(1\rightarrow4)$ linkage. The pre-existing glycogen primer comprises **glycogenin** (a 37-kD protein)

linked through a tyrosine residue to the anomeric carbon of a glucose molecule, a reaction catalyzed by a tyrosine glucosyltransferase, followed by an additional 7 glucan residues. There appears to be a 1:1 ratio of glycogenin to glycogen synthase. **Branching enzyme** can then move a terminal segment of about 10 glucose units to create branches by creating an $\alpha(1\rightarrow6)$ branching linkage.



Glycogenolysis

Glycogenolysis is the breakdown of glycogen. **Glycogen phosphorylase** removes single glucose units from the non-reducing ends of branched glycogen. It cannot break an $\alpha(1\rightarrow6)$ branching linkage. Phosphorylase uses inorganic phosphate (P_i) directly and the reaction is a phorolysis rather than a hydrolysis. Most importantly, the liberated glucose units are in the form glucose-1-phosphate and ATP is not utilize! The absence of phosphorylase leads to McArdles' Disease and decreased exercise ability. Inborn errors in glycogen metabolism are referred to as **glycogenoses**. **Phosphoglucomutase** converts the G-1-P to G-6-P, which can then enter the glycolytic pathway. **Debranching enzyme** removes branches by transferring a short branching segment from a C6 position to a C4 end. This circumvents the inability of phosphorylase to remove branching. The absence of this enzyme leads to Cori's disease in which branching created in glycogen synthesis can never be removed. Thus the liver becomes larger and larger with increased glycogen stores that cannot be utilized as the branching enzyme creates more branches.

Hormones, cAMP, and protein kinases

Hormone receptors are specialized proteins capable of binding hormone molecules with very high specificity and affinity. These proteins are found in small amounts in target cells, often on the cell surface. The water-soluble hormones such as epinephrine (adrenaline) and glucagon cannot readily pass through the cell membrane.

The binding of epinephrine or glucagon to their specific receptors on the target cell membrane stimulates the formation of the intracellular messenger; 3', 5'-cyclic adenosine monophosphate (cyclic AMP, also abbreviated **cAMP**). The formation of this **second messenger** is catalyzed by **adenylate cyclase**:

$$Mg^{2+}ATP \Leftrightarrow cAMP + PP_i$$

The reaction is pulled further to the right by the breakdown of **pyrophosphate**, PP_i. Adenylate cyclase is tightly bound to the plasma membrane.

Intracellular cAMP levels are controlled by modifying its rate of synthesis and its rate of degradation. Adenylate cyclase is responsible for cAMP synthesis and it is stimulated by the binding of epinephrine (adrenaline) or glucagon to a receptor on the outer surface of the plasma membrane of a cell. cAMP is degraded by **phosphodiesterase** to AMP by the hydrolysis of the 3' phosphodiester linkage.

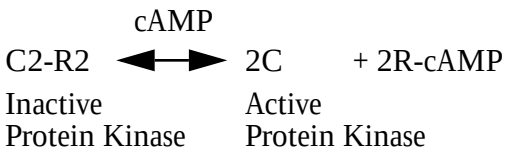
The concentration of cAMP in liver cells increases rapidly upon binding of epinephrine or glucagon. Muscle cells, which have receptors for epinephrine but not glucagon, show a similar response to epinephrine. Once the hormone is removed or destroyed, the cAMP levels drop rapidly to low levels due to phosphodiesterase activity.

$$cAMP + H_2O \Leftrightarrow AMP$$

Caffeine and theophylline, alkaloids found in small amounts in coffee and tea, prolong and intensify the activity of epinephrine. These compounds inhibit phosphodiesterase activity and lead to higher cAMP levels in cells stimulated by epinephrine.

Cyclic AMP-dependent **protein kinase**s are the key enzymes in linking cyclic AMP to the physiological changes that occur in the target cells. These protein kinases are allosteric proteins. They contain two types of subunits, a catalytic (C) subunit and a regulatory (R) subunit which inhibits the catalytic subunit. The allosteric modulator of protein kinase is cAMP. Cyclic AMP binds to a specific site on the regulatory subunit, causing the inactive CR complex to dissociate, yielding an R-cAMP complex and a free C-subunit that is now cata-

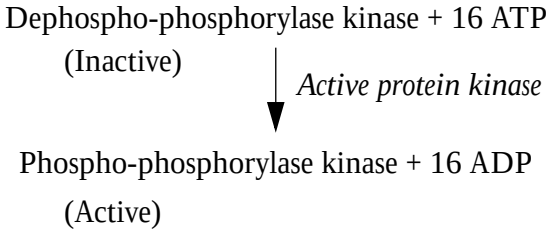
lytically active.



Protein kinase can phosphorylate a number of proteins such as phosphorylase kinase, glycogen synthase, inhibitor protein, histones, ribosomal proteins, membrane proteins of fat cells, and mitochondrial and lysosomal membrane proteins.

In liver, protein kinase A phosphorylates **phosphofructokinase-2** and **fructose-2,6-bisphosphatase.** These are actually the same protein with two different activities. The first is inactivated, and the second is activated upon phosphorylation. This leads to a decrease in **fructose-2,6-bisphosphate**, which is an allosteric activator of phosphofructokinase-1, so glycolysis is shut down when cAMP levels are increased in the liver. In muscle, phosphorylation of phosphofructokinase-2 and fructose-2,6-bisphosphatase leads to the opposite effect, so glycolysis is stimulated with increased cAMP.

Phosphorylase kinase is the enzyme responsible for activating phosphorylase and thus stimulating the mobilization of glycogen. Phosphorylase kinase is a large protein (over 1 million molecular weight) with 16 subunits. It exists in two forms; an inactive form with no phosphate attached and an active phosphorylated form.



Each of the 16 subunits becomes phosphorylated by ATP at a serine residue through the action of protein kinase.

The active phosphorylase kinase catalyzes the phosphorylation of **phosphorylase b** to give **phosphorylase a**.

Phosphorylase b + 4 ATP
"Inactive" (Dependent on allosteric activators)
↓ *phosphorylase kinase*
Phosphorylase a + 4 ADP
"Active" (Independent of allosteric activators)

Phosphorylase b has enzyme activity. Unlike phosphorylase a, however, its activity is dependent on allosteric effectors as shown in the following table:

Table 3: Characteristics of Phosphorylases

Tissue	Enzyme	Characteristics
MUSCLE	phosphorylase a	active as is
		composed of 4 subunits (tetramer)
	phosphorylase b	activated by AMP and P_i
		composed of 2 subunits (dimer)
		repressed by ATP and G6P
LIVER	phosphorylase a	dimer
	phosphorylase b	dimer
		weakly activated by AMP

Amplification Cascade

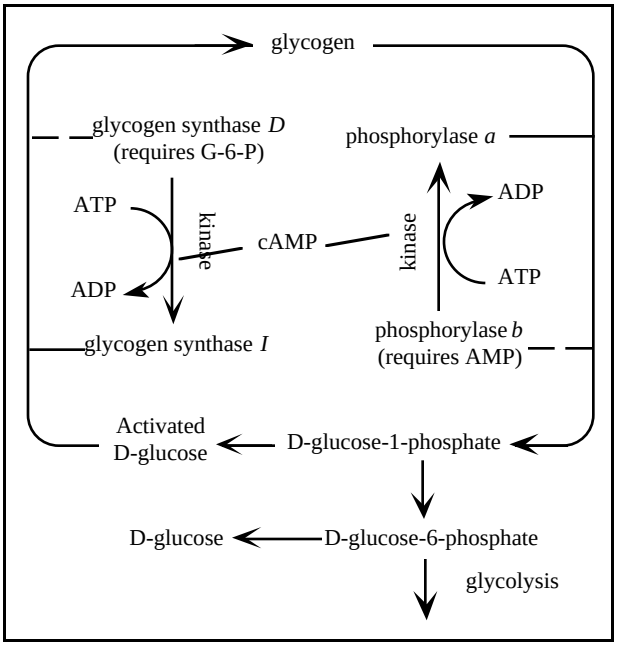
Epinephrine arriving at the surface of the muscle cell at a concentration of 10^{-8} to 10^{-10} M becomes bound to the specific epinephrine receptor sites on the outer surface of the cell membrane. The ability to bind hormone at these concentrations requires some of the tightest binding known. Its binding causes a local conformational change in the membrane receptor, resulting in the activation of adenylate cyclase, which is located on the inner surface of the plasma membrane. The activated adenylate cyclase converts ATP into cyclic AMP, which may attain a peak concentration of about 10^{-6} within the cell. The cyclic AMP then binds to the regulatory subunit of the protein kinase A and releases the catalytic subunit in an active form. The catalytic subunit then catalyzes the phosphorylation of the inactive form of phosphorylase kinase at the expense of ATP to yield active phosphorylase kinase. This enzyme now catalyzes the phosphorylation of

inactive phosphorylase b to yield active phosphorylase a, which in turn catalyzes breakdown of glycogen to yield glucose-1-phosphate, from which glucose-6-phosphate is formed. Ca^{2+}, which stimulates muscle contraction, can also bind to a subunit of phosphorylase kinase (**calmodulin**) and activate the enzyme.

Each step in this cascade is catalytic and thus results in a large amplification of the incoming signal, namely, the binding of a relatively small number of epinephrine molecules to the cell surface. Although there are many steps in this cascade of enzymes acting on enzymes, it can reach peak activity in a matter of only seconds.

Epinephrine not only stimulates glycogen breakdown but also inhibits glycogen synthesis. The binding of epinephrine to a muscle (or liver) cell and the subsequent formation of cyclic AMP promotes the phosphorylation of glycogen synthase by protein kinase A. The active or dephosphorylated form of glycogen synthase is converted to its phosphorylated, inactive form. Six serine residues of the glycogen synthase molecule are phosphorylated at the expense of ATP. The inhibition of glycogen synthase is thus brought about by a chain of events triggered by the same stimulus that causes acceleration of glycogen breakdown.

So long as epinephrine is secreted into the blood by the adrenal medulla, the adenylate cyclase system remains activated, maintaining cyclic AMP at a high concentration. However, once epinephrine secretion stops, the level of bound epinephrine on the cell membrane decreases. Cyclic AMP is then no longer formed, and the remaining molecules are destroyed by phosphodiesterase. The protein kinase A subunits now reassociate into a complex which has no catalytic activity. The phosphorylated form of phosphorylase kinase then undergoes dephosphorylation, as does phosphorylase a itself, by the action of phosphoprotein phosphatase. In this way, the glycogenolytic system returns the normal resting states; simultaneously, glycogen synthase is reactivated by dephosphorylation.



The return of the system to the normal resting state is rapid for the following reason. Phosphoprotein phosphatase activity is inhibited by phosphorylated **inhibitor protein (IP)**. However, IP does not inhibit the action of the phosphatase on IP itself.

$$IP_b \xrightarrow[\text{Phosphatase}]{\text{Protein kinase}} IP_a \text{ (active form)}$$

Thus, in order to maintain a high concentration of active inhibitor protein, signal must be continuously applied to maintain active protein kinase. (Since IP is continually being deactivated.) As soon as the stimulatory signal is removed, the IP returns to the inactive form and the phosphatases are no longer inhibited, i.e. the system is designed to deactivate itself upon removal of the stimulatory signal.

Module II: Mitochondria, the Tricarboxylic Acid Cycle, the Respiratory Chain, and Oxidative Phosphorylation

Objectives:

1. When given the name of an enzyme of the tricarboxylic acid cycle, write the reaction catalyzed by that enzyme, including the coenzymes required, their reaction, if any, and structure of the substrate(s) and product(s).

2. Pyruvate dehydrogenase and the TCA cycle are regulated by acetyl CoA, NADH, GTP, AMP, ADP and succinyl CoA. Rationalize why these particular compounds might be used for this purpose.

3. Write structures for ATP and ADP.

4. Describe the function of the TCA cycle. What metabolic pathways (e.g., carbohydrate, lipid, etc.) is the TCA cycle coupled to?

5. Describe the effect the following inhibitors on both ATP production and electron transport: rotenone, amytal, antimycin A, cyanide, CO, oligomycin, 2,4-dinitrophenol, valinomycin, gramicidin. What is the difference between uncoupler and ETC inhibitor?

6. Identify the components of the respiratory chain. On a diagram, indicate the coupling sites for ATP formation and the points of action of each of the inhibitors listed in 6. Justify the P/O ratios for NADH, $FADH_2$ and metabolites that lead to the formation of these reduced coenzymes.

7. Describe the chemiosmotic mechanism for oxidative phosphorylation.

8. What is substrate-level phosphorylation and how is it different from oxidative phosphorylation?

9. Why is there a difference in the amount of ATP generated from the aerobic oxidation of one molecule of glucose and one glucose residue in glycogen?

10. Show how oxidation of one molecule of acetyl coenzyme A can yield 12 ATP molecules.

11. How do the concentrations of ATP, ADP, and P_i differ during aerobic and anaerobic metabolism?

12. Describe the relationship of the following coenzymes to the B vitamins:
    coenzyme A
    FAD
    FMN
    lipoic acid
    NAD
    TPP

13. Use the above objectives and previous objectives to solve new problems. For examples, see the problem set for this Problem Unit.

14. Define, describe and be able to identify the correct and incorrect use of each of the terms in the **Nomenclature and Vocabulary** list.

Nomenclature and Vocabulary:

2,4-dinitrophenol
α-ketoglutarate dehydrogenase
a-ketoglutarate
acetaldehyde
acetyl CoA
aconitase
amytal
antimycin A
chemiosmotic coupling
citrate synthase
citric acid cycle
citrate
CO
CO_2
coenzyme A
coenzyme Q
coupling site
cristae
cyanide
cytochrome aa3
cytochrome b
cytochrome c1
cytochrome c
cytochrome oxidase
cytochromes
electron transport chain
ethanol
F1F0 ATPase
FAD
FADH2
flavoprotein
FMN
fumarase
fumarate

gramicidin	GTP
heme	ionophore
iron-sulfur proteins	isocitrate
isocitrate dehydrogenase	lipoic acid
malate	malate dehydrogenase
mitochondrial inner membrane	mitochondrial matrix
mitochondrial outer membrane	multienzyme complex
nigericin	non-heme-iron protein
oligomycin	oxaloacetate
oxidative phosphorylation	P/O ratio
protoporphyrin IX	proton concentration gradient
proton motive force	proton pump
pyruvate dehydrogenase complex	reducing equivalents
respiratory chain	rotenone
substrate-level phosphorylation	succinate
succinate dehydrogenase	succinyl CoA
thiamine pyrophosphate (TPP)	ubiquinone
uncoupling agent	valinomycin

STUDY GUIDE - II

The main function of the **Tricarboxylic Acid (TCA), Krebs, or Citric Acid Cycle**, as it is variously called, is to produce the reduced coenzymes NADH and $FADH_2$. These compounds are used to supply reducing equivalents for the respiratory chain, which in turn leads to ATP formation for the cell's energy needs. The production of NADH and $FADH_2$ (hydrogen carriers) is accomplished by coupling the reduction of NAD^+ and FAD with the oxidation of intermediates of the TCA cycle.

The TCA cycle plays a central role in the catabolism of many compounds. The food we eat consists mainly of carbohydrates, fats, and proteins. Each of these is eventually degraded to less complex molecules (catabolism) which are then oxidized to carbon dioxide and reducing equivalents in the TCA cycle. Carbohydrates are degraded to a three carbon compound, pyruvate. (At physiological pH pyruvic acid occurs as the carboxylate anion). Fatty acids from lipids are broken down to produce reducing equivalents and acetyl CoA, a two-carbon derivative with the skeleton of acetic acid. Proteins are

metabolized to amino acids which are converted to simpler α-keto acids. These can enter the TCA cycle and eventually yield reducing equivalents and CO_2. Our discussion for the present will be limited to carbohydrates and the TCA cycle, but you should be aware that the TCA cycle is a focal point of much of metabolism.

Carbohydrates (e.g., glucose) are converted to pyruvate by glycolysis. Glycolysis takes place in the soluble cytoplasm outside the mitochondria. The TCA cycle enzymes and the respiratory chain occur inside the mitochondria. Pyruvate, unlike other intermediates of glycolysis, can enter the mitochondria. (It is worth noting that the other intermediates of glycolysis are phosphorylated and hence are highly polar and, therefore, unable to cross the mitochondrial membrane by passive transport. There are no active transport systems for these phosphorylated intermediates.)

Mitochondria

The mitochondrion is an intracellular organelle which houses all of the enzymes that are involved in oxidative metabolism of sugars (and fatty acids). It is the machine which utilizes oxygen and thus is responsible for our reliance on oxygen. It is also the site of CO_2 production. The mitochondrion is composed of two membranes. The **outer membrane** is highly permeable due to the presence of a protein called porin that creates pores permitting passage of molecules on the order of 10,000 Daltons or less. The **inner membrane** is highly convoluted leading to the appearance of "fingers" in electron microscopic images. These are the so-called **cristae**. The space between the fingers, i.e. the inner space of the mitochondrion or **matrix**, is a highly crowded aqueous gel with less than 50% water. This is the location of the TCA cycle enzymes. The oxidative phosphorylation proteins reside in the inner mitochondrial membrane. The convolutions presumably increase the surface area of the inner membrane in increase the proximity of the TCA cycle proteins to the oxidative phosphorylation machinery, thereby increasing the speed by which pyruvate can be oxidized and ATP can be produced.

ATP

The ultimate purpose of the complete oxidation of carbohydrate via glycolysis and the TCA cycle to carbon dioxide and water is to transfer the chemical energy of the sugar to adenosine triphosphate (ATP).

ATP contains a high energy bond between its second and third phosphates. A high energy bond is one that releases more than 4-5 kcal/mole of energy upon hydrolysis. The standard state free energy of hydrolysis of the terminal phosphate of ATP is -7.3 kcal/mol. However, standard state free energies are those that would be observed if all of the products and reactants are at 1.0 M concentration. Since

this is not the situation *in vivo* **(see Table 1 in Module I)**, this is not the energy of hydrolysis in a cell. The actual free energy is given by

$$\Delta G = \Delta G^\circ + RT \ln \frac{[ADP][P]}{[ATP]}$$

where ΔG° is the standard state free energy, R is the gas constant (1.99 cal/deg/mol), T is the temperature in Kelvin (i.e. 273.15 plus the temperature in Celsius), and [ATP], [ADP], and [P] are the concentrations of ATP, ADP, and P_i in the cell. In most cells, the concentrations of ATP, ADP, and P_i are such that the ΔG is on the order of -10 to -13 kcal/mol.

When a chemical change takes place, the maximum energy available for useful work is the decrease in free energy (ΔG) which accompanies the change, under the conditions of temperature and concentration at the time of the change. The free energy that is released by ATP hydrolysis may be captured for useful work in the cell, e.g., synthesis of a new bond, active transport, transmission of an impulse along a nerve, and muscular movement.

As we have seen, there are many phosphorylated compounds involved in metabolism. Phosphorylated proteins were discussed in the previous section under the regulation of glycogenolysis. Monophosphate esters such as these are low energy compounds and are not important in energy transfer. The greater the release of free energy generated during the hydrolytic release of P_i, the greater the free energy stored in the original bond and the greater the phosphate group transfer potential, i.e., if $X\sim P_i$ has a greater phosphate potential than ATP, then the following reaction is possible: $X\sim P_i + ADP \rightarrow X + ATP$, while the reverse does not occur to as great an extent. If $Y\text{-}P_i$ has a lower phosphate group transfer potential than ATP, the following reaction is possible: $ATP + Y \rightarrow ADP + Y\text{-}P_i$, and the reverse is not favored.

Thus the phosphate group transfer potential indicates the direction of flow of phosphate groups from donors of high transfer potential to an acceptor which forms a compound of lower phosphate group transfer potential. ATP has an intermediate value in the scale of phosphate group transfer potential. The ATP-ADP system can therefore accept P_i from high potential systems and transfer P_i to acceptor systems of low potential.

Enzymes using or making ATP always employ Mg^{2+}. ATP and ADP can pass freely in and out of mitochondria *via* an adenine nucleotide translocase which is an antiport transport protein for ATP and ADP. (In tightly coupled mitochondria, the rate of ADP entry controls the rate of oxygen consumption.)

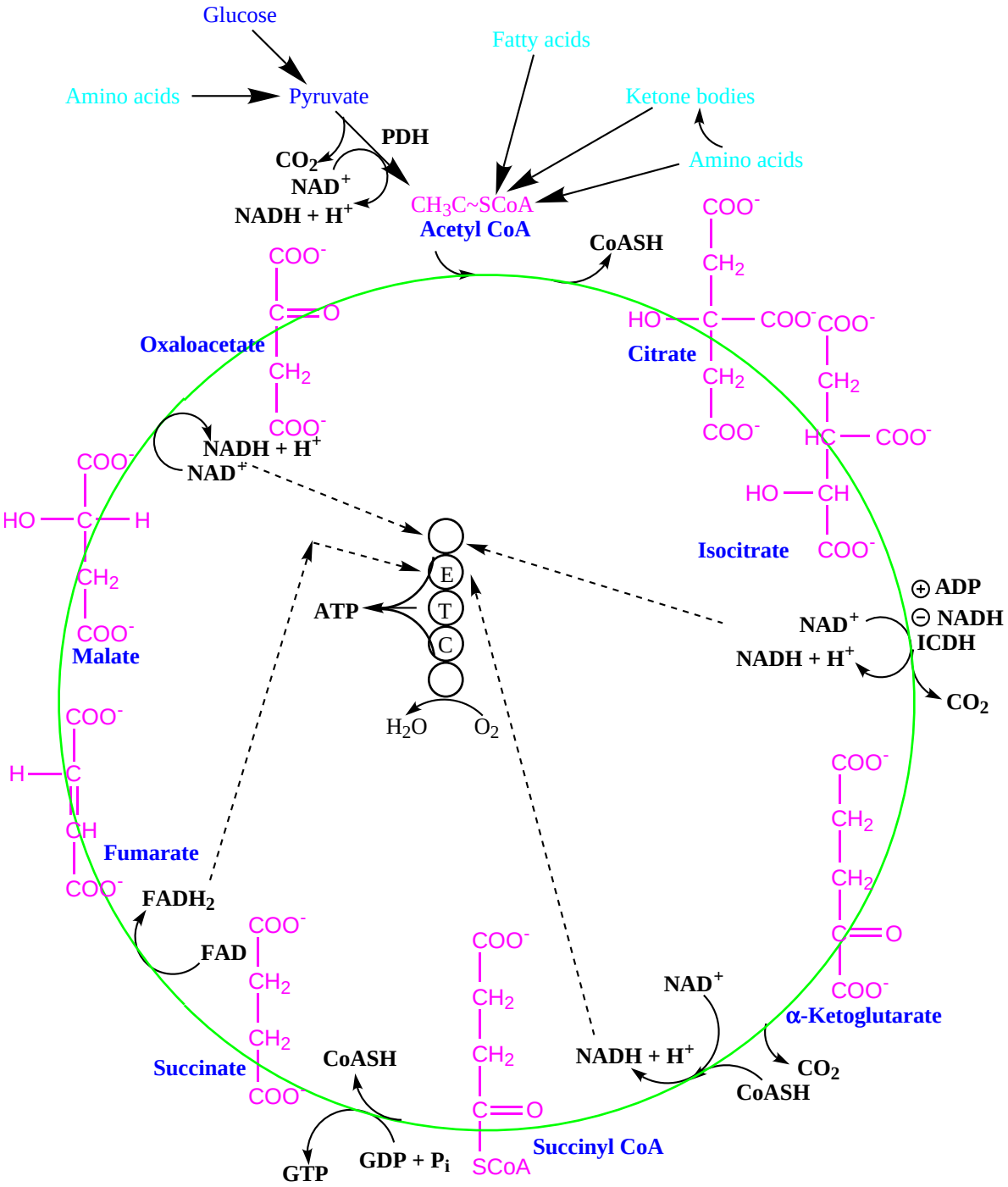
Pyruvate dehydrogenase

Pyruvate dehydrogenase links glycolysis to the TCA cycle. It is a large **multienzyme complex** made up of 5 enzymatic activities (in three different proteins) and uses 5 coenzymes: **thiamine pyrophosphate**, **lipoic acid**, FAD, NAD, **Coenzyme A**. It is a key utilizer of a number of vitamins. Thiamine pyrophosphate is the coenzyme for most oxidative decarboxylations. Hence, it is sometimes called cocarboxylase. Lipoic acid is a cofactor, typically associated with thiamine pyrophosphate, that functions in redox reactions while attached to an enzyme by an amide linkage to the ε-amino group of a lysine residue.

Pyruvate dehydrogenase performs the oxidation of pyruvate to **acetyl CoA** with the liberation of **CO_2**. Note that this is the first point in the "burning" of sugar where CO_2 appears. Essentially the three carbon fragments produced by aldolase have now been converted to an acetyl group attached to Coenzyme A and free CO_2. The acetyl CoA will now enter the TCA cycle to be further oxidized.

TCA Cycle

The TCA (tricarboxylic acid) cycle consists of a closed circle of reactions starting with using oxaloacetate and acetyl CoA to form citrate and ending with reformation of oxaloacetate. The two carbons of the acetyl group in acetyl CoA are liberated as CO_2. The reducing equivalents obtained from the oxidation are passed on to the electron transport chain to be converted into ATP.



Citrate synthase synthesizes **citric acid (citrate)** by coupling the acetyl group of acetyl CoA to **oxaloacetate**. The oxaloacetate is the final species in the **citric acid cycle**. Thus by coupling it to acetyl CoA the cycle is completed and started by citrate synthase.

Aconitase converts citrate to isocitrate (sometimes called aconitic

acid, thus the name of the enzyme).

Isocitrate dehydrogenase oxidizes **isocitrate** to α-ketoglutarate with the concomitant release of CO_2. This is the second molecule of CO_2 released in the breakdown of the three carbon fragments of glucose. The reducing equivalents from the oxidation are captured in NADH.

α-ketoglutarate is similar to pyruvate and **α-ketoglutarate dehydrogenase** catalyses an oxidation similar to that catalyzed by pyruvate dehydrogenase yielding succinyl CoA, the final molecule of CO_2, and reducing equivalents are captured in NADH.

Succinyl CoA synthase converts **succinyl CoA** to succinic acid and the energy of the CoA derivative is captured by the coupled synthesis of **GTP**, the guanosine analog of ATP. This is another example of **substrate level phosphorylation** since the formation of GTP does not require the electron transport chain of the mitochondria.

Succinate dehydrogenase differs from the other enzymes in the TCA cycle in that it is an integral membrane protein in the mitochondria. It is also a **flavoprotein**. The reducing equivalents are captured in **$FADH_2$**, not NADH, and are fed directly into the electron transport chain. The product of the oxidation of **succinate** is fumarate.

Fumarase catalyzes the isomerization of **fumarate** to malate. There is no energy change associated with this reaction. This is simply a conversion necessary for the last oxidation.

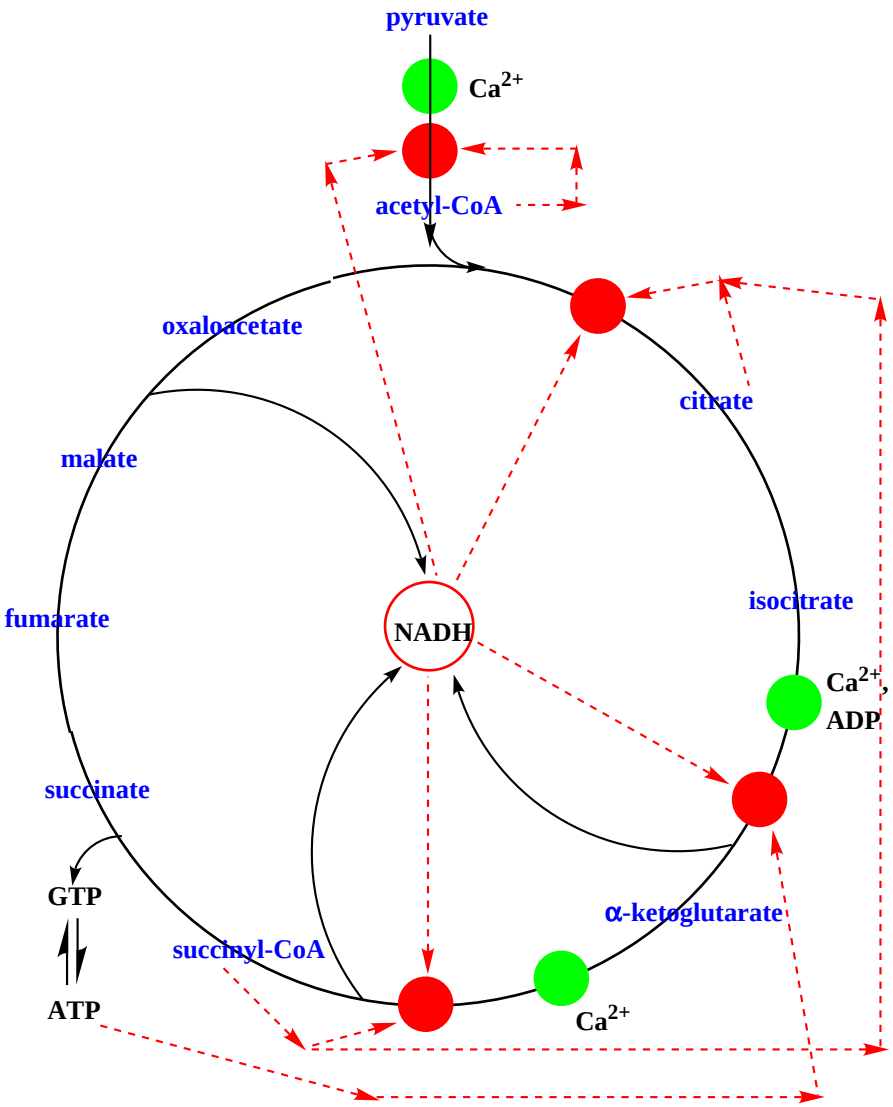
Malate dehydrogenase oxidizes **malate** to oxaloacetate. The reducing equivalents are put into NADH. The resulting oxaloacetate is now ready to react with another incoming acetyl CoA to begin the cycle again.

Note that there is no oxygen is utilized in the TCA cycle. Oxygen enters oxidative metabolism at the very last step (**see cytochrome oxidase below**).

Regulation of the TCA cycle

There are four primary points of regulation of the citric acid cycle (see the figure below; red circles are points of inhibition, red dashed lines signify inhibitory intermediates of the pathway, green circles are activators). One is prior to entry at the pyruvate dehydrogenase complex. The specific pyruvate dehydrogenase component of this complex is inhibited by GTP and activated by AMP. The enzyme complex is also controlled by covalent modification. It is inactive when the pyruvate dehydrogenase component is phosphorylated by a specific kinase which is part of the complex. The enzyme complex is

inactive until the phosphoryl group is removed by a specific phosphatase. The kinase is inhibited by pyruvate and ADP. Thus, increased levels of pyruvate act to activate the dehydrogenase activity by inhibiting the kinase. Finally, the products, acetyl coenzyme A and NADH inhibit the complex directly by product inhibition.



Citrate synthase provides another control point. It is inhibited by citrate and to a lesser extent by NADH. Succinyl-CoA competes with acetyl-CoA for the enzyme. **Please note** that Devlin (p. 233) indicates that the purified enzyme is inhibited by ATP, NADH, succinyl-CoA, and long-chain acyl-CoA derivatives.

Another regulatory enzyme is isocitrate dehydrogenase. ADP is an allosteric activator of this enzyme, while ATP inhibits the enzyme. NADH inhibits it by directly displacing NAD^+.

The last point of control is α-ketoglutarate dehydrogenase, which is inhibited by GTP (ATP), NADH (product inhibition), and succinyl-CoA. This is another multienzyme complex and some of its components are the same as those in the pyruvate dehydrogenase complex.

Figure 6.23 in Devlin (p. 239) illustrates some of these controls. The cycle, whose main purpose is to produce ATP when coupled with the respiratory chain and oxidative phosphorylation, is inhibited by the high-energy compounds GTP and ATP, by the product that feeds into the respiratory chain (NADH) and by specific intermediates (acetyl-CoA and succinyl-CoA); it is stimulated by the products of ATP utilization (ADP and AMP) and by pyruvate which feeds into the cycle.

Among inhibitors of the TCA cycle is the arsenite ion which inhibits the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes. (See Clinical Correlation 7.2 in Devlin, p.283)

Stoichiometry of the TCA cycle

The citric acid cycle satisfies stoichiometric requirements for the complete oxidation of pyruvate by oxygen to carbon dioxide and water, viz.

Oxidation of pyruvate

$$CH_3\text{-}CO\text{-}COO^- + 5\,[O] + H^+ \Leftrightarrow 3\,CO_2 + 2\,H_2O$$

The uptake of 5 atoms of oxygen corresponds to the removal of 5 pairs of electrons during operation of the cycle. Later the electrons are transferred to oxygen. Note that oxygen is not directly utilized in the TCA cycle. Rather, it acts as the electron acceptor at the end of the electron transport chain. For each atom of oxygen reduced, one molecule of water is formed. Because 3 molecules of water are taken up during operation of the cycle, the net change corresponds to the formation of 2 moles of water per mole of pyruvate oxidized.

The Respiratory Chain

The **respiratory chain (or electron transport chain (ETC))** is a group of proteins located in the inner membrane of the mitochondria which channel **reducing equivalents** to oxygen. Reducing equivalents are the electrons (and sometimes protons) which are lost by a substrate when it is oxidized and, thus, are capable of reducing another compound.

Electrons flow through the respiratory chain from a high energy level to a low energy level, much as water flows downhill. The electron transport chain serves as a conduit, or pipe, through which the reducing equivalents may flow, and simultaneously capture a portion of the

energy, similar to a turbine in a hydroelectric plant which converts the downhill flow of water to electricity. In this case, the flow of reducing equivalents is coupled to pumping protons across a membrane to create a proton concentration gradient that can be utilized elsewhere to do work and synthesize ATP. A pair of electrons and two protons are removed from a substrate by a dehydrogenase, resulting in oxidation of the substrate. The electrons and one proton (from NADH) are passed together through the first part of the chain to **coenzyme Q (ubiquinone)**. Then the electrons are passed through the cytochrome system, finally reducing an atom of oxygen. The reduction of oxygen by the reducing equivalents results in the formation of water:

$$[O] + 2\ e^- + 2\ H^+ \Leftrightarrow H_2O$$

There are three questions that need to be considered:

a) where do the reducing equivalents which feed the respiratory chain come from?;

b) what are the members of the respiratory chain which channel the reducing equivalents?; and

c) what reactions are involved that permit the capture of energy?

The origin of reducing equivalents

The product of glycolysis is pyruvate which is transported into the mitochondria. Pyruvate releases energy during its oxidation to CO_2 in the tricarboxylic acid cycle. The reducing equivalents then enter the respiratory chain.

Biological oxidations, like any other oxidations, involve the loss of electrons. Frequently, protons are lost at the same time, i.e., hydrogen atoms are removed. The enzymes that catalyze these reactions are, therefore, called dehydrogenases. There must be an acceptor for the electrons. When something is oxidized, something else must be reduced. The actual electron acceptor in the dehydrogenases is one of several coenzymes (usually NAD^+ or FAD).

Dehydrogenases catalyze the oxidation of substrates

Many oxidation enzymes use NAD^+ as a coenzyme. For example, the enzyme **alcohol dehydrogenase** has NAD^+ as a coenzyme and mediates the following reaction.

$$CH_3\text{-}CH_2OH \Leftrightarrow CH_3\text{-}CHO + 2\ H^+ + 2\ e^-$$

ethanol (an alcohol) **acetaldehyde** (an aldehyde)

In this reaction, ethanol looses two hydrogen atoms - two protons and two electrons. These are the reducing equivalents (for further details, **see discussion of this in Module 1**). One proton and two electrons are transferred to NAD^+ to give NADH, and H^+ is left over in solution. The overall reaction can be represented as:

$$\text{Ethanol} + NAD^+ \Leftrightarrow \text{Acetaldehyde} + NADH + H^+$$

NAD^+ (NADH) is a pyridine nucleotide. It is non-covalently bound to the dehydrogenase and may dissociate to transfer the reducing equivalents to other sites, most importantly to the electron transport chain.

Flavoproteins

A **flavoprotein** is a protein whose coenzyme is either FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide). FAD or FMN may be covalently bound or loosely bound to enzymes, depending on the particular enzyme. When FAD is reduced to $FADH_2$, the conversion involves the net addition of 2 hydrogen atoms (i.e., 2 H).

Cytochromes and what they do

Cytochromes are proteins that are found in virtually all aerobic organisms. They contain a prosthetic group which is related to the porphyrin ring system. The porphyrin molecule is a flat, conjugated, aromatic and extremely stable ring system that can chelate metal ions. They are highly colored compounds typically being yellow or orange. It is the metal that participates in transferring reducing equivalents in the cytochromes.

A variety of substituents are found around the perimeter of the rings. In protoporphyrin there are 3 types of substituents on the ring which allow 15 isomers. One isomer is called **protoporphyrin IX**. Protoporphyrin IX is part of the prosthetic group of cytochrome *b* (as well as hemoglobin and myoglobin). The protoporphyrin ring in cytochrome *c* is linked covalently to cysteine residues in the protein. A complex between a protoporphyrin and Fe^{2+} is called **heme**. A complex between a protoporphyrin and Fe^{3+} is called hemin. Hemoglobin (the protein which transports oxygen in the blood) is a heme compound, i.e., the Fe is in the 2+ state.

While the iron in hemoglobin remains as Fe^{2+} during its function, when the cytochromes are oxidized, the iron changes from 2+ to 3+. This may be represented as:

$$\text{Cyt } (Fe^{2+}) \Leftrightarrow \text{Cyt } (Fe^{3+}) + e^-$$

There are several cytochromes in the respiratory chain, viz. ***b*$_1$, *c*$_1$, *c*, *a*, and *a*$_3$**. Thus, the oxidized form of cytochrome c_1 is represented as Cyt $c_1(Fe^{3+})$ while the reduced form of cytochrome c_1 is represented as Cyt $c_1(Fe^{2+})$.

Non-heme iron proteins

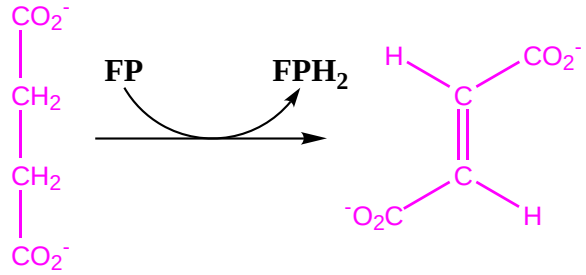
Non-heme iron proteins contain iron which is not associated with a porphyrin ring system. The iron is attached to the protein through iron sulfur clusters, typically [2Fe-2S] or [4Fe-4S] clusters attached to the protein by coordination with the sulfur of cysteine sulfhydryl groups. Such proteins are often referred to as **iron-sulfur proteins**. The iron atoms can transfer electrons by existing in either oxidized or reduced states. They are usually closely associated with flavoproteins.

Four complexes in the Chain

There are four protein complexes which make up the electron transport chain. Complex I is the NADH dehydrogenase complex which receives the reducing equivalents from NADH (from the TCA cycle). It is a flavoprotein containing both **FMN** and **non-heme iron (iron-sulfur) centers**. The reducing equivalents exit the complex and are passed to CoQ, which is freely soluble in the mitochondrial membrane and diffuses to Complex III. Complex II is the succinate dehydrogenase discussed below. The reducing equivalents from Complex II are also passed to CoQ. Complex III is a cytochrome *b* and c_1 complex, which accepts the electrons from ubiquinone (CoQ). The electrons are passed from Complex III to cytochrome *c*, a protein loosely associated with the membrane. Cytochrome *c* acts as an electron carrier to transport the electrons to Complex IV, also known as cytochrome oxidase and contains cytochrome aa_3. **Cytochrome oxidase** catalyzes the reduction of O_2 to H_2O. This is the only point where O_2 is utilized in oxidative metabolism of sugars.

The respiratory chain may be entered at two points

Succinate feeds reducing equivalents directly into the chain via the following reaction:



succinate **fumarate**

This bypasses the NADH dehydrogenase reaction. In this case reducing equivalents enter the respiratory chain through a flavoprotein and only 2 ATP molecules are made rather than 3. The same is true of reducing equivalents from NADH that enter the respiratory chain via the glycerol phosphate shuttle.

Oxidative Phosphorylation

Oxidative phosphorylation is the process by which ATP is generated as a result of the flow of reducing equivalents from NADH through the respiratory chain. This is one of the most important ATP producing reactions in higher organisms. The free energy released by the respiratory chain is used to convert ADP plus P_i to ATP

The oxidation of NADH by the respiratory chain can be written as follows:

$$NADH + H^+ + 1/2\ O_2 \Leftrightarrow NAD^+ + H_2O + \text{free energy}$$

The net phosphorylation of ADP is given by:

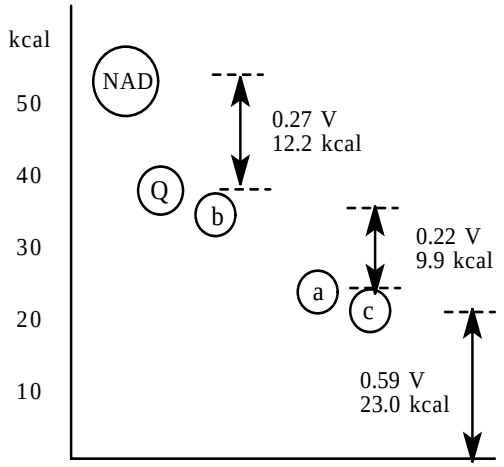
$$\text{free energy} + P_i + ADP \Leftrightarrow ATP$$

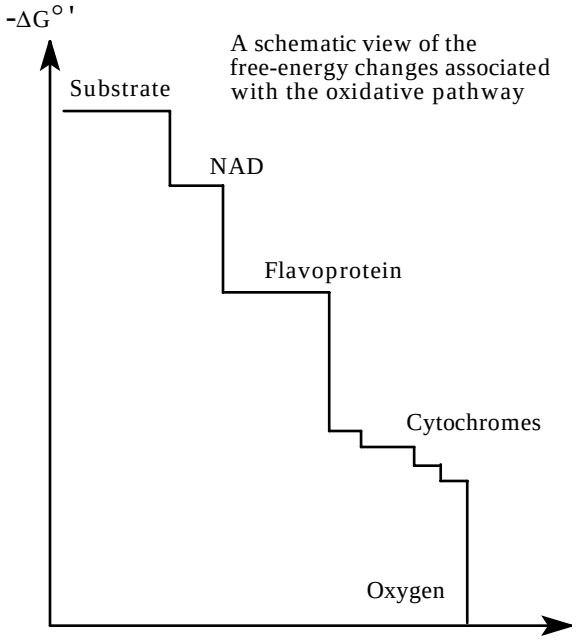
These two processes are tightly coupled in oxidative phosphorylation.

The respiratory chain is composed of three **coupling sites**. A coupling site is a location in the respiratory chain where the transfer of reducing equivalents is coupled to the generation of ATP. (Note: ATP is not generated at the coupling site.)

$NAD^+ \Leftrightarrow Fp$	- Site 1
Cyt *b* $\Leftrightarrow$ Cyt c_1	- Site 2
Cyt $aa_3 \Leftrightarrow O_2$	- Site 3

The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Each of the three segments yields sufficient energy to generate a molecule of ATP from ADP and phosphate





A schematic view of the free-energy changes associated with the oxidative pathway

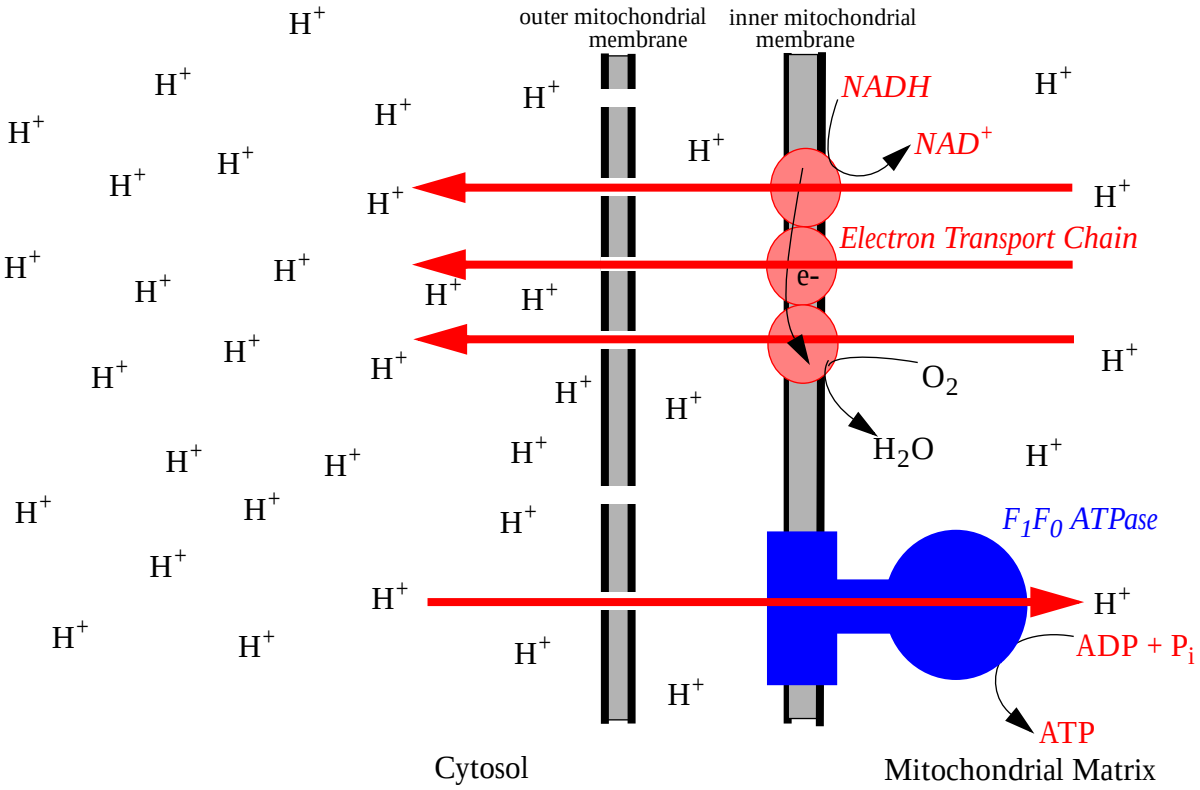
There are two electron carriers that bridge these sites – **CoQ** between site 1 and site 2 and cyt *c* between site 2 and site 3. O_2 is the ultimate electron acceptor after site 3.

When all three coupling sites are utilized, the **maximum** yield of ATP per 1/2 O_2 used is 3. The ratio (P_i converted to ATP) / (1/2 O_2 [flux of 2e]) is called the **P/O ratio**.

Note that the P/O ratio is 2 (**maximum** yield of ATP per 1/2 O_2 used is 2) for the oxidation of succinate since the reducing equivalents enter the respiratory chain after the first coupling site.

The efficiency of oxidative phosphorylation is not the same for each coupling site. The diagrams above show the decline in free energy as electron pairs flow down the respiratory chain to oxygen and the three segments that yield sufficient energy to generate a molecule of ATP.

Chemiosmotic Coupling of the Electron Transport Chain to the F_1F_0 ATPase



Oxidative phosphorylation is coupled to the electron transport chain via a **proton concentration gradient** using a **chemiosmotic coupling mechanism** (see figure above). The three **"coupling sites"** in the respiratory chain actually contain **proton pumps** such that electron transport through the site results in a pumping of protons across the mitochondrial membrane from the matrix into the cytosolic space. This results in a proton concentration gradient or **proton motive force** across the inner mitochondrial membrane. The

increased concentration of protons in the cytosol creates an osmotic force or potential across the membrane attempting to drive the protons back to equilibrium (i.e. equal concentrations on both sides). The situation is similar to a battery in that the energy of the reducing equivalents is stored in a proton concentration gradient. Maintaining the concentration gradient is similar to the charging of a battery. The drive to dissipate the non-equilibrium state across the membrane pushes protons through any available pore in the inner mitochondrial membrane. The most important here is the **F_1F_0 ATPase**. A proton passing through the ATPase is tighty coupled to the synthesis of ATP. This is the site of ATP synthesis in oxidative phosphorylation, a site distinct from the electron transport chain and the TCA cycle, but coupled to them via the proton concentration gradient across the mitochondrial membrane. ATPase are normally thought of as enzymes which break down ATP to do work. This one functions in the "reverse" direction to use the energy of proton flux to make ATP.

Coupled and uncoupled mitochondria

The flux of reducing equivalents through a coupling site leads to proton pumping across the membrane, and ultimately causes P_i + ADP to form ATP. Since the flux of reducing equivalents is geared to the formation of ATP, a restriction at any part of the flow, or a lack of any single necessary component (such as ADP), could stop the process. If ADP is left out and the available ATP is consumed, will there be increased uptake of oxygen to produce more ATP? The answer is normally "no", even though all other components are present (substrate, oxygen, mitochondria, buffer, P_i), because the cycle needs ADP to allow a flux of reducing equivalents. Such mitochondria are called tightly coupled.

The coupling mechanism imposes a restraint on the flux of reducing equivalents. Reducing equivalents cannot flow through the ETC if all of the necessary components are not available further along in the mechanism. Thus, if ADP or P_i are diminished there will be no uptake of oxygen since there is no flow of protons through the F_1F_0 ATPase, the proton gradient is maximally charged, and so the flux of reducing equivalents through the respiratory chain comes to a halt.

2,4-Dinitrophenol (DNP) is a classic **uncoupling agent**. DNP functions by allowing H^+'s to pass through the membrane and bypass the F_1F_0 ATPase. It is observed that when DNP is added to a system such as that described in the preceding paragraph, there is a sudden burst of respiration (oxygen uptake). Clearly, in the presence of DNP, the obligatory formation of ATP from ADP plus P_i no longer imposes a restraint on the flux of reducing equivalents through the respiratory chain, i.e., the flux of reducing equivalents is no longer

coupled to the formation of ATP. The energy (chemical potential) generated by the flow of reducing equivalents can no longer be used to join P_i to ADP. Such mitochondria are said to be **uncoupled** and DNP is called an uncoupling agent. In other words, in the presence of DNP, the transfer of electrons from NADH or $FADH_2$ to oxygen is no longer coupled to the production of ATP, and no ATP is made.

Ionophores

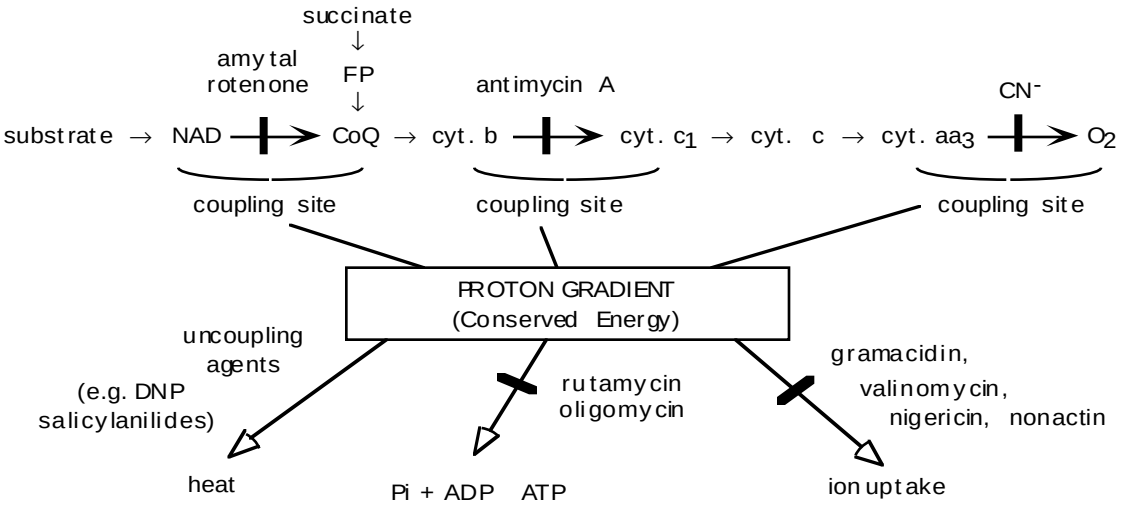
Since the protons used to create the chemiosmotic force across the inner mitochondrial membrane have a positive charge, there is an electric component to the energy difference that makes the outside positively charged relative to the inside of the mitochondrion. **Ionophores** can perturb or dissipate the electric component of the chemiosmotic gradient by transporting ions across the mitochondrial membrane. **Valinomycin** is an ionophore with high specificity for K^+. Note that if there is pre-existing K^+ gradient, valinomycin will generate a potential across the membrane, i.e. it can be electrogenic. **Nigericin** can move K^+ and H^+ ions in opposite directions, i.e. it is an electroneutral antiporter. **Gramicidin** forms a channel in the membrane with little specificity. It therefore can dissipate both a pH and charge gradient across the membrane.

Inhibition of ATP formation

Besides uncoupling agents, there are also respiratory inhibitors and inhibitors of phosphorylation. Respiratory inhibitors are compounds that prevent the flow of electrons through the chain. **Cyanide** and **carbon monoxide (CO)** are two; they bind to cyt aa_3 and prevent the flow of electron from cyt aa_3 to oxygen. Another is the antibiotic **antimycin** which blocks the flux of reducing equivalents from cyt *b* to cyt c_1. Other respiratory inhibitors are **rotenone** and **amytal** which block site I.

Inhibitors of phosphorylation block oxidative phosphorylation. Two of these are the antibiotics **oligomycin** and rutamycin. These function by acting at the F_1F_0 ATPase level preventing its function.

These relationships are diagrammed in the Figure below.



Efficiency of Mitochondrial Reactions

The citric acid cycle is combined with electron transport and oxidative phosphorylation to provide most of the energy used by the cell. Hence, the mitochondria are often called the "powerhouses of the cell". Evidence of this is their high concentration in muscle and around the base of the flagellum in sperm where they supply the flagellum with energy in the form of ATP for propulsion of the spermatozoan.

In the mitochondria, there is a close association of enzymes of the tricarboxylic acid cycle with enzymes involved in reoxidation of reduced coenzymes and prosthetic groups, i.e., components of electron transfer chain from NADH and $FADH_2$ to oxygen. The energy is transferred to ATP. Consequently, only catalytic amounts of the various cofactors are needed for synthesis of relatively large amounts of ATP in a tightly coupled mitochondria.

The energy liberated in the course of the removal and transfer of electrons during oxidation of a substrate is either stored temporarily in a high-energy bond (ATP) or directly dissipated as heat. Heat cannot be used to do useful work in a living cell because to do so it would have to flow from a body of higher temperature to one of lower temperature. Living cells clearly operate at constant temperature.

The transfer of electrons from substrate to oxygen liberates much more energy than this, so electrons are transferred in a series of steps rather than in one step. Each step permits the formation of a high-energy phosphate bond by a process that couples electron transfer to oxidation phosphorylation. By this means, a large proportion of the

liberated energy of the substrate is stored and relatively little is "wasted" as heat. This stepwise system is very characteristic of biological process. It allows energy production to occur very efficiently so that a high percentage of the energy can be recovered as useful work.

The stoichiometry of ATP formation from acetyl-coenzyme A and from pyruvate

Twelve ATP molecules are produced from each acetyl-coenzyme A molecule that enters the TCA cycle. Their origin is shown in the table below.

Table 1: ATP Formation from Acetyl CoA

Reaction Type	Reaction	Cofactor reduced	Yield ATP/ mole acetyl-CoA
substrate-level phosphorylation	succinyl-CoA → succinate	none	1 (as GTP)
electron transport (oxidative phosphorylation)	isocitrate → α-ketoglutarate	NAD	3
	α-ketoglutarate → succinyl-CoA	NAD	3
	succinate → fumarate	FAD	2
	malate → oxaloacetate	NAD	3
TOTAL			12

Catabolism of carbohydrate produces pyruvate. Two moles of pyruvate are made for each mole of glucose entering the pathway of glycolysis. The conversion of these two moles of pyruvate into two moles of acetyl-CoA is accompanied by the reduction of two moles of NAD. Its reoxidation in the electron transport chain results in the production of 6 (2 x 3) moles of ATP. Thus, each two moles of pyruvate (produced from one mole of glucose) that enter mitochondria results in the production of 30 moles of ATP. Six of these originate from the oxidation of pyruvate and the remaining 24 from the TCA cycle.

How much ATP is produced by the complete oxidation of glucose, via glycolysis and the TCA cycle?

Table 2: Total ATP Production

Reaction	ATP Formation	
	substrate level	oxidative phosphorylation
glycolysis	2	
2 NADH		4 or 6*
pyruvate → acetyl CoA		
2 NADH		6
citric acid cycle	2	
6 NADH		18
2 $FADH_2$		4
subtotal	4	32 or 34*
TOTAL = 36 or 38* ATP		

*In muscle and nerve cells, the NADH molecules produced in glycolysis, which occurs outside mitochondria in the cytoplasm, yield only 2 molecules of ATP each rather than 3 because the reducing equivalents must enter the mitochondria by a the glycerol phosphate shuttle. In liver and heart cells, the malate-aspartate shuttle allows about 6 ATP molecules to be formed from reoxidation of the 2 NADH molecules formed in glycolysis. These shuttles are outlined in Devlin's text (p. 267).

38 ATP x 11 kcal/mole of ATP = 418 kcal

Total amount of energy released on oxidation of 1 mole of glucose = 686 kcal

$$\frac{418,000 \text{cal}}{686,000 \text{cal}} \times 100 = 61\% \text{ efficiency.}$$

How much ATP really forms?

This Study Guide (and Devlin) have indicated that 36 or 38 molecules of ATP are produced from the complete oxidation of one mole of glucose. However, recent texts indicate modern P/O ratios of 2.5 for $NADH + H^+$ and 1.5 for $FADH_2$, which suggest that 29.5 or 31 molecules of ATP are produced when the glycerol phosphate or malate-aspartate shuttles, respectively, are used.

By reviewing Salway (pp. 14-21), we see that the key lies with the transport of 4 H^+ for every ATP produced. The operation of complexes I-IV results in the production of 10 H^+, while the running of the ATPase requires 4 H^+ per ATP synthesized. This gives a P/O ratio of 10/4 = 2.5 ATP. If we consider that one proton is required for the glutamate/aspartate carrier, the P/O ratio becomes 9/4 = 2.25 ATP.

Bypassing complex I by shuttling reducing equvalents ($FADH_2$) through complex II, generates 6 H^+. The P/O ratio is 6/4 = 1.5 ATP. No protons are diverted for carriers.

Enzyme Localization

Mitochondria do a number of other things in addition to those already described. To allow them to carry out their varied reactions and to maintain such a high degree of efficiency, they have a unique and highly organized structure. The location of all mitochondrial enzymes has not been determined but more than 50 have been localized. Those that have been discussed here are given below:

Table 3: Mitochondrial Enzyme Localization

Outer membrane	Intermembrane space	Inner membrane	Matrix
monoamine oxidase	adenylate kinase	succinate dehydrogenase	citrate synthase
kynurenine hydroxylase	nucleoside diphosphate kinase	respiratory chain components (cytochromes *b*, *c*, c_1, *a*, a_3)	aconitase

Table 3: Mitochondrial Enzyme Localization

Outer membrane	Intermembrane space	Inner membrane	Matrix
nucleoside diphosphate kinase		mitochondrial α-glycerol phosphate dehydrogenase (flavoprotein)	isocitrate dehydrogenase
phospholipase A		F_1-ATPase	fumarase
fatty acyl-CoA synthetases		NADH dehydrogenase	malate dehydrogenase
NADH: cytochrome c reductase (rotenone insensitive)		β-hydroxybutyrate dehydrogenase	pyruvate dehydrogenase
choline phosphotransferase		carnitine: acyl-CoA transferase	α-ketoglutarate dehydrogenase
		adenine nucleotide translocase	succinyl-CoA synthetase
		mono, di, and tricarboxylate translocase	malate dehydrogenase
		glutamate-aspartate translocase	fatty acid oxidation system
			glutamate dehydrogenase
			glutamate-oxaloacetate transaminase
			ornithine transcarbamoylase

Pyruvate dehydrogenase and α-ketoglutarate have been located in the matrix.

The enzymes of the respiratory chain and oxidative phosphorylation are located on the inner membrane, while many of the enzymes of the TCA cycle, but not all, are located in the matrix.

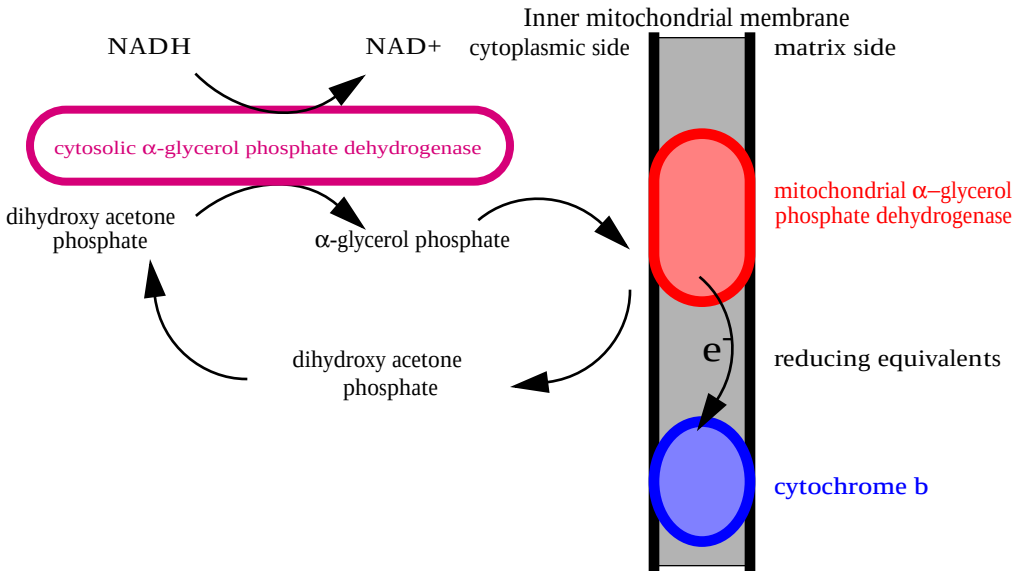
None of the enzymes of the TCA cycle, the respiratory chain, or oxidative phosphorylation have been found in the outer membrane or the intermembrane space (outer compartment).

See Devlin, pp. 238-246 for a description of mitochondrial structure and transport systems.

Glycerol Phosphate Shuttle

The α-glycerol phosphate shuttle operates in muscle and nerve to move reducing equivalents from the cytoplasm (generated by glycolysis) into mitochondria. An outline is given in the figure below. The reducing equivalents from NADH are transferred to α-glycerol phosphate by reducing dihyroxyacetone phosphate using the cytosolic glycerol phosphate dehydrogenase. α-glycerol phosphate then diffuses to the inner mitochondrial membrane where there is a membrane bound mitochondrial glycerol phosphate dehydrogenase. The previous reaction is reversed so that now the glycerol phosphate is oxidized and the reducing equivalents are injected into the electron transport chain at site II. This recycles the dihydroxyacetone phosphate permitting more NADH to be oxidized and more reducing equivalents to be moved into the mitochondria. Note that by entering the electron transport chain at site II, the traditional P/O ratio is decreased to 2 maximum, i.e. only 2 ATP are made per NADH oxidized. Recall that the modern P/O ratio is 6/4 = 1.5 for the glycerol phosphate shuttle.

α-glycerol phosphate shuttle

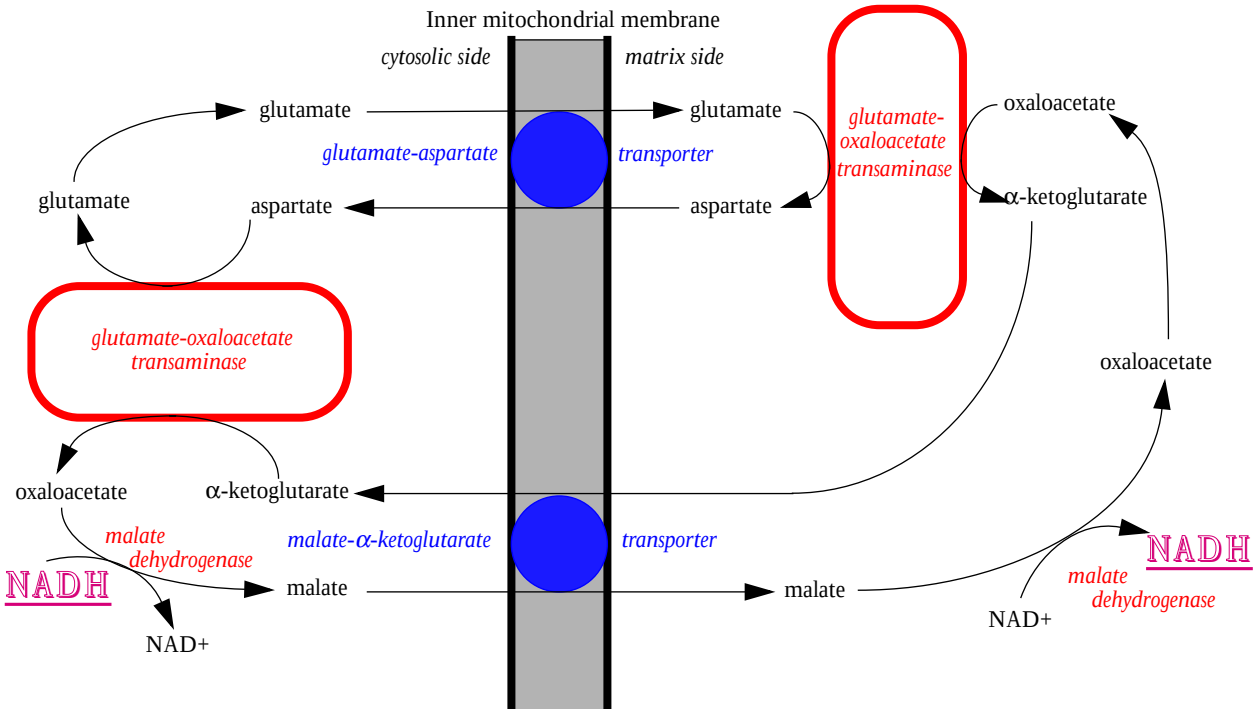


Malate-Aspartate Shuttle

The malate-aspartate shuttle functions in liver and heart to move reducing equivalents from the cytoplasm into the mitochondria. Two different transporters exchange glutamate with aspartate and malate with α-ketoglutarate. A transaminase in the mitochondrial matrix permits the transfer of an amino group from glutamate to oxaloacetate to make α-ketoglutarate and aspartate. The reverse process occurs in the cytosol. Malate dehydrogenases in both the cytosol and

matrix permit the interconversion of malate and oxaloacetate. The net reaction is the movement of reducing equivalents in NADH from the cytosol to the matrix where it can be utilized at the electron transport chain. Since the product is NADH in the matrix, this can enter site I in the electron transport chain and give a maximum of 3 ATP's per NADH, or a P/O ratio of 3. Recall that the modern P/O ratio is 10/4 = 2.5.

Malate-Aspartate Shuttle



Module III: Other Pathways of Carbohydrate Metabolism.

Objectives:

1. Describe the function of lactate formation. Describe the Cori cycle and give its function.
2. Describe the hexose monophosphate pathway with particular reference to the oxidative and regenerative phases. Identify the principal and secondary function of the HMP pathway.
3. Discuss, in general terms, drug-induced erythrocyte glucose-6-phosphate dehydrogenase deficiency.
4. Describe the structural and functional differences of the pyridine nucleotide, NAD and NADP. How do they differ energetically?
5. Describe and explain the clinical symptoms of galactosemia. Describe how galactosemia is managed. Give the pathway for galactose metabolism. Identify the enzyme that is defective or absent in the two forms of galactosemia.
6. Describe and explain the clinical symptoms of fructose intolerance. Describe the management of fructose intolerance. Identify the enzyme that is defective or absent in patients who are fructose intolerant.
7. Account for the presence of ethanol in the blood of a normal individual. Describe the routes of ethanol elimination from the body. Prioritize these in terms of importance.
8. Use the above objectives and previous objectives to solve new problems.
9. Define and use correctly the terms in the **Nomenclature and Vocabulary** list. Be able to answer questions such as those in the Practice Exam which pertain to this Problem Unit.

Nomenclature and Vocabulary:

6-phosphogluconate dehydrogenase
aldolase A, B, and C
Cori cycle
erythrose-4-P
fructokinase
fructose-6-P
fructose-1-P
fructose intolerance
G6PDH deficiency
galactokinase

galactose-1-phosphate uridyl transferase **galactose-1-P**
galactosemia **glucose-6-phosphate dehydrogenase**
HMP **pentose phosphate epimerase**
pentose phosphate pathway **respiratory quotient**
ribose-5-phosphate isomerase **ribulose**
seduheptulose-7-P **transaldolase**
transketolase **UDP galactose**
UDP-glucose **UDP-galactose-4-epimerase**
xylulose

STUDY GUIDE - III

Hexose Monophosphate Pathway

The **hexose monophosphate (HMP)** pathway is also called the **pentose phosphate pathway or shunt**.

The overall reaction of the HMP pathway is as follows:

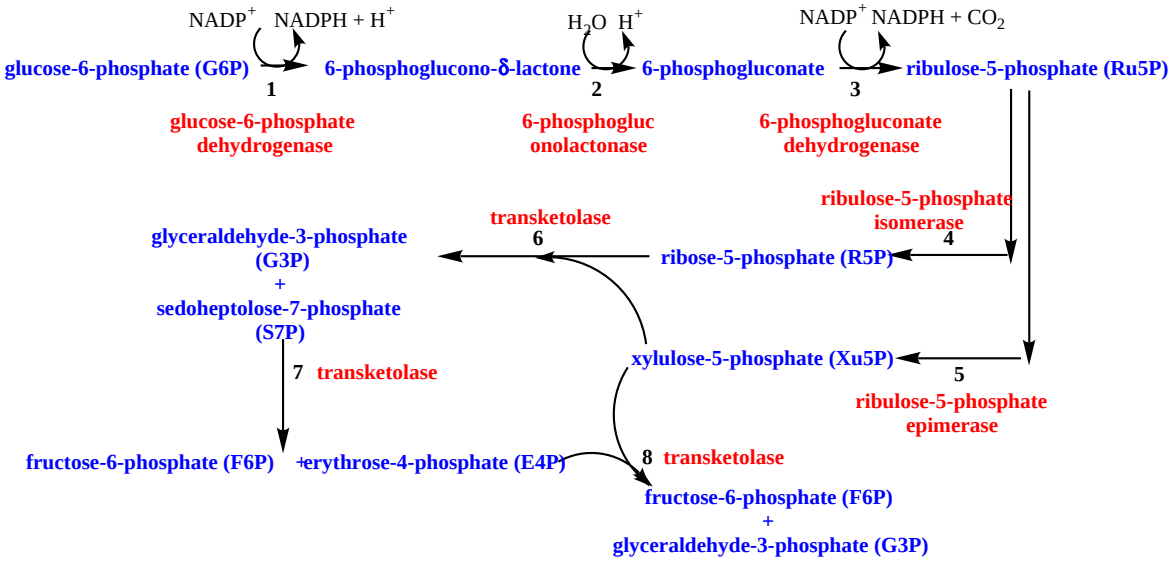
$$G\text{-}6\text{-}P + 12\ NADP^+ \Leftrightarrow 6\ CO_2 + 12\ NADPH + 12\ H^+ + P_i$$

The pathway should be viewed in two stages:

1) There are two oxidative steps that produce all the NADPH. (The pathway can be balanced most easily by starting with 6 moles of glucose-6-phosphate.) Thus, in the two oxidative steps, 6 moles of hexose phosphate (6 x C_6 = 36C) are converted into 6 moles of pentose phosphate (6 x C_5 = 30C) and 6 moles of carbon dioxide.

2) In the regenerative or shuffling phase, the 6 moles of pentose phosphate (30C) are converted into 5 moles of hexose phosphate (30C). Thus, the net reaction is the conversion of 1 mole of hexose phosphate into 6 moles of CO_2.

The regenerative phase merely involves a reshuffling of the carbons in six 5-carbon sugars to give five 6-carbon sugars.



Oxidative Phase of HMP Pathway

The first reaction in the oxidative phase is the oxidation of glucose-6-phosphate by **glucose-6-phosphate dehydrogenase** to give 6-phosphogluconolactone. The reducing equivalents are passed to $NADP^+$ to give NADPH, which is similar to NADH except for an additional phosphate (**see NAD structure above**). **Lactonase** opens the lactone ring to give 6-phosphogluconate. We can see now that the oxidation of the aldose aldehyde has produced an acid. **6-phosphogluconate dehydrogenase** then oxidizes the acid to ribulose-5-phosphate. The oxidation of the acid leads to release of CO_2 and shortening of the hexose to a pentose along with capture of the reducing equivalents into NADPH. These three reactions essentially constitute the energy production part of the HMP pathway.

Interconversion reactions

The remaining reactions in the HMP pathway take six ribulose-5-phosphates and convert them to 5 glucose-6-phosphates, permitting another oxidative cycle. In addition, some of the sugars made in the shuffling can be used for synthetic purposes, e.g. ribose-5-phosphate is needed for nucleotide synthesis.

ribose-5-phosphate isomerase

ribulose-5-phosphate ⇔ ribose-5-phosphate

phosphopentose epimerase

ribulose-5-phosphate ⇔ **xylulose**-5-phosphate

transketolase

xylulose-5-phosphate + ribose-5-phosphate ⇔

sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate

transaldolase

xylulose-5-phosphate + ribose-5-phosphate ⇔
fructose-6-phosphate + **erythrose-4-phosphate**

transketolase

erythrose-4-phosphate + xylulose-5-phosphate ⇔
fructose-6-phosphate + glyceraldehyde-3-phosphate

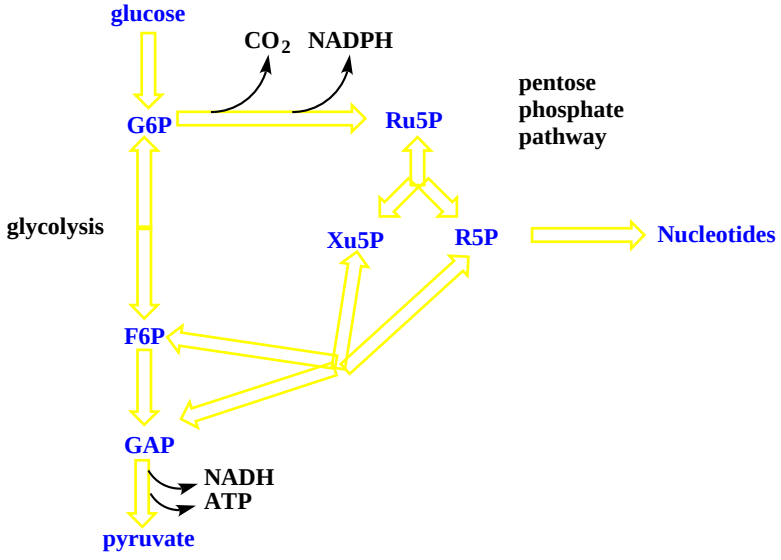
glucose phosphate isomerase

fructose-6-phosphate ⇔ glucose-6-phosphate

Function of the HMP Pathway

Note that although glucose can be completely oxidized to CO_2 via this pathway, there is no ATP production anywhere! Reducing power is needed in the body for more that ATP production. For example, it is necessary for the biosynthesis of molecules such as fatty acids and steroid hormones (adrenal cortex). Most of this reducing power is produced by the hexose monophosphate pathway in the form of NADPH. The extra phosphate on the **structure of NADPH** is a "flag" indicating that this is to be used for biosynthesis. This permits separate control of ATP production and anabolism. In some tissues, the HMP pathway is more significant for the oxidation of glucose than the glycolytic pathway and the citric acid cycle. The HMP pathway is most active in those tissues in which there is greatest fatty acid synthesis such as in adipose tissues and lactating mammary glands.

A secondary function of the HMP pathway is the production of D-ribose-5-phosphate for the synthesis of nucleotides.



NAD vs NADP

The structures of the pyridine nucleotides nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) were given in the **glycolysis module**. NAD and NADP have very similar standard free energies of oxidation and reduction, but dehydrogenases that use NADP as coenzyme cannot use NAD, and vice versa. Therefore, the two pyridine nucleotides can be used in separately controlled sequences involving the same sorts of reactions. In general, NADP is used as an electron carrier for reductive synthesis (anabolic reactions), such as the biosynthesis of fatty acids and cholesterol. NAD, in general, is used in energy production, i.e., catabolic reactions that generate reducing equivalents for the respiratory chain. There are exceptions to these rules, but they hold most of the time.

The essential point is that the ratio of concentrations of the oxidized and reduced forms of these two pyridine nucleotides can be maintained at different levels because they participate in different reactions using different enzymes. In the liver, the ratio of NAD^+:NADH is approximately 4:1, while the ratio of $NADP^+$:NADPH is approximately 1:3. Other things being equal, these differences will have the following results. Dehydrogenases with a specificity for NAD can readily oxidize their substrates and dehydrogenases with a specificity for NADP will tend to reduce their substrates.

Drug-Induced Symptoms of Erythrocyte Glucose-6-Phosphate Dehydrogenase (G6PDH) Deficiency

Red cells contain an active HMP pathway to produce NADPH. NADPH is a coenzyme of glutathione reductase and its primary role is to keep glutathione in erythrocytes in a reduced state. Reduced glutathione, in turn, prevents oxidation of hemoglobin to methemoglobin and also maintains the erythrocyte membrane. Insufficient reduced glutathione leads to lysis of the erythrocyte and hemolytic anemia.

A deficiency in **glucose-6-phosphate dehydrogenase (G6PDH)** in erythrocytes can have significant effects. Hemolysis of red blood cells can occur in a person with such a deficiency when exposed to drugs such as antimalarials and sulfa antibiotics. The syndrome is sometimes called primaquine sensitivity because it was recognized during studies of the antimalarial drug primaquine.

Because of a deficiency of G6PDH in red cells, there is a limited production of NADPH. Though there is evidence of other abnormalities in these cells, the afflicted individuals have no clinical symptoms unless exposed to primaquine or another of the group of drugs and other substances known to have this effect.

When primaquine is administered daily to sensitive persons, an acute hemolytic crisis develops; it begins on the second or third day and lasts six to ten days. From 30 to 50% of the circulating red cells may be destroyed in this period. The patient becomes jaundiced, with blackish urine and a serum bilirubin of 3-5 mg/100mL due to break-down of the released heme.

There is more than one genetic type of the disease. In African Americans, about 10% of sample populations have been found to be primaquine sensitive. In some patients the concentrations of G6PDH may be 30-50% of normal. A more severe form of sensitivity has been found in Sardinians, Greeks, Iranians, and Sephardic Jews (but not in Ashkenazi Jews from Germany, Poland, or Russia). There may be a complete absence of the enzyme in sensitive individuals.

The mutant gene is sex-linked and is partially dominant. Affected males have full expression by having the mutant gene on the X-chromosome (X_dY), while the female will have full expression only if she inherits the trait from both parents (X_dX_d). Most often she has only expression as a heterozygote (X_dX).

Galactosemias

Galactosemia implies an abnormal amount of galactose in the blood. Galactose arises from the hydrolysis of lactose, the major carbohydrate of milk. There are 3 steps in the pathway from galactose to glucose-1-phosphate. The first reaction is the phosphorylation of galactose to **galactose-1-phosphate** by **galactokinase**.

$$\text{galactose} + \text{ATP} \Leftrightarrow \text{gal-1-P} + \text{ADP}$$

In the second step **UDP-galactose** is formed by an interchange reaction catalyzed by **galactose-1-phosphate uridyl transferase**.

$$\text{gal-1-P} + \text{UDP-glucose} \Leftrightarrow \text{UDP-gal} + \text{glu-1-P}$$

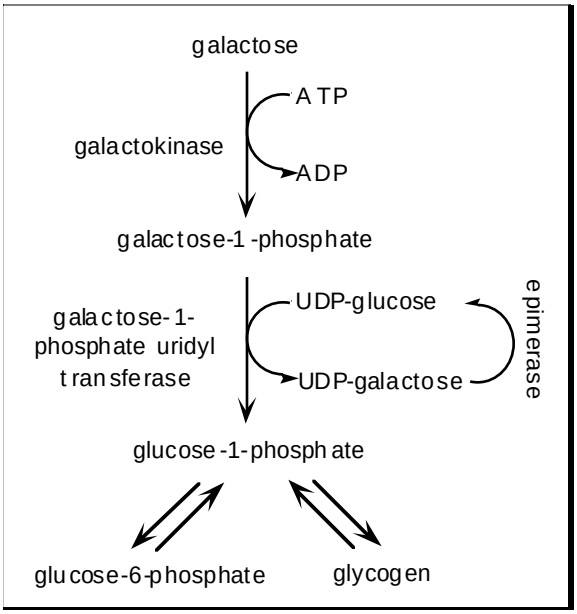
In the third reaction, UDP-galactose is converted to **UDP-glucose** by **UDP-galactose-4-epimerase** while attached to UDP. (Glucose and galactose are epimers; they differ only in the arrangement of atoms at C4 (**see structure above**))

$$\text{UDP-galactose} \Leftrightarrow \text{UDP-glucose}$$

The overall reaction is the sum of these three reactions is as follows:

$$\text{galactose} + \text{ATP} \Leftrightarrow \text{glucose-1-P} + \text{ADP}$$

A diagram of this pathway is given below.



Note that UDP-galactose is also important in the synthesis of lactose in lactating mammary gland. The epimerase permits the production of UDP-galactose from UDP-glucose, so that dietary galactose and the transferase are not required.

The absence of galactose-1-phosphate uridyl transferase causes galactosemia, a severe disease that is inherited as an autosomal recessive gene. One in 70,000 individuals suffers from this disorder. Afflicted infants fail to thrive or diarrhea occurs following milk consumption, and enlargement of the liver and jaundice are common. Also, many galactosemics are mentally retarded. Blood galactose level is elevated, and galactose is found in the urine. The concentration of galactose-1-phosphate is elevated in red cells. The absence of the transferase in red blood cells is a definitive diagnostic criterion.

Galactosemia is simply treated by the exclusion of galactose from the diet. Such a diet leads to regression of virtually all the clinical symptoms, expect for mental retardation, which may not be reversible. Continued galactose intake may lead to death. After these individuals have reached puberty, a new enzyme, UDP-galactose pyrophosphorylase, appears in the liver and dietary restrictions can be released. This enzyme catalyzes the reaction of galactose-1-phosphate with UTP to form UDP-Gal directly.

A second less common form of galactosemia is caused by a defect in galactokinase. These individuals accumulate galactose and its alcohol derivative, galactitol, in the blood. Galactitol causes cataracts to form in these individuals as well as those that lack the transferase. Other

than the formation of cataracts, this type of galactosemia does not have the severe symptoms associated with the absence of the transferase. It is also treated by eliminating galactose from the diet.

Fructose Intolerance

Fructose comprises a significant portion of the carbohydrate in the diet of most Americans (100g/day), both as the free sugar and as a moiety of sucrose. A large part of the ingested fructose is metabolized in the liver by the **fructose-1-phosphate** pathway. The first step is the phosphorylation of fructose to fructose-1-phosphate by **fructokinase**. Fructose-1-phosphate is then split into glyceraldehyde (not phosphorylated) and dihydroxyacetone phosphate by aldolase **(see below)**. Glyceraldehyde is then phosphorylated by triose kinase to give glyceraldehyde-3-phosphate.

Alternatively, fructose can be phosphorylated to **fructose-6-phosphate** by hexokinase. However, the affinity of hexokinase for glucose is twenty times what it is for fructose. In the liver, the glucose level is high; so there is little phosphorylation of fructose to fructose-6-phosphate in the liver. In other tissues, with low glucose levels (i.e., adipose tissue), the formation of fructose-6-phosphate an occur to an appreciable extent.

There are three structurally distinct forms of aldolase. **Aldolase A** is found predominantly in muscle, **aldolase B** in the liver, and **aldolase C** in the brain. Each of the aldolase isozymes catalyzes the aldolytic cleavage of both fructose bisphosphate and fructose-1-phosphate. However, they differ from on another in their detailed kinetics. Muscle extracts, which contain virtually only aldolase A, have an activity ratio of about 50:1 (i.e. there is a 50:1 preference for fructose bisphosphate). In contrast, in liver extracts which contain predominantly aldolase B, the activity ratio is 1:1.

Fructose intolerance is caused by a **deficiency in liver aldolase B**. This disorder is highly deleterious and results in hypoglucosemia and liver damage. This condition is usually first recognized in infancy when the child is put on foods that contain added sucrose.

When fructose is administered to individuals with defective aldolase B, F-1-P accumulates in their tissues, blood fructose levels are high, and fructose is excreted in the urine. The toxic effect of fructose is attributed to the increased concentration of F-1-P since a deficiency in fructokinase which also gives rise to increased fructose levels has no clinical symptoms.

Alcoholism

Alcohol is both a rapidly metabolized substance and a drug, depending on the amount consumed and the duration of exposure. Only 2-

10% of that absorbed is eliminated through the kidneys and lungs; the rest must be oxidized in the body. Organs that metabolize ethanol are the liver and, only to a small degree, the kidney. Therefore, direct effects are on the liver where marked metabolic imbalances are seen.

The physiological mechanism for alcohol metabolism starts functioning at very low concentrations and approaches its maximal rate at concentrations of about 10 mM (40 mg/100 mL of blood). Ethanol catabolism produces striking metabolic imbalances in the liver because of the following characteristics:

1. It produces a large caloric load, sometimes exceeding that of all other nutrients.
2. There is very little renal or pulmonary excretion.
3. It cannot be stored.
4. It is oxidized almost exclusively in the liver.
5. There is no feedback mechanism to adjust the rate of ethanol oxidation to the metabolic state of the hepatocyte.

The biochemistry of its catabolism can be summarized as follows:

$$\text{Ethanol} + NAD^+ \Leftrightarrow \text{acetaldehyde} + NADH + H^+$$

$$\text{Acetaldehyde} + NAD^+ \Leftrightarrow \text{acetate} + NADH + H^+$$

The $NADH:NAD^+$ ratio in the cytoplasm increases 3-4 times and in the mitochondria 2-3 times, producing a change in the concentrations of all metabolites that are dependent on the $NADH\text{-}NAD^+$ couple. The concentration of acetate in the blood increases to about 1 mM. The concentration of acetaldehyde in the blood increases to about 10 mM. The lactate:pyruvate ratio in the blood and liver increases, resulting in hyperlactacidemia. There is an increase in the concentration of α-glycerol phosphate in the liver that, along with the increase in NADH, results in enhanced lipogenesis. See Clinical Correlation 28.5 in Devlin (p.1128) for a discussion of the nutritional problems of the chronic alcoholic.

What is the role of glycogen in skeletal muscle metabolism?

Glycogen is a more readily available substrate for energy production in the working muscle than is exogenous glucose. You should recall that in glycolysis each D-glycopyranosyl unit of glycogen yields a net of 3 ATP molecules; whereas, glucose yields 2 ATP. Thus, in a sense, glucose has a slightly lower energy content than glycogen. Because an additional mole of ATP is required to convert glucose to glucose-6-

phosphate to the reaction catalyzed by glucokinase or hexokinase, the net production of ATP from glucose in glycolysis is one mole less than that from glycogen. The ability to perform heavy, prolonged work is directly related to the initial glycogen stores of skeletal muscle, which averages about 1.5 g/100 g of muscle. After 2 hours of work with 75% maximal oxygen uptake, the glycogen content approaches zero.

What is the role of glucose-6-phosphatase in skeletal muscle metabolism and liver?

Muscles do not contain glucose-6-phosphatase. They do contain phosphorylase, so glycogen can be phosphorolyzed to glucose-1-phosphate, which can be converted to glucose-6-phosphate; but glucose-6-phosphate cannot be converted to free glucose (which then might leave the cell.) Thus, the glycogen stores in muscle can be quite high, especially since muscle mass is about 20 times that of the liver in a normal adult. However, this store can never be used elsewhere in the body. It is committed to the muscle.

Liver (and kidney) contains glucose-6-phosphatase; so in the liver, glycogen can be converted into glucose (in a **process** involving the endoplasmic reticulum and vesicle formation and fusing with the plasma membrane) which can be transported via the cardiovascular system to other tissues, such as brain and skeletal muscles, where it can be used as an energy source. The glycogen content of the liver is 50-100 g.

Regulation of Blood Glucose Levels

Blood glucose can form glycogen in both the liver and muscles. The formation of glycogen is induced by insulin, which is released in response to a rise in blood glucose. The formation of glucose from glycogen is induced by epinephrine (adrenaline) and glucagon, the latter being released in response to a fall in blood glucose levels. Thus, glycogen stored in the liver serves to maintain blood glucose levels.

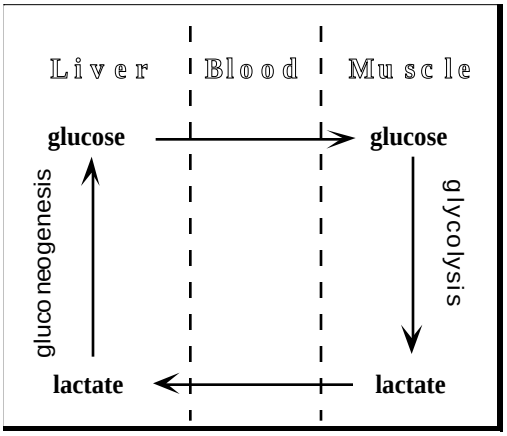
The central nervous system (CNS), which has a very low content of glycogen, depends largely on blood glucose as an energy source. In the human body, approximately 60% of the glucose produced by the liver is utilized by the brain, which needs 110-145 gm/day.

With a sugar content of approximately 1 g/liter, the total glucose content of the blood is 5-6 g. Since as much as 3 g of carbohydrate may be utilized during every minute of heavy exercise in a person with high aerobic power, the available glucose in the blood can cover only 2 minutes of work. If half of the blood sugar was used, leaving 0.5 g/liter, severe symptoms of hypoglycemia would develop. However, skeletal muscles continue to use carbohydrate even when blood sugar (glucose) falls to critically low levels due to its own stores.

To prevent a marked decrease in blood sugar levels, some control mechanism must operate. One point of control is at the level of transport of glucose into muscle cells. The two principle control mechanisms rely on insulin and hexokinase. In one, the permeability of the muscle cell membrane to glucose depends on the plasma insulin concentration, which decreases during heavy exercise. In the other, hexokinase in the cells traps glucose as glucose-6-phosphate after it has entered; but hexokinase is allosterically inhibited by the catabolism of glycogen. As long as sufficient glycogen is present and G-6-P levels are maintained at sufficient levels, hexokinase is not required and is turned off. Therefore, glycogen, which has a higher net energy yield, is a more readily available energy source in a working muscle cell than is exogenous glucose. This of course, is advantageous to the CNS.

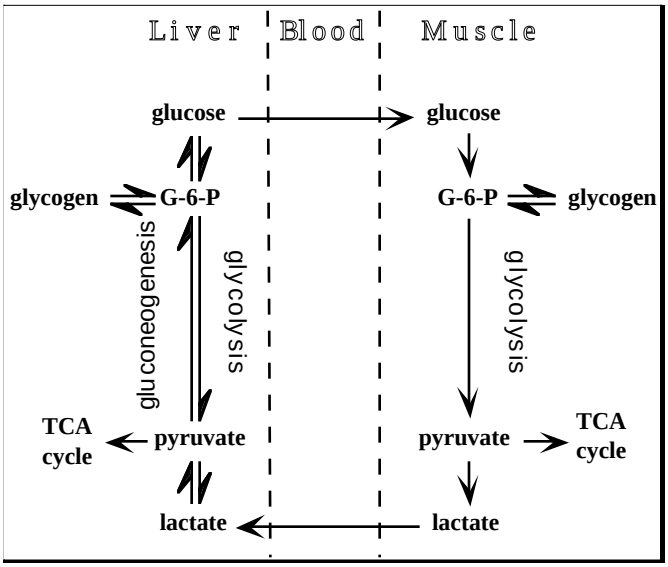
The Cori cycle

When oxygen is in short supply, some of the glucose needed for energy by muscle cells is converted into lactate (lactic acid) to provide some ATP without the aid of oxygen. In the liver, this lactate can be converted back into glucose by gluconeogenesis. Gluconeogenesis refers to the net synthesis of glucose from noncarbohydrate sources. We have discussed the steps of gluconeogenesis in a previous module. The complete process whereby glucose is converted to lactate to produce ATP in the working muscle and lactate is converted back to glucose in the liver is called the **Cori cycle**, which can be diagrammed as follows:



In the liver, 15% of lactate is oxidized to CO_2 and water, first by oxidation to pyruvate and then by oxidation of the pyruvate in the citric acid cycle. This provides the ATP required to convert the remaining 85% to glucose by gluconeogenesis. Glycolysis plus the Cori cycle produces 37% as much ATP as glycolysis plus the citric acid cycle. However, the resynthesis of glucose from lactic acid is a slow process.

A more complete depiction of the relationship of carbohydrate metabolism in the liver and muscle is given below.



Fat as an energy source in skeletal muscle

In some ways, fat is a better way to store chemical energy than is carbohydrate. Because the release of energy is due to oxidation and because carbohydrate is already partially oxidized (i.e., contains -CHOH- groups), fat (which contains mostly -CH2- groups) releases more energy upon oxidation. Fat contains as much as 90% carbon and hydrogen and has an energy density of about 9 Kcal/g. Carbohydrates contain only about 46% carbon and hydrogen and have an energy density of about 4 Kcal/g. The energy density difference is even greater because glycogen is hydrated while fat is not. This hydration reduces the energy of glycogen to about 1 Kcal/g.

Heart muscle uses fat almost exclusively as an energy source. This will be discussed in the next Problem Unit.

Fat or carbohydrate as an energy source

Factors that affect the participation of fat versus carbohydrates in muscle metabolism are as follows:

1. Intensity of work in relation to the person's total work capacity, i.e., his/her physical conditioning, or stated in another way, whether or not the work or exercise can be accomplished under aerobic conditions.

2. Duration of the work or exercise

3. Diet

Comparison of oxidation of fat and carbohydrates

Fat:

$$CH_3(CH_2)_{14}CO_2H + 23\ O_2 \Leftrightarrow 16\ CO_2 + 16\ H_2O + 130\ ATP$$

The **respiratory quotient** (RQ) is:

$$RQ = \frac{CO2\ produced}{O2 consumed} = \frac{16}{23} = 0.7$$

moles of ATP/g of fat = 130/256 = 0.51

moles of ATP/mole of O_2 = 130/23 = 5.65

Carbohydrate:

$$C_6H_{10}O_5 + 6\ O_2 \rightarrow 6\ CO_2 + 5\ H_2O + 37\ ATP$$

RQ = 1.00

moles of ATP/g of glucose unit of glycogen = 37/162 = 0.23

moles of ATP/mole of O_2 = 37/6 = 6.17

Carbohydrate can also produce ATP anaerobically:

$$C_6H_{10}O_5 \Leftrightarrow 2\ lactate + 3\ ATP$$

moles of ATP/g of glucose unit of glycogen = 3/162 = 0.019

When a young person of good physical condition springs into action, the high-energy phosphate (ATP + creatine phosphate) stored in the muscle sustains strong contraction for less than one minute in full emergency effort. At this point glycogen reserves are used preferentially, i.e., about 90% of the ATP is supplied by oxidation of glycogen. Therefore, the availability of a glycogen reserve controls more intense short-term activity. However, the oxidation of glycogen steadily declines and the oxidation of fat increases until it accounts for 80% or more of the total ATP produced. This shift accounts for the increased oxygen consumption because fatty acids can only be oxidized aerobically, i.e., via beta-oxidation and the citric acid cycle, and because oxidation of fatty acids inherently requires more oxygen. (The consumption of oxygen may increase 16-fold or greater in the first five minutes of intense activity.)

After a long period of light or moderate work or exercise, energy is supplied by fat and carbohydrate (mainly glycogen) in about equal amounts. As the muscular work continues, fat is used in increasing amounts as an energy source. If the exercise is of moderate intensity so that the metabolism is aerobic, about 50-60% of the energy is supplied by fat.

As explained above, during heavy muscular work, where the metabolism is in part anaerobic, the major energy source must be glycogen. In other words, the more inadequate the oxygen supply, the higher the carbohydrate (glycogen) utilization. Assuming 7.3/kcal of usable chemical energy for muscle contraction per mole of ATP (which is undoubtedly a minimum value), in the catabolism of carbohydrate to CO_2 and water, 1 liter of oxygen will provide at least 2.02 kcal of usable energy as ATP while, in the complete catabolism of a fatty acid (for example, palmitic acid), 1 liter of oxygen will provide at least 1.85 kcal of usable energy as ATP. In other words, there is about 9% higher energy yield in terms of ATP per liter of oxygen used when carbohydrate is catabolized than when fat is catabolized. The circulatory capacity to transport oxygen during heavy exercise averages about 3.0 liters/min in a young adult male. In summary, endurance during heavy exercise is determined by the size of the glycogen stores and the oxygen delivery rate.

Glycogen stores in skeletal muscles determine the person's ability to sustain prolonged heavy exercise. Assuming a glycogen concentration of 1.5-2.0% at the beginning of work or exercise, the glycogen utilization at a work level of 25-30% of the patient's maximum aerobic power would be sufficient to allow him to continue for 8-10 hours before depletion of the glycogen. At a work load demanding 75-85% of the maximum, the same amount of glycogen would be catabolized within 1.5 hours. (This calculation assumes complete utilization of glycogen, which may never occur.)

Skeletal muscle can also use 3-hydroxybutyrate and acetoacetate (ketone bodies) very efficiently. However, these are not major substrates during activity.

Cardiac muscle, which is rich in myoglobin and mitochondria, mainly utilizes aerobic reactions to make ATP. Relatively little glucose is used by cardiac muscle.

The effect of diet on the energy metabolism of skeletal muscle

A low-carbohydrate diet tends to favor the utilization of fat as an energy source, but this will reduce the capacity for prolonged heavy work. A carbohydrate-rich diet enhances the storage of glycogen, which improves endurance. It is possible to increase the glycogen content of skeletal muscles by a carbohydrate-rich diet to about 2.5 g/100 g of muscle, thus improving performance in heavy, prolonged exercise (i.e., carbo-loading).

What is oxygen debt?

During recovery from work, oxygen consumption remains high for a period of time and then gradually declines over an extended period as ADP concentration falls. The extra consumption over the resting

level during recovery is sometimes referred to as an oxygen debt. The increased oxygen consumption during recovery represents a continued demand for high-energy phosphate for replenishment of the creatine phosphate and glycogen stores, i.e., the oxygen that is needed past the time activity ceases is used for the restore the normal phospho-creatine concentration and to convert the remaining lactate to glycogen.

Summary

Stores of ATP and creatine phosphate will sustain intense muscular activity for less than one minute. Then carbohydrate (glycogen) metabolism starts to replenish the ATP. It takes a while longer for the respiratory and cardiovascular systems to respond so that initially the metabolism is, in part anaerobic. Lactic acid production begins in about 15 seconds when about 50% of the creatine phosphate is gone. Lactic acid production rate peaks in about 35 seconds.

As the respiratory and cardiovascular systems begin to respond, the percentage of carbohydrate (glucose units of glycogen) converted into lactate declines and the percentages completely oxidized to CO_2 and water in mitochondria increases. Glycogen initially supplies 90% of the energy. Therefore, the availability of glycogen determines the intensity of short term activity. (A 1.5-2.0% concentration of glycogen and utilization at 25-30% of maximum aerobic power would allow work for 8-10 hours; a 1.5-2.0 concentration of glycogen and utilization at 75-85% of maximum aerobic power would allow work for 1.5 hours.) The RQ sometimes goes over 1.00 during this period, probably because of a release of CO_2 due to the lactic acid in the blood.

Carbohydrate gives more ATP per liter of oxygen, but fat gives more ATP per gram. Therefore, fat is a better energy store but requires more oxygen for its oxidation. Fats are not stored in muscle but in adipose tissue and must be mobilized and transported to muscle for use. An initial drop in fatty acid metabolism by skeletal muscle is followed by a gradual rise until it supplies 60% of the energy utilized.

Muscle uses little blood glucose for rapid energy. Available blood glucose could cover only about 2 minutes of work; 60% of glucose produced by the liver is used in the brain. Control of glucose metabolism in skeletal muscle cells is at the level of transport into the cells.

Muscles able to work over long periods (smooth muscle, cardiac muscle) have many mitochondria, more myoglobin, and little glycogen. Muscles used for short bursts of activity have more ATP and creatine phosphate and fewer mitochondria.

APPENDIX I: Using Acrobat Reader with pdf Files

Portable Document Format (PDF) files can be read by Acrobat Reader, a free program which can be downloaded from the Adobe Web site (**http://www.adobe.com/acrobat**). If Acrobat Reader is installed on your system, it will automatically open simply by double-clicking on the pdf file that you wish to read.

Acrobat Window

The document will be displayed in the center of your window and an index will appear at the left side of the screen. Each entry in the index is a hypertext link to the associated topic in the text.

Using hypertext links in a pdf document is exactly like that in a web page or html document. When you place the cursor over a hypertext link, it changes to a hand with the index finger pointing to the underlying text. Clicking the mouse causes the text window to jump to that location. The index does not change. Magnification may need to be adjusted using the menu option in the lower part of the screen to optimize the view and readability. The best magnification is usually around 125%.

Subheadings in the index can be viewed by clicking on the open diamonds to the left of appropriate entries to cause them to point downwards. Clicking again will close the subheadings lists.

Hypertext links

Hypertext links in the text (not in the index) are indicated by blue underlined text. The cursor should change to a hand with the index finger pointing to this text when it passes over it. Clicking will cause the text page to move to the associated or linked text which will be highlighted in red underlined text. Red underlined text is not a hyperlink, only a destination.

How to back up to a previous window:

If you wish to return to a previous text window after following a hypertext link, use the double solid arrow key at the top of the Acrobat window (or use the key equivalent "command - "). Acrobat keeps a record of your last 20 or so windows so that multiple steps back can be made my repeating the command.

Links to web sites

A number of url links to web sites are located in the pdf file and appear in blue underlined type starting with http:// (e.g. **http://**

www.som.siu.edu). Clicking on these should open a web browser such as Netscape and take you to those web sites. You may need to resize the Acrobat Window to view the web browser window displayed underneath it.

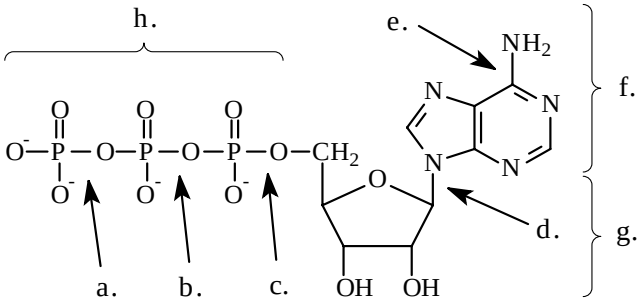
COMMENTS

I hope that you find this pdf file useful. Comments on how to make it better would be greatly appreciated. Please notify me in person or by email (enieder@som.siu.edu) of any errors so that they can be removed. The online version on the Biochem server can be easily updated.

PROBLEM SET

The following set of problems is given as a self-study aid. It is not intended to be an exhaustive survey of the Problem Unit.

1.



Hydrolysis of ATP to yield energy usable for maintenance of life most often involves the breaking of which bond in the above structure?

a. a
b. b
c. c
d. d
e. e

(answer)

2. The adenine ring in ATP is indicated in the above drawing by:

a. e
b. f
c. g
d. h

(answer)

3. Glycolysis is which of the following?

a. a catabolic process
b. an anabolic process
c. an anaplerotic process

(answer)

4. For the reaction

$$\text{Glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP}$$

$\Delta G^{o'}$ = -4 Kcal/mole. This implies that the preferred direction of the reaction is:

a. to the right

b. to the left

c. the reaction is at equilibrium as written

(answer)

5. Organic reduction normally involves:

a. an increase in energy

b. a decrease in energy

c. no change in energy

(answer)

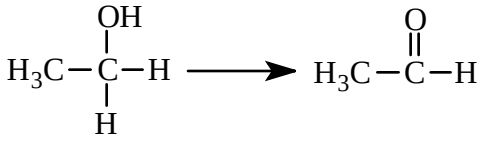
6. For a reaction with a very large $\Delta G^{o'}$, e.g., +15 Kcal/mole, the velocity of the reaction is very large.

a. True

b. False

(answer)

7. The following reaction (not balanced)



represents which of the following?

a. a hydrolysis

b. a reduction

c. an oxidation

d. nonsense

(answer)

8. Which of the following are "high energy" compounds important in bioenergetics and the transferal of energy.

1. ATP
2. $NADH + H^+$
3. $NADPH + H^+$
4. creatine

a. 1
b. 1, 2
c. 1, 2, 3
d. 1, 2, 3, 4
e. 1, 2, 4

(answer)

9. Enzymes are important because they:

1. direct the flow of energy in certain paths.
2. increase the velocity of a reaction.
3. may be regulated.
4. decrease the relative free energies of the reactants and products.
5. are composed of protein.

a. 1
b. 1, 2
c. 1, 2, 3
d. 2, 3, 4
e. 3, 4, 5
f. 1, 3, 5

(answer)

10. Which of the following determines the amount of work required to synthesize ATP in a living cell, and therefore the amount of work obtainable from ATP for processes such as muscle contraction.

a. $\Delta G'$
b. $\Delta G^{o'}$
c. K
d. ΔS

(answer)

11. How many reducing ends does glycogen have?
    a. 0
    b. 1
    c. 2
    d. a very large number
    (answer)

12. Glucose-6-phosphate is a reasonable inhibitor of liver glucokinase.
    a. True
    b. False
    (answer)

13. Citrate is a reasonable inhibitor of phosphofructokinase 1.
    a. True
    b. False
    (answer)

14. Ca^{2+} is a reasonable activator of phosphorylase kinase.
    a. True
    b. False
    (answer)

15. AMP is a reasonable inhibitor of phosphorylase.
    a. True
    b. False
    (answer)

16. AMP is a reasonable activator of isocitrate dehydrogenase.
    a. True
    b. False
    (answer)

17. Acetyl CoA is a reasonable activator of pyruvate kinase.
    a. True
    b. False
    (answer)

18. If hexokinase has a K_m of 1×10^{-4} M for glucose, it will be functioning at full velocity, for all practical purposes, at (assume high

[ATP])

a. 10^{-6} M glucose

b. 10^{-4} M glucose

c. 10^{-2} M glucose

(answer)

19. Lactic dehydrogenase functions most importantly in:

a. aerobic metabolism

b. anaerobic metabolism

(answer)

20. When a cell is switched from aerobic metabolism to anaerobic metabolism, the ATP concentration changes roughly from 4.2 mM to:

a. 10 mM

b. 4 mM

c. 0.1 mM

(answer)

21. The reaction which irreversibly commits sugar to the glycolytic pathway is catalyzed by:

a. hexokinase or glucokinase

b. phosphofructokinase

c. phosphoglucomutase

d. glucose phosphate isomerase

e. aldolase

(answer)

22. NAD^+ contains which of the following?

a. thiamine

b. lipoic acid

c. niacin

d. riboflavin

e. CoA

(answer)

23. Phosphoglycerate kinase functions in carbohydrate metabolism to produce ATP via:

a. oxidative phosphorylation

b. substrate level phosphorylation
c. oxidative decarboxylation
d. phosphorolysis

(answer)

24. Glucose-1-phosphate is produced from glycogen via:
    a. oxidative phosphorylation
    b. substrate phosphorylation
    c. glycogen kinase activity
    d. phosphorolysis

    (answer)

25. Which of the following is important in transferring energy from the glycolytic pathway to the TCA cycle?
    a. $NADH + H^+$
    b. $FADH_2$
    c. citrate
    d. acetyl CoA
    e. GTP

    (answer)

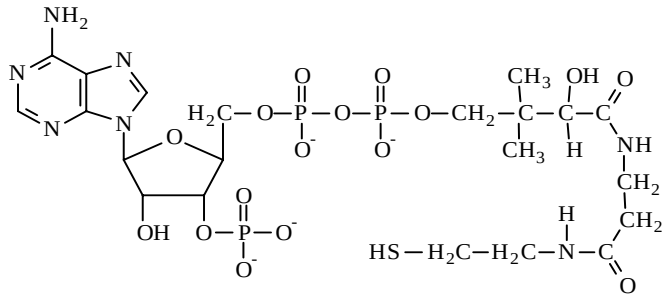
26. Which enzyme is deficient in the liver in cases of fructose intolerance?
    a. hexokinase
    b. aldolase
    c. glucokinase
    d. phosphofructokinase
    e. triose kinase

    (answer)

27. Riboflavin is a part of the structure of which of the following?
    a. FAD
    b. NAD^+
    c. CoA
    d. ATP
    e. UTP

    (answer)

28.



The above is the structure of:

a. CoA

b. thiamine pyrophosphate

c. FAD

d. lipoic acid

(answer)

29. A deficiency in UDP-glucose: galactose-1-phosphate uridylyl-transferase will lead to an accumulation of:

a. galactose

b. glucose

c. glycogen

d. UDP

e. UTP

(answer)

30. Cori's, McArdle's, von Gierke's and Andersen's diseases are all examples of:

a. glycogenolysis

b. gluconeogenesis

c. glycogenosis

d. glycogenesis

(answer)

31. Hormones such as epinephrine and glucagon function by entering the target cell and activating adenylate kinase directly.

a.True

b.False

(answer)

32. Caffeine functions by inactivating:
    a. adenylate kinase
    b. cAMP
    c. phosphodiesterase
    d. phosphorylase

    (answer)

33. Glycogen synthase is _____ by phosphorylation.
    a. activated
    b. inhibited

    (answer)

34. A thiamine deficiency will affect the activity of which of the following directly?
    a. isocitrate dehydrogenase
    b. lactate dehydrogenase
    c. pyruvate dehydrogenase

    (answer)

35. The TCA cycle is important for which of the following?

    1. carbohydrate metabolism
    2. lipid metabolism
    3. amino acid metabolism

    a. 1
    b. 2
    c. 3
    d. 1, 2
    e. 1, 2, 3
    f. 2, 3

    (answer)

36. Electron transport and oxidative phosphorylation require the presence of mitochondria.
    a. True
    b. False

    (answer)

37. In the chemiosmotic mechanism of oxidative phosphorylation, electrons are pumped across a membrane to drive the reducing equivalents through the three complexes of the electron trans-

port chain.

a. True

b. False

(answer)

38. If site I is inhibited by rotenone, then all further electron transport must come to a stop.

a. True

b. False

(answer)

39. Oligomycin interacts directly with:

a. complex I

b. complex II

c. complex III

d. F_1F_0 ATPase

e. NADH dehydrogenase

(answer)

40. Cyanide interacts directly with:

a. complex I

b. complex II

c. complex IV

d. F_1F_0 ATPase

e. NADH dehydrogenase

(answer)

41. Uncouplers are substances which allow electron transport but ATP is not made.

a. True

b. False

(answer)

42. Under anaerobic conditions a cell may obtain energy from all of the following except for:

a. ATP

b. creatine phosphate

c. glycogen

d. fatty acids

e. glucose

(answer)

43. Glycogen yields _____ ATP(s) per glucopyranosyl unit passing through the glycolytic pathway.

a. 1
b. 2
c. 3
d. 4
e. 5

(answer)

44. Glucose yields_____ ATP(s) per mole when passing through the glycolytic pathway.

a. 1
b. 2
c. 3
d. 4
e. 5

(answer)

45. The glycogen level in skeletal muscle is a function of:

1. amount of exercise
2. length of time since last meal
3. conditioning
4. diet

a. 1
b. 1, 2
c. 1, 2, 3
d. 2, 3, 4
e. 1, 2, 3, 4
f. 1, 2, 4

(answer)

46. Lactate does not accumulate in cardiac muscle which is functioning continuously because:

a. lactate inhibits glucokinase
b. lactate inhibits lactate dehydrogenase
c. pyruvate inhibits lactate dehydrogenase
d. pyruvate activates citrate synthase

(answer)

47. The normal blood-level of ethanol is approximately 1 mM. Where is this predominantly derived from?

a. glycolysis in the brain
b. glycolysis by the flora in the lumen of the gut
c. from ingestion of alcoholic beverages

(answer)

48. Sugar phosphates are oxidized via the hexose monophosphate pathway to:
    a. provide energy in the form of ATP.
    b. synthesize NADPH + H^+
    c. synthesize pyruvate and acetyl CoA
    d. synthesize fructose-1,6-bisphosphate

(answer)

49. A patient who has ingested a large amount of methanol will begin to show which of the following in his blood as a result of alcohol dehydrogenase activity?
    a. acetaldehyde
    b. acetic acid
    c. pyruvate
    d. formaldehyde
    e. ethanol

(answer)

50. The patient described in #49 should be administered which of the following to prevent death?
    a. acetaldehyde
    b. acetic acid
    c. pyruvate
    d. formaldehyde
    e. ethanol

(answer)

51. Ethanol is removed from the body primarily by:
    a. exhaling it from the lungs.
    b. excreting it in the urine.
    c. metabolism in the liver.

(answer)

52. In terms of carbohydrate metabolism the most active organ in the body is:

a. heart
b. skeletal muscle
c. brain
d. smooth muscle

(answer)

53. The standard free energy difference between NADPH and $NADP^+$ is:
    a. about the same as
    b. less than
    c. greater than

that for NADH and NAD^+?

(answer)

54. Lactose is synthesized in human mammary gland by lactose synthetase. Uridine diphosphate galactose is joined to glucose to form lactose. Can a mother homozygous for galactosemia (i.e., lacking uridyl transferase) produce lactose?

    Explain your answer.

    (answer)

55. Explain the observation of hypophosphatemia (i.e., low blood phosphate) in a patient experiencing fructose intolerance, i.e., lacking aldolase B.

    (answer)

56. Explain the role of lactate dehydrogenase in efficient anaerobic metabolism.

    (answer)

57. What is the major regulatory step in glycolysis. How is this reaction controlled?

    (answer)

58. Discuss standard state free energies and free energies in a cell. Are they different, and if so, why are they different?

    (answer)

59. Explain drug induced erythrocyte glucose-6-phosphate dehydrogenase deficiency.

    (answer)

60. What is the biochemical importance of the following coenzymes or vitamins?

    A. thiamine

    B. lipoic acid

    C. pantothenic acid

    D. riboflavin

    E. niacin

    (answer)

61. Von Gierke's disease results from a deficiency in glucose-6-phosphatase. Explain what the ramifications of this disease might be.

    (answer)

62. Explain why 50% more energy in the form of ATP is obtained from glycolysis when starting with glycogen than when starting with glucose.

    (answer)

63. Explain how epinephrine is able to activate phosphorylase and simultaneously inhibit glycogen synthase.

    (answer)

64. Describe the role of a membrane in oxidative phosphorylation.

    (answer)

65. Which of the following enzymes generate high-energy phosphate at the substrate level?

    a. Phosphofructokinase
    b. Phosphoglycerate kinase
    c. Lactate dehydrogenase
    d. Aldolase
    e. Phosphoglyceromutase

    (answer)

66. All of the following are required for the conversion of glucose to acetyl-CoA in striated muscle EXCEPT which one?

    a. The action of lactate dehydrogenase (LDH).
    b. The action of phosphofructokinase 1 (PFK1).
    c. Phosphorylation of glucose to glucose-6-phosphate.

d. Cleavage of fructose-1,6-bisphosphate to triose phosphates.

e. NAD.

(answer)

67. Administration of primaquine to humans with a deficiency of glucose-6-phosphate dehydrogenase produces anemia. The best reason given below for this anemia is:

a. inability to decompose peroxide via glutathione peroxidase because of lack of a physiological reductant for glutathione.

b. excess ATP production in the absence of an alternate pathway for utilization of glucose-6-phosphate.

c. lower than normal NADH production rates.

d. inhibition of glycolysis by primaquine.

e. inability to produce nucleotides and thus limiting DNA production and causing a slowing of erythrocyte production.

(answer)

68. All of the following occur in the hexose monophosphate pathway for glucose metabolism, EXCEPT:

a. reduction of NAD.

b. formation of C-7 sugar phosphates.

c.formation of C-5 sugar phosphate precursor of nucleotides.

d. release of C-1 of glucose as carbon dioxide.

e. reduction of NADP.

(answer)

69. During strenuous exercise, the NADH produced in the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase in skeletal muscle has to be reoxidized in order for glycolysis to continue. The primary enzyme involved in this reoxidation is:

a. malate dehydrogenase.

b. lactate dehydrogenase.

c. pyruvate dehydrogenase.

d. isocitrate dehydrogenase.

e. glucose-6-phosphate dehydrogenase.

(answer)

70. In oxidative phosphorylation, an uncoupling agent causes which of the following?

a. Both respiration and phosphorylation to decrease

b. Phosphorylation to remain constant and respiration to decrease
c. Respiration to increase and phosphorylation to decrease
d. Respiration and phosphorylation to increase
e. The P/O ratio to go up.

(answer)

71. In the electron transport scheme below, which electron carrier is missing?

$$NADH \rightarrow FMNH_2 \rightarrow ? \rightarrow \text{cytochromes} \rightarrow 0_2$$

a. Vitamin K
b. Vitamin C
c. Ferridoxin
d. Ubiquinone
e. NADPH

(answer)

72. Which of the following is the first enzyme unique to the pentose phosphate pathway?
a. Lactonase
b. 6-Phosphogluconate dehydrogenase
c. Transaldolase
d. Glucose-6-phosphate dehydrogenase
e. Phosphoglucoisomerase

(answer)

73. Cyanide is toxic because it inhibits:
a. succinate dehydrogenase.
b. NADH dehydrogenase.
c. phosphorylase.
d. pyruvate dehydrogenase.
e. cytochrome oxidase.

(answer)

74. All of the following will inhibit electron transport in mitochondria EXCEPT:
a. amytal.
b. antimycin A.

c. dinitrophenol.

d. cyanide.

e. rotenone.

(answer)

75. Oligomycin interferes with synthesis of "high energy" compounds by:

a. dissociating cytochrome components from mitochondrial membranes.

b. uncoupling electron transfer between NADH and a flavoprotein.

c. preventing formation of a proton gradient across the inner mitochondrial membrane.

d. inhibiting the activity of ATP synthesis.

e. catalyzing degradation of a "high energy" chemical intermediate.

(answer)

76. The products of oxidation of one mole of glucose 6-phosphate through the oxidative portion of the pentose phosphate pathway are:

a. 2 moles of reduced NAD, one mole of ribulose 5-phosphate and one mole of carbon dioxide.

b. 2 moles of reduced NADP, one mole of ribulose 5-phosphate and one mole of carbon dioxide.

c. 2 moles of reduced NADP, one mole of xylulose 5-phosphate and one mole of carbon dioxide.

d. 2 moles of reduced NADP, one mole of ribose 5 phosphate and one mole of carbon dioxide.

e. one mole of fructose 6-phosphate and five moles of carbon dioxide.

(answer)

77. What is the terminal cytochrome in the mitochondrial electron transport process leading to reduction of oxygen to form water?

a. Cytochrome c_1

b. Cytochrome aa_3

c. Cytochrome *d*

d. Cytochrome *b*

e. None of these.

(answer)

78. If both pyruvate and succinate are present in a mitochondrial reaction mixture and rotenone is added to the reaction mixture, the P/O ratio observed will be:

    a. 0.
    b. 1.
    c. 2.
    d. 3.
    e. 4.

(answer)

79. If the following compounds are added to a suspension of mitochondria metabolizing succinate as a substrate, the system that will produce the most heat is:

    a. 2,4-dinitrophenol and cyanide.
    b. 2,4-dinitrophenol and rotenone.
    c. rotenone and ADP.
    d. 2,4-dinitrophenol and antimycin A.

(answer)

80. NADH can reduce coenzyme Q through the mediation of an enzyme associated with which of the following?

    a. cytochrome *c*
    b. lipoic acid
    c. iron-Porphyrin
    d. cytochrome *a*
    e. FMN

(answer)

81. In order to reverse glycolysis, which one of the following enzymes must be present in addition to the regular glycolytic enzymes?

    a. fructose-1,6-bisphosphate phosphatase
    b. phosphofructokinase
    c. pyruvate kinase
    d. aldolase
    e. glyceraldehyde-3-phosphate dehydrogenase

(answer)

82. Electrons on the way to molecular oxygen are first transferred from NADH to:
    a. a quinone.
    b. a cytochrome.
    c. lipoic acid.
    d. a flavin.
    e. ferredoxin.

    (answer)

83. Which of the following is an iron-porphyrin-protein found as a component of the mitochondrial respiratory chain?
    a. catalase
    b. myoglobin
    c. coenzyme Q
    d. cytochrome *c*
    e. flavoprotein

    (answer)

84. The enzymes responsible for electron transfer to oxygen are located in the mitochondrial:
    a. matrix.
    b. inner membrane.
    c. space between inner and outer membranes.
    d. outer membrane.

    (answer)

85. The oxidation and reduction of NAD occurs on which of the following?
    a. the adenine ring
    b. the phosphodiester bonds
    c. the side-chain nitrogen of nicotinamide
    d. the pyridine ring of nicotinamide
    e. a ribose hydroxyl

    (answer)

86. Phosphorylase is activated directly by:
    a. epinephrine.
    b. phosphorylase kinase.
    c. phosphorylase phosphatase.

d. cAMP.

e. glucagon.

(answer)

87. The enzyme in the citric acid cycle that converts GDP + P_i → GTP is:

a. fumarase.

b. isocitrate dehydrogenase.

c. malate dehydrogenase.

d. succinate dehydrogenase.

e. succinyl-CoA synthetase.

(answer)

88. All of the following steps in the oxidation of pyruvate to carbon dioxide and water eventually result in the generation of 3 ATP from ADP by oxidative phosphorylation EXCEPT which one?

a. pyruvate dehydrogenase

b. isocitrate dehydrogenase

c. α-ketoglutarate dehydrogenase

d. malate dehydrogenase

e. succinate dehydrogenase

(answer)

89. If electrons from NADH are diverted to an artificial electron acceptor so that no electrons enter the electron transport chain from NADH, how many ATP's will be formed when acetyl-CoA is completely oxidized in such a system?

a. None

b. One

c. Two

d. Three

e. Four

(answer)

90. A simple explanation of the action of an uncoupler of mitochondrial oxidative phosphorylation is that it is any agent that:

a. blocks the oxidation of reduced cytochrome *c* by cytochrome oxidase.

b. prevents formation of ATP by interacting with and blocking proton transport through the proton translocating ATPase.

c. acts to conduct protons back into the mitochondrial matrix without accompanying formation of ATP.

d. blocks proton conductance and slows oxygen uptake rates.

(answer)

91. The synthesis and degradation of glycogen in muscle is not a futile (ATP-hydrolyzing) cycle because:

a. glycogen synthase and phosphorylase are simultaneously activated.

b. when glycogen synthase is inactivated, phosphorylase is simultaneously activated.

c. glycogen binds Mg^{2+}, thereby lowering the concentration of $MgATP^{2-}$.

d. cyclic AMP activates adenylate kinase.

e. of the Pasteur effect.

(answer)

92. Which one of the following is the most correct?

a. NAD is used in glycolysis and in the pentose shunt.

b. NAD is used in glycolysis, but not in the pentose shunt.

c. NADP is used in glycolysis and in the pentose shunt.

d. NADP is used in glycolysis, but not in the pentose shunt.

e. NAD and NADP are both used in oxidative phosphorylation.

(answer)

93. The hexose monophosphate shunt pathway is utilized for all of the following EXCEPT which one?

a. formation of ATP

b. generation of NADPH

c. ribose-5-phosphate synthesis

d. ribose-5-phosphate degradation

e. synthesis of pentoses

(answer)

94. Which of the following is a tricarboxylic acid?

a. oxaloacetate

b. succinate

c. α-ketoglutarate

d. citrate

e. acetate

(answer)

95. Which one of the following substances can be formed directly by a carbon dioxide fixation reaction?

a. glucose

b. glutamine

c. lactic acid

d. pyruvic acid

e. oxaloacetic acid

(answer)

96. The reduced coenzyme of glycerol phosphate dehydrogenase, located in the mitochondrial membrane, is oxidized by:

a. NADH dehydrogenase.

b. coenzyme Q (ubiquinone).

c. cytochrome *c*.

d. cytochrome oxidase.

e. cytochrome *a*.

(answer)

97. The glycerol phosphate shuttle functions in:

a. anaerobic glycolysis for regeneration of NAD.

b. lipid catabolism.

c. aerobic glycolysis to transport NADH equivalents resulting from glycolysis into mitochondria.

d. triglyceride synthesis.

(answer)

Answer the following questions using the key outlined below:

A. If 1, 2, and 3 are correct

B. If 1 and 3 are correct

C. If 2 and 4 are correct

D. If only 4 is correct

E. If all four are correct

98. Which of the following are functions of the TCA cycle?
    1. The generation of NADH and reduced flavins.
    2. The formation of α-ketoglutarate.
    3. The oxidation of acetyl-CoA produced from glycolysis and fatty acid oxidation.
    4. The utilization of excess ATP generated by glycolysis.

    (answer)

99. Which of the following metabolic processes in the cell proceed by different pathways in the forward and reverse directions, under physiological conditions?
    1. Glucose ⇔ glucose-6-phosphate.
    2. Fructose 6-phosphate ⇔ fructose-1,6-bisphosphate.
    3. Phosphoenolpyruvate ⇔ pyruvate.
    4. 1,3-Bisphosphoglycerate ⇔ 3-phosphoglycerate.

    (answer)

100. The hexose monophosphate shunt:
    1. can convert glucose to carbon dioxide in the absence of oxygen.
    2. can provide five carbon sugars when the cell's needs for nucleotides is high.
    3. would have a diminished role in the metabolism of glucose in individuals with glucose-6-phosphate dehydrogenase deficiency.
    4. can provide the cell with NADH needed in the synthesis of fatty acids.

    (answer)

ANSWERS

1.	a	**2.**	b
3.	a	**4.**	a
5.	a	**6.**	b
7.	c	**8.**	c
9.	c	**10.**	a

11. a
12. b
13. a
14. a
15. b
16. a
17. b
18. c
19. b
20. b
21. b
22. c
23. b
24. d
25. d
26. b
27. a
28. a
29. a
30. c
31. b
32. c
33. b
34. c
35. e
36. a
37. b
38. b
39. d
40. c
41. a
42. d
43. c
44. b
45. e
46. c
47. b
48. b
49. d
50. e
51. c
52. c
53. a

54. yes. UDP-glucose can be converted to UDP-galactose by an epimerase.

55. Phosphate would increasingly be tied up in F-1-P.

56. Regenerate NAD for glyceraldehyde-3-P dehydrogenase activity.

57. Phosphofructokinase is activated by AMP, inhibited by ATP and citrate.

58. Standard state free energies are a 1 M concentrations, a non-physiological condition.

59. Decreased NADPH levels due to low levels of glucose-6-P dehydrogenase. NADPH is required for drug metabolism. The dehydrogenase deficiency is not apparent until challenged by a drug.

60. a. pyruvate dehydrogenase
b. "
c. "
d. FAD and FMN requiring enzymes (e.g. succinate dehydrogenase)
e. NAD and NADP requiring enzymes (e.g. lactate dehydrogenase)

61. Glucose cannot be released from the liver, kidney, and intestinal epithelium. This results in increased glycogen levels.

62. Phosphorylase uses P_i directly to produce G-1-P.

63. Phosphorylase is activated by phosphorylation and glycogen synthetase is inhibited.

64. As an impermeable barrier to H^+, it allows the creation of a proton motive force.

65. b **66.** a

67. a **68.** a

69. b **70.** c

71. d **72.** d

73. e **74.** c

75. d **76.** b

77. b **78.** c

79. b **80**. e

81. a

82. d

83. d

84. b

85. d

86. b

87. e

88. e

89. b

90. c

91. b

92. b

93. a

94. d

95. e

96. b

97. c

98. a

99. a

100.a