KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore - 641021.

(For the candidates admitted from 2017 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **METABOLISM OF CARBOHYDRATES AND LIPIDS**

SEMESTER : **III**

SUBJECT CODE: **17BCU301** **CLASS** : **II B.Sc., BC**

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

S. No	Duration of period	Topics covered	Books referred	Page No	Web page referred
		UNIT-I			
1	1	Autotrophs, heterotrophs, Metabolic pathways, Catabolism, anabolism	T1	23; 27-28	
2	1	ATP as energy currency Reducing power of the cell	T1 T1	482-483 245-248	
3	1	Glycolysis - a universal pathway, reactions of glycolysis,	T1	522-534	
4	1	Fermentation, fates of pyruvate	R1	536-540	
5	1	Feeder pathways for glycolysis, galactosemia	T1	534-537 234-235	
6	1	Gluconeogenesis- Synthesis of glucose from non-carbohydrate sources	T1 R1	543-549 543-570	
7	1	Reciprocal regulation of glycolysis and gluconeogenesis	T1	544-576	
8	1	Pentose phosphate pathway and its importance	R1	571-580	
9	1	Revision and QP discussion			
	Total number of hours planned for Unit I: 09				
		UNIT-II			
1	1	Glycogen metabolism: Glycogenesis	T1	547-553	
2	1	Glycogenolysis	R1	554-562	
3	1	Regulation of glycogen metabolism, glycogen storage diseases	T1	565-567	W1
4	1	Citric acid cycle: Production of acetyl CoA,	T1	601-610	

		Reactions of citric acid cycle	R1	592-611	
5	1	Anaplerotic reactions, amphibolic role	R1	612-614	-
6	1	Regulation of citric acid cycle	R1	614-617	
7	1	Glyoxalate pathway	T1	608-610	
8	1	Coordinated regulation of glyoxalate and citric acid pathways	T1 R1	616-618 620-624	-
9	1	Revision and QP discussion			-
	Total number of hours planeed for Unit II: 09				
		UNIT-III			
1	1	Synthesis of carbohydrates- Calvin cycle	T1	752-760	-
		Regulation of calvin cycle		760-762	
2	1	Regulated synthesis of starch, sucrose	T1	623-626	-
3	1	Regulated synthesis of sucrose	T1	348-350	-
4	1	Photorespiration	T1	766 - 769,	-
5	1	C4 pathway	T1	770-772	
6	1	CAM pathway	T1	773-774	
7	1	Synthesis of cell wall polysaccharides	T1	775-780	
8	1	Integration of carbohydrate metabolism in plant cell.	R1	143-159	
9	1	Revision and discussion of possible question			-
	Total number of hours planned for Unit III: 09				
		UNIT-IV: Fatty acid oxidation and synthesis			
1	1	Digestion, mobilization and transport of cholesterol and triacyl glycerols	T1 R1	632-637 700-702	
2	1	Fatty acid transport to mitochondria	R1	721-722	
3	1	β oxidation of even numbered, saturated and unsaturated fatty acids	R1	714-720	
4	1	oxidation of odd numbered and branched chain fatty acids,	R1	772-775	
5	1	Regulation of fatty acid oxidation	T1	642-645	-
6	1	Peroxisomal oxidation, ω oxidation	T1 R1	647-650 700-702	-
7	1	Ketone bodies metabolism, ketoacidosis.	T1 R1	647-650 697-700	-
8	1	Fattyacid synthase complex, Synthesis of saturated,even numbered fattyacid	T1	637-639	-
9	1	Synthesis of unsaturated, odd numbered fattyacid	T1	640-645	-
	Total number of hours planned for Unit IV: 09				

UNIT-V: Biosynthesis of eicosanoids, cholesterol, steroids, isoprenoids and membrane lipids					
1	1	Synthesis of prostagladins, leukotrienes and thromboxanes	R1 T1	725-736 800-816	-
2	1	Synthesis of cholesterol, regulation of cholesterol synthesis	R1 T1	1157-1159 825-827	
3	1	Synthesis of steroids and isoprenoids	R1	1160-1164	-
4	1	Synthesis of membrane phospholipids in prokaryotes and eukaryotes, Respiratory distress syndrome	R1 R1 T1	296,306; 729 808-811	
5	1	Biosynthesis of triacylglycerol, biosynthesis of plasmalogens,	R1 T1	735 804, 813	
6	1	sphingolipids and glycolipids, lipid storage diseases.	R1	737-742	
7	1	Starve-feed cycle, Well-fed state, early fasting state, fasting state, Early re-fed state	R2	1263-1265	
8	1	Energy requirements, reserves and caloric value	R2	1265-1267	
9	1	Homeostasis-five phases of glucose homeostasis	-	-	W2
	Total number of hours planned for Unit V:09				
Previous year end semester examination question paper discussion					
1	1	Previous year End Semester Exam- QP discussion			
2	1	Previous year End Semester Exam- QP discussion			
3	1	Previous year End Semester Exam- QP discussion			
Total number of hours planned for this course: 48					

SUGGESTED READINGS:

T1-Lehninger: Principles of Biochemistry (2013) 6th ed., Nelson, D.L. and Cox, M.M., W.H. Freeman and Company (New York), ISBN:13:978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

R1- Textbook of Biochemistry with Clinical Correlations (2011) 7th ed., Devlin, T.M., John Wiley & Sons, Inc. (New Jersey), ISBN:978-0-470-28173-4.

R2: Biochemistry (2012) 7th ed., Berg, J.M., Tymoczko, J.L. and Stryer L., W.H. Freeman and Company (New York), ISBN:10:1-4292-2936-5, ISBN:13:978-1-4292-2936-4.

W1- https://ghr.nlm.nih.gov/condition/glycogen-storage-disease

W2- http://www2.csudh.edu/nsturm/CHE452/24_Glucose%20Homeostas.htm

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SUBJECT : METABOLISM OF CARBOHYDRATES AND LIPIDS

SEMESTER : III

SUBJECT CODE: 17BCU301 **CLASS : II B.Sc. BC**

SCOPE

To give an insight on various metabolic pathways, that involves energy in metabolic pathways.

OBJECTIVES

To understand the metabolic pathways of carbohydrates, lipids, metabolism.

Unit 1

Basic design of metabolism : Autotrophs, heterotrophs, metabolic pathways, catabolism, anabolism, ATP as energy currency, reducing power of the cell.

Glycolysis: Glycolysis - a universal pathway, reactions of glycolysis, fermentation, fates of pyruvate, feeder pathways for glycolysis, galactosemia.

Gluconeogenesis and pentose phosphate pathway : Synthesis of glucose from non-carbohydrate sources, reciprocal regulation of glycolysis and gluconeogenesis, pentose phosphate pathway and its importance.

Unit 2

Glycogen metabolism: Glycogenesis and glycogenolysis, regulation of glycogen metabolism, glycogen storage diseases.

Citric acid cycle : Production of acetyl CoA, reactions of citric acid cycle, anaplerotic reactions, amphibolic role, regulation of citric acid cycle, glyoxalate pathway, coordinated regulation of glyoxalate and citric acid pathways.

Unit 3

Synthesis of carbohydrates : Calvin cycle, regulation of calvin cycle, regulated synthesis of starch and sucrose, photorespiration, C4 and CAM pathways, synthesis of cell wall polysaccharides, integration of carbohydrate metabolism in plant cell.

Unit 4

Fatty acid oxidation : Digestion, mobilisation and transport of cholesterol and triacyl glycerols, fatty acid transport to mitochondria, β oxidation of saturated, unsaturated, odd and even numbered and branched chain fatty acids, regulation of fatty acid oxidation, peroxisomal oxidation, ω oxidation, ketone bodies metabolism, ketoacidosis.

Fatty acid synthesis : Fatty acid synthase complex. Synthesis of saturated, unsaturated, odd and even chain fatty acids and regulation.

Unit 5

Biosynthesis of Eicosanoids, cholesterol, steroids and isoprenoids : Synthesis of prostagladins, leukotrienes and thromboxanes. Synthesis of cholesterol, regulation of cholesterol synthesis. Synthesis of steroids and isoprenoids.

Biosynthesis of membrane lipids

Synthesis of membrane phospholipids in prokaryotes and eukaryotes, respiratory distress syndrome, biosynthesis of triacylglycerol, biosynthesis of plasmalogens, sphingolipids and glycolipids, lipid storage diseases.

Starve-feed cycle

Well-fed state, early fasting state, fasting state, early re-fed state, energy requirements, reserves and caloric homeostasis, five phases of glucose homeostasis.

SUGGESTED READINGS:

Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed., W.H. Freeman and Company (New York), ISBN:13:978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

Devlin, T.M., (2011). Textbook of Biochemistry with Clinical Correlations 7th ed., John Wiley & Sons, Inc. (New Jersey), ISBN:978-0-470-28173-4.

Berg, J.M., Tymoczko, J.L. and Stryer L., (2012). Biochemistry 7th ed., W.H. Freeman and Company (New York), ISBN:10:1-4292-2936-5, ISBN:13:978-1-4292-2936-4.

UNIT-I
SYLLABUS

Basic design of metabolism : Autotrophs, heterotrophs, metabolic pathways, catabolism, anabolism, ATP as energy currency, reducing power of the cell.
Glycolysis: Glycolysis - a universal pathway, reactions of glycolysis, fermentation, fates of pyruvate, feeder pathways for glycolysis, galactosemia.
Gluconeogenesis and pentose phosphate pathway: Synthesis of glucose from non-carbohydrate sources, reciprocal regulation of glycolysis and gluconeogenesis, pentose phosphate pathway and its importance.

Basic design of metabolism

The concepts of conformation and dynamics dealing with the specificity and catalytic power of enzymes, the regulation of their catalytic activity, and the transport of molecules and ions across membranes—enable us to now raise questions fundamental to biochemistry like,*How does a cell extract energy and reducing power from its environment? How does a cell synthesize the building blocks of its macromolecules and then the macromolecules themselves?*

These processes are carried out by a highly integrated network of chemical reactions that are collectively known as *metabolism*.

More than a thousand chemical reactions take place in even as simple an organism as *Escherichia coli*. The array of reactions may seem overwhelming at first glance. However, closer scrutiny reveals that metabolism has a *coherent design containing many common motifs*. These motifs include the use of an energy currency and the repeated appearance of a limited number of activated intermediates. In fact, a group of about 100 molecules play central roles in all forms of life. Furthermore, although the number of reactions in metabolism is large, the number of *kinds* of reactions is small and the mechanisms of these reactions are usually quite simple. Metabolic pathways are also regulated in common ways. The purpose of this chapter is to introduce some general principles and motifs of metabolism to provide a foundation for the more detailed studies to follow.

Cells Transform Different Types of Energy:

Living organisms require a continual input of free energy for three major purposes: (1) the performance of mechanical work in muscle contraction and other cellular movements, (2) the active transport of molecules and ions, and (3) the synthesis of macromolecules and other biomolecules from simple precursors. The free energy used in these processes, which maintain an organism in a state that is far from equilibrium, is derived from the environment.

The First Law of Thermodynamics states that energy can be neither created nor destroyed. The amount of energy in the universe is constant. Nevertheless, energy can be converted from one form into another.

Photosynthetic organisms, or ***phototrophs,*** use the energy of sunlight to convert simple energy-poor molecules into more-complex energy-rich molecules that serve as fuels. In other words, photosynthetic organisms transform light energy into chemical energy. Indeed, this transformation is ultimately the primary source of chemical energy for the vast majority of organisms, human beings included. ***Chemotrophs,*** which include animals, obtain chemical energy through the oxidation of foodstuffs generated by phototrophs.

Chemical energy obtained from the oxidation of carbon compounds may be transformed into the unequal distribution of ions across a membrane, resulting in an ion gradient. This gradient, in turn, is an energy source that can be used to move molecules across membranes, that can be converted into yet other types of chemical energy, or that can convey information in the form of nerve impulses. In addition, chemical energy can be transduced into mechanical energy. We convert the chemical energy of a fuel into structural alterations of contractile proteins that result in muscle contraction and movement. Finally, chemical energy powers the reactions that result in the synthesis of biomolecules.

At any given instant in a cell, thousands of energy transformations are taking place. Energy is being extracted from fuels and used to power biosynthetic processes. These transformations are referred to as *metabolism* or *intermediary metabolism*.

AUTOTROPHS

Autotrophs are organisms that can produce their own food from the substances available in

their surroundings using light (photosynthesis) or chemical energy (chemosynthesis). Heterotrophs cannot synthesize their own food and rely on other organisms — both plants and animals — for nutrition. Technically, the definition is that autotrophs obtain carbon from inorganic sources like carbon dioxide (CO2) while heterotrophs get their reduced carbon from other organisms. Autotrophs are usually plants; they are also called "self feeders" or "primary producers".

Types of Autotrophs

Scientists classify autotrophs according to how they obtain their energy. Types of autotrophs include photoautotrophs, and chemoautotrophs.

Photoautotrophs

Photoautotrophs are organisms who get the energy to make organic materials from sunlight. Photoautotrophs include all plants, green algaes, and bacteria which perform photosynthesis.

All photoautotrophs perform photosynthesis – a word that comes from the root words “light” and “to make.” Photoautotrophs capture photons from the Sun and harvest their energy, using it to perform important biochemical processes such as making ATP.

Photoautotrophs make more than just fuel and organic compounds for heterotrophs like ourselves. Many photoautotrophs take carbon from the atmosphere and use it to make sugars and other molecules that store the Sun’s energy in their molecular bonds. To do this, they take in molecules of CO2, which is created by nonliving geological processes, and release molecules of O2 – also known as the oxygen we need to breathe!

Chemoautotrophs

Chemoautotrophs are organisms that obtain energy from inorganic chemical processes. Today, chemoautotrophs are most commonly found in deep water environments which receive no sunlight. Many need to live around deep sea volcanic vents, which produce enough heat to allow metabolism to occur at a high rate.

Chemoautotrophs use volatile chemicals such as molecular hydrogen, hydrogen sulfide, elemental sulfur, ferrous iron, and ammonia as their energy sources. This makes them well-suited to live in places that would be toxic to many other organisms, as well as places without sunlight.

Chemoautotrophs are usually bacteria or archae bacteria, as their metabolisms are usually not efficient enough to support multicellularity.

Examples of Autotrophs

Plants

Plants, with very few exceptions (such as the venus fly trap which can eat insects) are photoautotrophs. They produce sugars and other essential ingredients for life by using their pigments, such as chlorophyll, to capture photons and harness their energy. When plants are consumed by animals, animals are then able to use that energy and those organic materials for themselves.

Green Algae

Green algaes, which may be familiar to you as pond scum, are also photoautotrophs. Green algae may in fact bear a great resemblance to the first common life form on Earth – cyanobacteria, a green bacteria that grew in mats and began the process of turning Earth into a world with an oxygen atmosphere.

"Iron Bacteria" – Acidithiobacillus ferrooxidans

The bacterium *Acidithiobacillus ferrooxidans* obtains energy from ferrous iron. In the process, it converts the iron atoms from a molecular form where they cannot be dissolved in water to a molecular form where they can.

As a result, *Acidithiobacillus ferrooxidans* has been used to extract iron from ores that could not be extracted through conventional means.

The field of biohydrometallurgy is the study of using living organisms to obtain metals by dissolving them in water, where they can be further processed.

HETEROTROPH

It is an organism that ingests or absorbs organic carbon (rather than fix carbon from inorganic sources such as carbon dioxide) in order to be able to produce energy and synthesize compounds to maintain its life. Ninety-five percent or more of all types of living organisms are heterotrophic, including all animals and fungi and some bacteria and protists

Heterotrophs may be subdivided according to their energy source. If the heterotroph uses chemical energy, it is a chemoheterotroph (e.g., humans and mushrooms). If it uses light for

energy, then it is a photoheterotroph (e.g., green non-sulfur bacteria).Heterotrophs are one of the two types of organotrophs, the other being lithotrophs. Organotrophs exploit reduced carbon compounds as energy sources, like carbohydrates, fats, and proteins from plants and animals. Photoorganoheterotrophs, such as Rhodospirillaceae and purple non-sulfur bacteria synthesize organic compounds using sunlight coupled with oxidation of inorganic substances, including hydrogen sulfide, elemental sulfur, thiosulfate, and molecular hydrogen. They use organic compounds to build structures. They do not fix carbon dioxide and apparently do not have the Calvin cycle. Chemolithoheterotrophs can be distinguished from mixotrophs (or facultative chemolithotroph), which can use either carbon dioxide or organic carbon as the carbon source.

COMPARISION BETWEEN AUTOTROPHS AND HETEROTROPHS

Properties	Autotrophs	Heterotrophs
Food	Produce their own food for energy	They eat other organisms to get proteins and energy
Definition	An organism that is able to form nutritional organic substances from simple inorganic substances such as carbon dioxide.	Heterotrophs cannot produce organic compounds from inorganic sources and therefore rely on consuming other organisms in the food chain.
Examples	Plants, algae, and some bacteria	Herbivores, omnivores, and carnivores
Types	Photoautotroph, Chemoautotroph	Photoheterotroph, Chemoheterotroph
Food chain level	Primary	Secondary and tertiary

OVERVIEW OF INTERMEDIARY METABOLISM

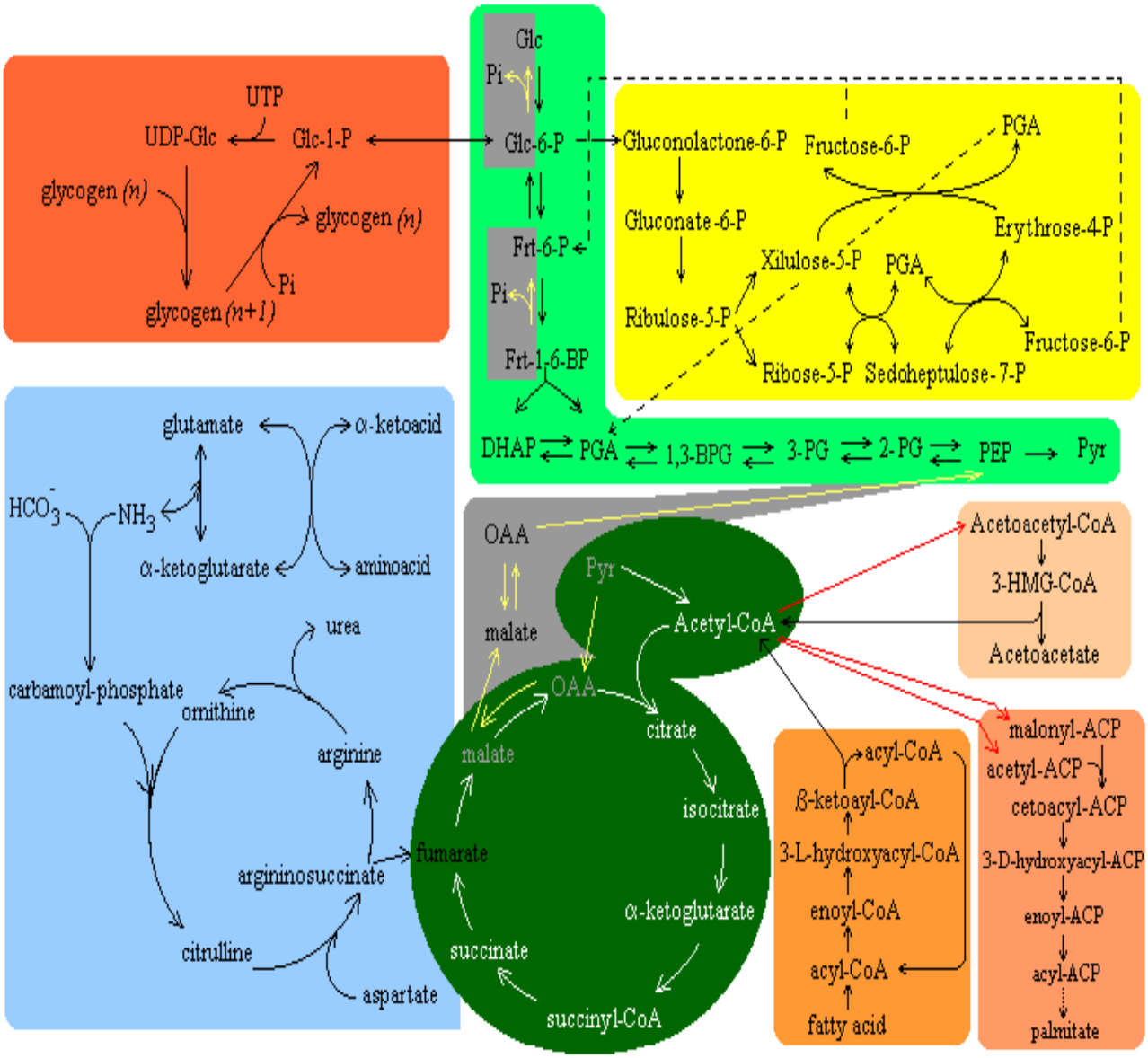
Intermediary metabolism is the fate of dietary carbohydrate, fat, and protein after digestion and absorption. The major intermediary metabolites are glucose, fatty acids, glycerol, and amino acids.

THE BASIC METABOLIC PATHWAYS

There is a very large number of metabolic pathways. In humans, the most important

metabolic pathways are:

- Glycolysis - glucose oxidation in order to obtain ATP
- Citric acid cycle (Krebs' cycle) - acetyl-CoA oxidation in order to obtain GTP and valuable intermediates.
- Oxidative phosphorylation - disposal of the electrons released by glycolysis and citric acid cycle. Much of the energy released in this process can be stored as ATP.
- Pentose phosphate pathway - synthesis of pentoses and release of the reducing power needed for anabolic reactions.
- Urea cycle - disposal of NH_4^+ in less toxic forms
- Fatty acid β-oxidation - fatty acids breakdown into acetyl-CoA, to be used by the Krebs' cycle.
- Gluconeogenesis - glucose synthesis from smaller percursors, to be used by the brain.



Generate energy from fuel molecules to feed all cells of the body. Some pathways are catabolic (break down molecules) and some are anabolic (produce fuel molecules). Metabolism comprises the entire set of chemical reactions that occur in a living organism that allow it to reproduce, develop, maintain its structure and respond to the environment. These chemical reactions form an intricate network of pathways and cycles in which the flow of reaction products (metabolites) is determined by many regulatory mechanisms. Traditionally, metabolism

is subdivided into catabolism, the breaking down of complex molecules, and anabolism, processes related to the synthesis of complex organic substances.

Catabolism

Catabolism is the set of metabolic processes that break down large molecules. These include breaking down and oxidizing food molecules. The purpose of the catabolic reactions is to provide the energy and components needed by anabolic reactions. The exact nature of these catabolic reactions differ from organism to organism and organisms can be classified based on their sources of energy and carbon (their primary nutritional groups), as shown in the table below. Organic molecules are used as a source of energy by organotrophs, while lithotrophs use inorganic substrates and phototrophs capture sunlight as chemical energy. However, all these different forms of metabolism depend on redox reactions that involve the transfer of electrons from reduced donor molecules such as organic molecules, water, ammonia, hydrogen sulfide or ferrous ions to acceptor molecules such as oxygen, nitrate or sulfate.In animals these reactions involve complex organic molecules being broken down to simpler molecules, such as carbon dioxide and water. In photosynthetic organisms such as plants and cyanobacteria, these electron-transfer reactions do not release energy, but are used as a way of storing energy absorbed from sunlight

Anabolism

Anabolism is the set of constructive metabolic processes where the energy released by catabolism is used to synthesize complex molecules. In general, the complex molecules that make up cellular structures are constructed step-by-step from small and simple precursors. Anabolism involves three basic stages. Firstly, the production of precursors such as amino acids, monosaccharide, isoprenoids and nucleotides, secondly, their activation into reactive forms using energy from ATP, and thirdly, the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids.

ATP AS A ENERGY CURRENCY

ATP is called the energy currency of the cell, and of life, because it is the energy molecule that all cells need in order to do anything within the human body. The molecule is used like a battery within cells and allows the consumption of one of its phosphorous molecules.

The enzymatic removal of a phosphate group from ATP to form ADP releases a huge amount of energy which is used by the cell in several metabolic processes as well as in the synthesis of macromolecules such as proteins. The removal of a second phosphate group from ATP results in further energy release and the formation of adenosine monophosphate (AMP).

Functions of ATP in cells

ATP finds use in several cellular processes. Some important functions of ATP in the cell are briefly discussed below:

Active Transport

ATP plays a critical role in the transport of macromolecules such as proteins and lipids into and out of the cell. The hydrolysis of ATP provides the required energy for active transport mechanisms to carry such molecules across a concentration gradient. Transport of molecules into the cell is called endocytosis whilst transport out of the cell is known as exocytosis.

Cell Signaling

ATP has key functions both in intracellular and extracellular signaling. It is easily recognized by purinergic receptors in mammalian tissues - its release from synapses and axons activates purinergic receptors that modulate calcium and cyclic AMP levels inside the cell.

Structural Maintenance

ATP plays a very important role in preserving the structure of the cell by helping the assembly of the cytoskeletal elements. It also supplies energy to the flagella and chromosomes to maintain their appropriate functioning.

Muscle contraction

ATP is critical for the contraction of muscles; it binds to myosin to provide energy and facilitate its binding to actin to form a cross-bridge. ADP and phosphate are then released and a new ATP molecule binds to myosin. This breaks the cross-bridge between myosin and actin filaments, thereby releasing myosin for the next contraction.

In the central nervous system, adenosine modulates neural development, the control of immune systems, and of neuron/glial signaling.

ATP is also involved in signal transduction - its phosphate groups are used up by kinases in phosphate transfer reactions which activate a cascade of protein kinase reactions.

Synthesis of DNA and RNA

During DNA synthesis, ribonucleotide reductase (RNR) reduces the sugar residue from ribonucleoside diphosphates to form deoxyribonucleoside diphosphates such as dADP.

Thus, RNR regulation helps keep the balance of deoxynucleotides (dNTPs) in the cell. Low concentrations of dNTPs inhibit DNA synthesis and repair whilst high levels are shown to be mutagenic because DNA polymerase tends to add the wrong dNTP during DNA synthesis.

The adenosine from ATP is a building block of RNA and is directly added to RNA molecules during RNA synthesis by RNA polymerases. The removal of pyrophosphate provides the energy required for this reaction.

REDUCING POWER OF THE CELL

Living organisms carry out a diverse set of tasks, such as building and maintaining physical structures, moving, synthesizing macromolecules, maintaining electrochemical gradients, and maintaining a constant body temperature. All of these processes require energy. One fundamental problem living organisms face is how to obtain energy to carry out these tasks.

Redox potentials are used to characterize the free energy cost and direction of reactions involving electron transfer, one of the most ubiquitous and important of biochemical reactions. Such reduction-oxidation reactions are characterized by a free energy change that shares some conceptual features with that used to describe pKa in acid-base reactions where proton transfer is involved rather than electron transfer. The redox potential, or more accurately the reduction potential, of a compound refers to its tendency to acquire electrons and thereby to be reduced.

Many biochemical reactions involve oxidation (elimination of electrons from a compound) & discount (addition of electrons to a compound); organisms shop electrons in coenzymes known as NAD (nicotinamide adenine dinucleotide) & NADP (nicotinamide adenine dinucleotide phosphate). those compounds capture electrons in the shape of hydrogen atoms from compounds which might be being oxidized, as a consequence forming NADH & NADPH).

NAD & NADP stores the cell's reducing electricity. Organisms will then use this decreasing electricity to build its cell additives.

GLYCOLYSIS

Glycolysis is an almost universal pathway for extraction of the energy available from carbohydrates, shared among prokaryotes and eukaryotes, aerobes and anaerobes alike. In anaerobes, glycolysis is the only significant source of energy from carbohydrates. In aerobic organisms, considerably more energy can be harvested downstream from glycolysis in the citric acid cycle. Glycolysis produces energy in the form of ATP and NADH. The glycolytic pathway consists of 10 enzyme-catalyzed steps. During glycolysis, glucose, a six-carbon carbohydrate, is oxidized to form two molecules of pyruvate, a three-carbon molecule. For each glucose molecule metabolized, the pathway produces two molecules of ATP and two molecules of NADH.

Glycolysis-A universal pathway

Glycolysis is not isolated from other metabolic pathways. Other molecules besides glucose can enter at a few points along the glycolytic pathway. For example, the product of glycogen breakdown, glucose-6-phosphate, can enter the glycolytic pathway at the second step. Glyceraldehyde-3-phosphate, which is produced by photosynthesis, is also a glycolytic intermediate, so it can be directed from this anabolic pathway into glycolysis when energy is needed. Additionally, intermediates can be drawn out of the glycolytic pathway when energy levels are high, for use in biosynthetic pathways. For instance, during active energy production pyruvate, the product of glycolysis, enters the citric acid cycle, but when energy is not needed pyruvate serves as a substrate in amino acid synthesis.

Reactions of glycolysis

It consist of two phases namely preparatory phase and pay off phase

a)Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates. The first step in glycolysis is phosphorylation of glucose by a family of enzymes called hexokinases to form glucose 6-phosphate (G6P). This reaction consumes ATP, but it acts to keep the glucose concentration low,

promoting continuous transport of glucose into the cell through the plasma membrane transporters. In addition, it blocks the glucose from leaking out – the cell lacks transporters for G6P, and free diffusion out of the cell is prevented due to the charged nature of G6P. Glucose may alternatively be formed from the phosphorolysis or hydrolysis of intracellular starch or glycogen. In animals, an isozyme of hexokinase called glucokinase is also used in the liver, which has a much lower affinity for glucose (Km in the vicinity of normal glycemia), and differs in regulatory properties. The different substrate affinity and alternate regulation of this enzyme are a reflection of the role of the liver in maintaining blood sugar levels.cofactors: $Mg2^{+}$.

G6P is then rearranged into fructose 6-phosphate (F6P) by glucose phosphate isomerase. Fructose can also enter the glycolytic pathway by phosphorylation at this point.The change in structure is an isomerization, in which the G6P has been converted to F6P. The reaction requires an enzyme, phosphohexose isomerase, to proceed. This reaction is freely reversible under normal cell conditions. However, it is often driven forward because of a low concentration of F6P, which is constantly consumed during the next step of glycolysis. Under conditions of high F6P concentration, this reaction readily runs in reverse.

The energy expenditure of another ATP in this step is justified in 2 ways: The glycolytic process (up to this step) is now irreversible, and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase 1 (PFK-1) is coupled to the hydrolysis of ATP, an energetically favorable step, it is, in essence, irreversible, and a different pathway must be used to do the reverse conversion during gluconeogenesis. This makes the reaction a key regulatory point (see below). This is also the rate-limiting step. Furthermore, the second phosphorylation event is necessary to allow the formation of two charged groups (rather than only one) in the subsequent step of glycolysis, ensuring the prevention of free diffusion of substrates out of the cell.

The same reaction can also be catalyzed by pyrophosphate-dependent phosphofructokinase (PFP or PPi-PFK), which is found in most plants, some bacteria, archea, and protists, but not in animals. This enzyme uses pyrophosphate (PPi) as a phosphate donor instead of ATP. It is a reversible reaction, increasing the flexibility of glycolytic metabolism. A rarer ADP-dependent PFK enzyme variant has been identified in archaean species.

Cofactors: $Mg2^{+}$.

Destabilizing the molecule in the previous reaction allows the hexose ring to be split by aldolase into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde. There are two classes of aldolases: class I aldolases, present in animals and plants, and class II aldolases, present in fungi and bacteria; the two classes use different mechanisms in cleaving the ketose ring.

Electrons delocalized in the carbon-carbon bond cleavage associate with the alcohol group. The resulting carbanion is stabilized by the structure of the carbanion itself via resonance charge distribution and by the presence of a charged ion prosthetic group. Triosephosphate isomerase rapidly interconverts dihydroxyacetone phosphate with glyceraldehyde 3-phosphate (GADP) that proceeds further into glycolysis. This is advantageous, as it directs dihydroxyacetone phosphate down the same pathway as glyceraldehyde 3-phosphate, simplifying regulation.

b)Pay-off phase

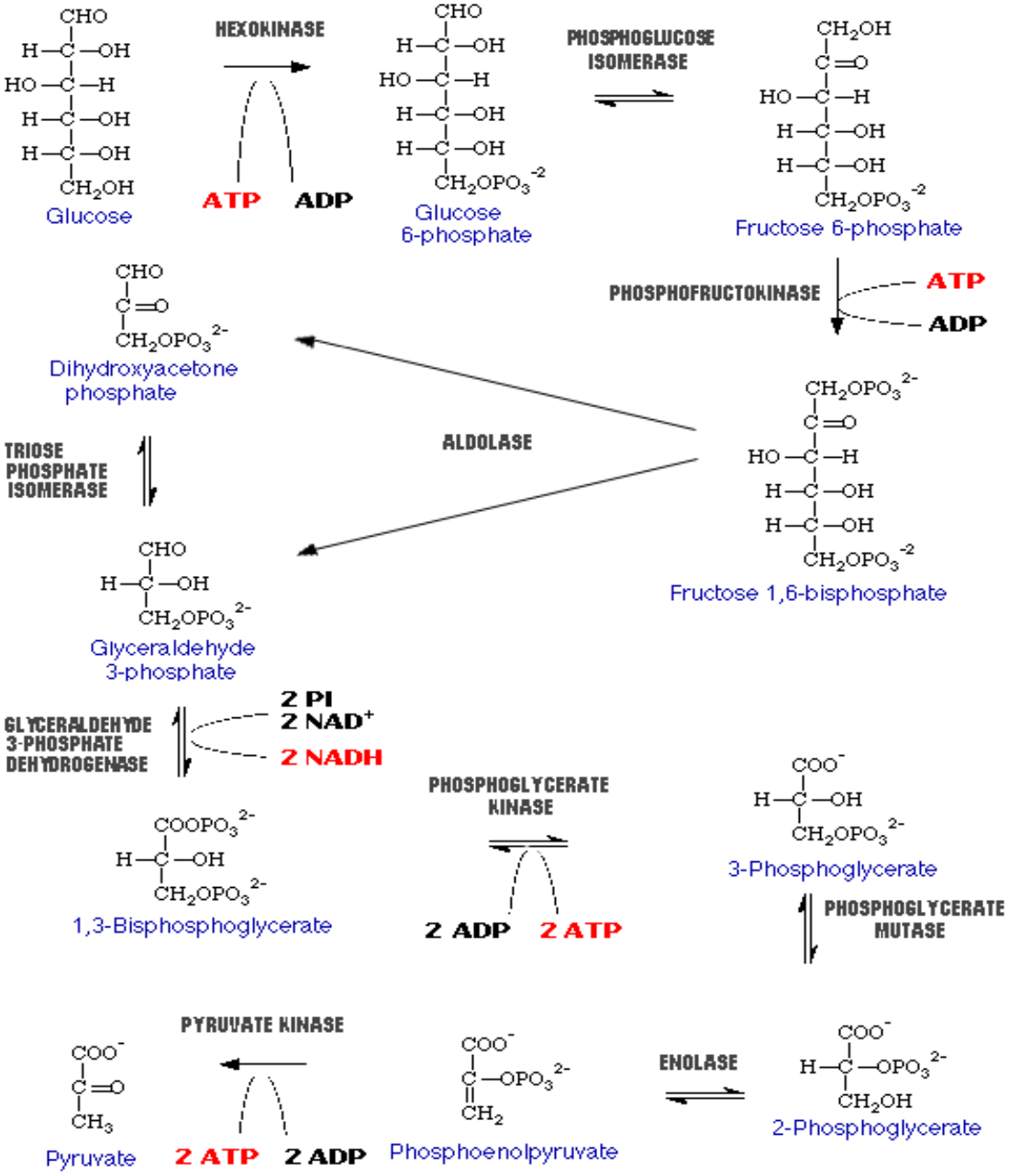
The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose. The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1, 3-bisphosphoglycerate. The hydrogen is used to reduce two molecules of NAD+, a hydrogen carrier, to give NADH + H^+ for each triose. Hydrogen atom balance and charge balance are both maintained because the phosphate (Pi) group actually exists in the form of a hydrogen phosphate anion (HPO42-), which dissociates to contribute the extra H+ ion and gives a net charge of -3 on both sides.

This step is the enzymatic transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP by phosphoglycerate kinase, forming ATP and 3-phosphoglycerate. At this step, glycolysis has reached the break-even point: 2 molecules of ATP were consumed, and 2 new molecules have now been synthesized. This step, one of the two substrate-level phosphorylation steps, requires ADP; thus, when the cell has plenty of ATP (and little ADP), this reaction does not occur. Because ATP decays relatively quickly when it is not metabolized, this is an important regulatory point in the glycolytic pathway. ADP actually exists as ADPMg-, and ATP as $ATPMg^2$-, balancing the charges at -5 both sides. A final substrate-level phosphorylation now

forms a molecule of pyruvate and a molecule of ATP by means of the enzyme pyruvate kinase. This serves as an additional regulatory step, similar to the phosphoglycerate kinase step.

Regulation

Glycolysis is regulated by slowing down or speeding up certain steps in the glycolysis pathway. This is accomplished by inhibiting or activating the enzymes that are involved. The steps that are regulated may be determined by calculating the change in free energy, G, for each step. If a step's products and reactants are in equilibrium, then the step is assumed not to be regulated. Since the change in free energy is zero for a system at equilibrium, any step with a free energy change near zero is not being regulated. If a step is being regulated, then that step's enzyme is not converting reactants into products as fast as it could, resulting in a build-up of reactants, which would be converted to products if the enzyme were operating faster. Since the reaction is thermodynamically favorable, the change in free energy for the step will be negative. A step with a large negative change in free energy is assumed to be regulated.



Post-glycolysis processes

The overall process of glycolysis is:

Glucose + 2 NAD^+ + 2 ADP + 2 Pi 2 Pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O

If glycolysis were to continue indefinitely, all of the NAD+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD+. How this is performed depends on which external electron acceptor is available.

Fermentation

In this pyruvate is converted to lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation:

Pyruvate + NADH + H^+ Lactate + NAD^+

This process occurs in the bacteria involved in making yogurt (the lactic acid causes the milk to curdle). This process also occurs in animals under hypoxic (or partially anaerobic) conditions, found, for example, in overworked muscles that are starved of oxygen, or in infarcted heart muscle cells. In many tissues, this is a cellular last resort for energy; most animal tissue cannot tolerate anaerobic conditions for an extended period of time. Some organisms, such as yeast, convert NADH back to NAD+ in a process called ethanol fermentation. In this process, the pyruvate is converted first to acetaldehyde and carbon dioxide, then to ethanol.

Lactic acid fermentation and ethanol fermentation can occur in the absence of oxygen. This anaerobic fermentation allows many single-cell organisms to use glycolysis as their only energy source. Anoxic regeneration of NADH is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes and is dominant from about 10–30 seconds during a maximal effort. It replenishes very quickly over this period and produces 2 ATP molecules per glucose molecule, or about 5% of glucose's energy potential (38 ATP molecules in bacteria). The speed at which ATP is produced is about 100 times that of oxidative phosphorylation. The pH in the cytoplasm quickly drops when hydrogen ions accumulate in the muscle, eventually inhibiting enzymes involved in glycolysis.

The burning sensation in muscles during hard exercise can be attributed to the production of hydrogen ions during a shift to lactic acid fermentation as oxygen is converted to carbon dioxide by aerobic respiration faster than the body can replenish it. These hydrogen ions form a part of lactic acid along with lactate. The body falls back on this less efficient but faster method of producing ATP under low oxygen conditions. This is thought to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere and thus would represent a more ancient form of energy production in cells. The liver later gets rid of this excess lactate by transforming it back into an important glycolytic intermediate called pyruvate; see Cori cycle. Fermenation of pyruvate to lactate is sometimes also called "anaerobic glycolysis", however, glycolysis ends with the production of pyruvate

regardless in the presence or absence of oxygen.

Anaerobic respiration

In the above two examples of fermentation, NADH is oxidized by transferring two electrons to pyruvate. However, anaerobic bacteria use a wide variety of compounds as the terminal electron acceptors in cellular respiration: nitrogenous compounds, such as nitrates and nitrites; sulfur compounds, such as sulfates, sulfites, sulfur dioxide, and elemental sulfur; carbon dioxide; iron compounds; manganese compounds; cobalt compounds; and uranium compounds.

Aerobic respiration

In aerobic organisms, a complex mechanism has been developed to use the oxygen in air as the final electron acceptor.

- First, pyruvate is converted to acetyl-CoA and CO2 within the mitochondria in a process called pyruvate decarboxylation.
- Second, the acetyl-CoA enters the citric acid cycle, also known as Krebs Cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a hydrogen ion gradient across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce about 2.5 ATP for every NADH oxidized in a process called oxidative phosphorylation.

Fates of pyruvate

The catabolic role of glycolysis with regard to converting potential chemical energy to usable chemical energy during the oxidation of glucose to pyruvate is evidenced. Many of the metabolites in the glycolytic pathway are also used by anabolic pathways, and, as a consequence, flux through the pathway is critical to maintain a supply of carbon skeletons for biosynthesis. In addition, not all carbon entering the pathway leaves as pyruvate and may be extracted at earlier stages to provide carbon compounds for other pathways. These metabolic pathways are all strongly reliant on glycolysis as a source of metabolites: and many more.

- Gluconeogenesis
- Lipid metabolism
- Pentose phosphate pathway

- Citric acid cycle, which in turn leads to:
- Amino acid synthesis
- Nucleotide synthesis
- Tetrapyrrole synthesis

From an anabolic metabolism perspective, the NADH has a role to drive synthetic reactions, doing so by directly or indirectly reducing the pool of NADP+ in the cell to NADPH, which is another important reducing agent for biosynthetic pathways in a cell.

Feeder Pathways for Glycolysis

In addition to glucose, many other carbohydrates ultimately enter the glycolytic pathway to undergo energy-yielding degradation. The most significant are the storage polysaccharides glycogen and starch, the disaccharides maltose, lactose, trehalose, and sucrose, and the monosaccharides fructose, mannose, and galactose. We shall now consider the pathways by which these carbohydrates can enter glycolysis.

Glycogen and Starch Are Degraded by Phosphorolysis

The glucose units of the outer branches of glycogen and starch gain entrance into the glycolytic pathway through the sequential action of two enzymes: glycogen phosphorylase (or the similar starch phosphorylase in plants) and phosphoglucomutase. Glycogen phosphorylase catalyzes the reaction in which an (α1→4) glycosidic linkage joining two glucose residues in glycogen undergoes attack by inorganic phosphate, removing the terminal glucose residue as α-D--glucose-1-phosphate. This phosphorolysis reaction that occurs during intracellular mobilization of glycogen stores is different from the hydrolysis of glycosidic bonds by amylase during intestinal degradation of glycogen or starch; in phosphorolysis, some of the energy of the glycosidic bond is preserved in the formation of the phosphate ester, glucose-1-phosphate. Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction; its phosphate group acts as a general acid catalyst, promoting attack by Pi on the glycosidic bond.

Glycogen phosphorylase (or starch phosphorylase) acts repetitively on the nonreducing ends of glycogen (or amylopectin) branches until it reaches a point four glucose residues away from an (α1→6) branch point. Here the action of glycogen or starch phosphorylase stops. Further degradation can occur only after the action of a **"debranching enzyme," oligo (α1→6) to**

(α1→4) glucantransferase, which catalyzes two successive reactions that remove branches. Glucose-1-phosphate, the end product of the glycogen and starch phosphorylase reactions, is converted into glucose-6-phosphate by **phosphoglucomutase**, which catalyzes the reversible reaction

Glucose-1-phosphate _____ glucose-6-phosphate

Phosphoglucomutase requires as a cofactor **glucose-1,6-bisphosphate**; its role is analogous to that of 2,3-bisphosphoglycerate in the reaction catalyzed by phosphoglycerate mutase. Phosphoglucomutase, like phosphoglycerate mutase, cycles between a phosphorylated and nonphosphorylated form. In phosphoglucomutase, however, it is the hydroxyl group of a Ser residue in the active site that is transiently phosphorylated in the catalytic cycle.

Other Monosaccharides Can Enter the Glycolytic Pathway

In most organisms, hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated derivative. D-Fructose, present in free form in many fruits and formed by hydrolysis of sucrose in the small intestine, can be phosphorylated by hexokinase, which acts on a number of different hexoses:

$$\text{Fructose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{fructose-6-phosphate} + \text{ADP}$$

In the muscles and kidney of vertebrates this is a major pathway. In the liver, however, fructose gains entry into glycolysis by a different pathway. The liver enzyme **fructokinase** catalyzes the phosphorylation of fructose, not at C-6, but at C-l:

$$\text{Fructose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{fructose-1-phosphate} + \text{ADP}$$

The fructose-1-phosphate is then cleaved to form glyceraldehyde and dihydroxyacetone phosphate by **fructose-1-phosphate aldolase.**Dihydroxyacetone phosphate is converted into glyceraldehyde-3phosphate by the glycolytic enzyme triose phosphate isomerase. Glyceraldehyde is phosphorylated by ATP and triose kinase to glyceraldehyde-3-phosphate:

$$\text{Glyceraldehyde + ATP} \xrightarrow{Mg^{2+}} \text{glyceraldehyde-3-phosphate + ADP}$$

Thus both products of fructose hydrolysis enter the glycolytic pathway as glyceraldehyde- 3-phosphate.

D-Galactose, derived by hydrolysis of the disaccharide lactose (milk sugar), is first phosphorylated at C-1 at the expense of ATP by the enzyme galactokinase:

$$\text{Galactose + ATP} \xrightarrow{\text{galactokinase}} \text{galactose-1-phosphate + ADP}$$

The galactose-1-phosphate is then converted into its epimer at C-4, glucose-1-phosphate, by a set of reactions in which uridine diphosphate (UDP) functions as a coenzymelike carrier of hexose groups.

There are several human genetic diseases in which galactose metabolism is affected. In the most common form of galactosemia, the enzyme UDP-glucose : galactose-1-phosphate uridylyltransferase is genetically defective, preventing the overall conversion of galactose into glucose. Other forms of galactosemia result when either galactokinase or UDP-glucose-4-epimerase is genetically defective.

D-Mannose, which arises from the digestion of various polysaccharides and glycoproteins present in foods, can be phosphorylated at C-6 by hexokinase:

$$\text{Mannose + ATP} \xrightarrow{Mg^{2+}} \text{mannose-6-phosphate + ADP}$$

Mannose-6-phosphate is then isomerized by the action of phosphomannose isomerase, to yield fructose-6-phosphate, an intermediate of glycolysis.

Dietary Disaccharides Are Hydrolyzed to Monosaccharides

Disaccharides cannot directly enter the glycolytic pathway; indeed they cannot enter cells without first being hydrolyzed to monosaccharides extracellularly. In vertebrates, ingested disaccharides must first be hydrolyzed by enzymes attached to the outer surface of the epithelial cells lining the small intestine (Fig. 14-14), to yield their monosaccharide units:

$$\text{Maltose} + H_2O \xrightarrow{\text{maltase}} 2\ \text{D-glucose}$$

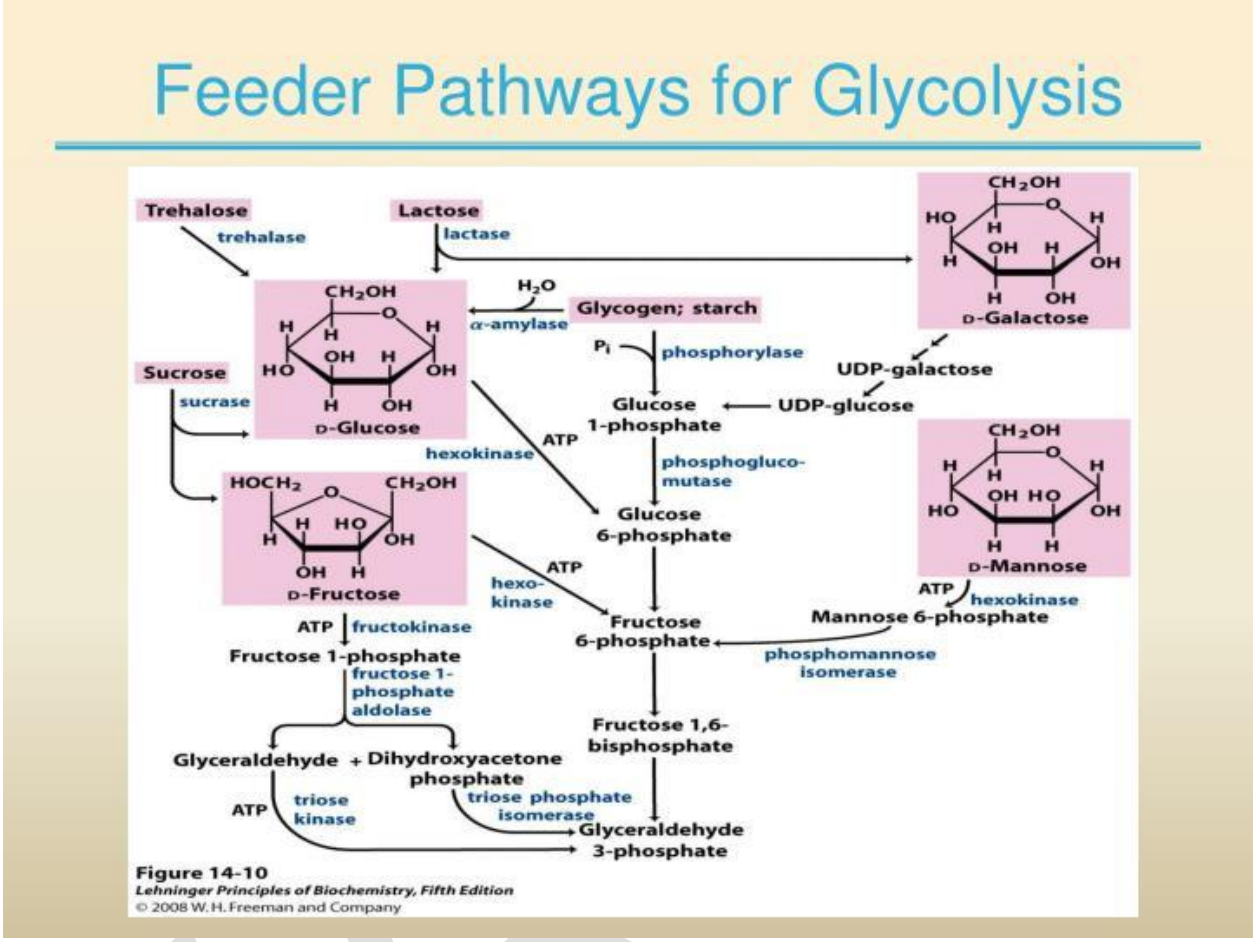
$$\text{Lactose} + H_2O \xrightarrow{\text{lactase}} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + H_2O \xrightarrow{\text{sucrase}} \text{D-fructose} + \text{D-glucose}$$

$$\text{Trehalose} + H_2O \xrightarrow{\text{trehalase}} 2\ \text{D-glucose}$$

The monosaccharides so formed are transported into the cells lining the intestine, from which they pass into the blood and are carried to the liver. There they are phosphorylated and funneled into the glycolytic sequence as described above.

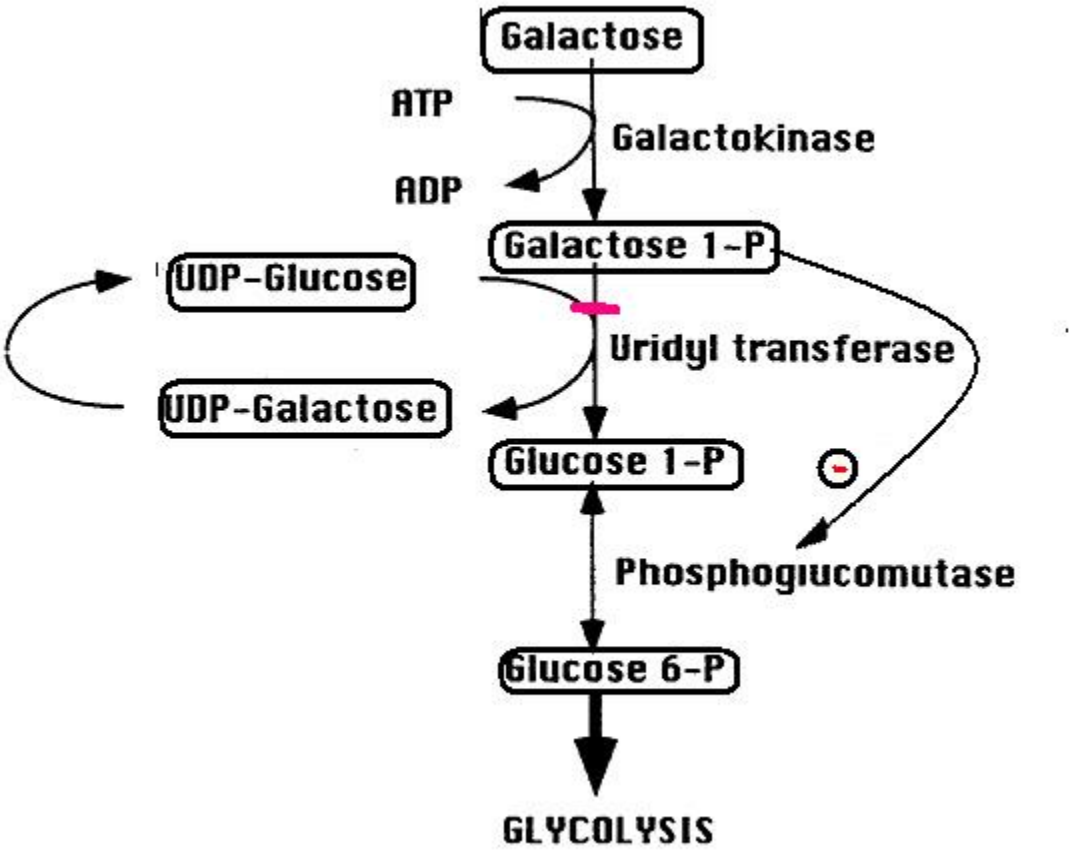
Lactose intolerance is a condition, common among adults of most human races except Northern Europeans and some Africans, in which the ingestion of milk or other foods containing lactose leads to abdominal cramps and diarrhea. Lactose intolerance is due to the disappearance after childhood of most or all of the lactase activity of the intestinal cells (Fig. 14-14b), so that lactose cannot be completely digested and absorbed. Lactose not absorbed in the small intestine is converted by bacteria in the large intestine into toxic products that cause the symptoms of the condition. In those parts of the world where lactose intolerance is prevalent, milk is simply not used as a food by adults. Milk products digested with lactase are commercially available in some countries as an alternative to excluding milk products from the diet. In certain diseases of humans, several or all of the intestinal disaccharidases are missing because of genetic defects or dietary factors, resulting in digestive disturbances triggered by disaccharides in the diet. Altering the diet to reduce disaccharide content sometimes alleviates the symptoms of these defects. The following Figure summarizes the feeder pathways that funnel hexoses, disaccharides, and polysaccharides into the central glycolytic pathway



Galactosemia

Galactosemia is a rare genetic metabolic disorder that affects an individual's ability to metabolize the sugar galactose properly. Galactosemia follows an autosomal recessive mode of inheritance that confers a deficiency in an enzyme responsible for adequate galactose degradation.

Galactose is converted into glucose by the action of three enzymes namely Galactose-1-phosphate uridylyltransferase , galactokinase, and epimerase. The disease is associated with deficiencies of each of these three enzymes:



Reduction to galactitol

In galactosemic patients, the accumulation of galactose becomes the substrate for enzymes that catalyze the polyol pathway of carbohydrate metabolism. The first reaction of this pathway is the reduction of aldoses, types of sugars including galactose, to sugar alcohols.[6] Recent data suggests that aldose reductase is the enzyme responsible for the primary stage of this pathway. Therefore, aldose reductase reduces galactose to its sugar alcohol form, galactitol. Galactitol, however, is not a suitable substrate for the next enzyme in the polyol pathway, polyol dehydrogenase. Thus, galactitol accumulates in body tissues and is excreted in the urine of galactosemic patients. Accumulation of galactitol has been attributed to many of the negative effects of galactosemia, and high concentrations of galactitol have been found in people with classic galactosemia (GALT deficiency or Galactose-1-phosphate uridylyltransferase deficiency), galactokinase deficiency, and epimerase deficiency.

Oxidation to galactonate

Accumulated galactose can also undergo an alternative reaction: Oxidation to galactonate. The mechanism of galactonate formation is still unclear. However, recent studies suggest that galactose dehydrogenase is responsible for converting galactose to galactonolactone, which then spontaneously or enzymatically converts to galactonate. Once formed, galactonate may enter the pentose phosphate pathway. Thus, Oxidation to galactonate serves as an alternate pathway for metabolizing galactose. This oxidative pathway renders accumulated galactonate less harmful than accumulated galactitol.

Diagnosis

Galactosemia test is a blood test (from the heel of the infant) or urine test that checks for three enzymes that are needed to change galactose sugar that is found in milk and milk products into glucose, a sugar that the human body uses for energy. A person with galactosemia doesn't have one of these enzymes. This causes high levels of galactose in the blood or urine.

Galactosemia is normally first detected through newborn screening, or NBS. Affected children can have serious, irreversible effects or even die within days from birth. It is important that newborns be screened for metabolic disorders without delay. Galactosemia can even be detected through NBS before any ingestion of galactose-containing formula or breast milk.

Treatment

The only treatment for classic galactosemia is eliminating lactose and galactose from the diet.

GLUCONEOGENESIS

Gluconeogenesis is the biosynthesis of new glucose, (i.e. not glucose from glycogen). This process is frequently referred to as endogenous glucose production (EGP). The production of glucose from other carbon skeletons is necessary since the testes, erythrocytes and kidney medulla exclusively utilize glucose for ATP production. The brain also utilizes large amounts of the daily glucose consumed or produced via gluconeogenesis. However, in addition to glucose, the brain can derive energy from ketone bodies which are converted to acetyl-CoA and shunted into the TCA cycle. The primary carbon skeletons used for gluconeogenesis are derived from pyruvate, lactate, glycerol, and the amino acids alanine and glutamine. The liver is the major site

of gluconeogenesis, however, as discussed below, the kidney and the small intestine also have important roles to play in this pathway. Synthesis of glucose from three and four carbon precursors is essentially a reversal of glycolysis

Precursor

In humans the main gluconeogenic precursors are lactate, glycerol (which is a part of the triacylglycerol molecule), alanine and glutamine. Altogether, they account for over 90% of the overall gluconeogenesis. Other glucogenic amino acid as well as all citric acid cycle intermediates, the latter through conversion to oxaloacetate, can also function as substrates for gluconeogenesis. In ruminants, propionate is the principal gluconeogenic substrate.

Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. Transamination or deamination of amino acids facilitates entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

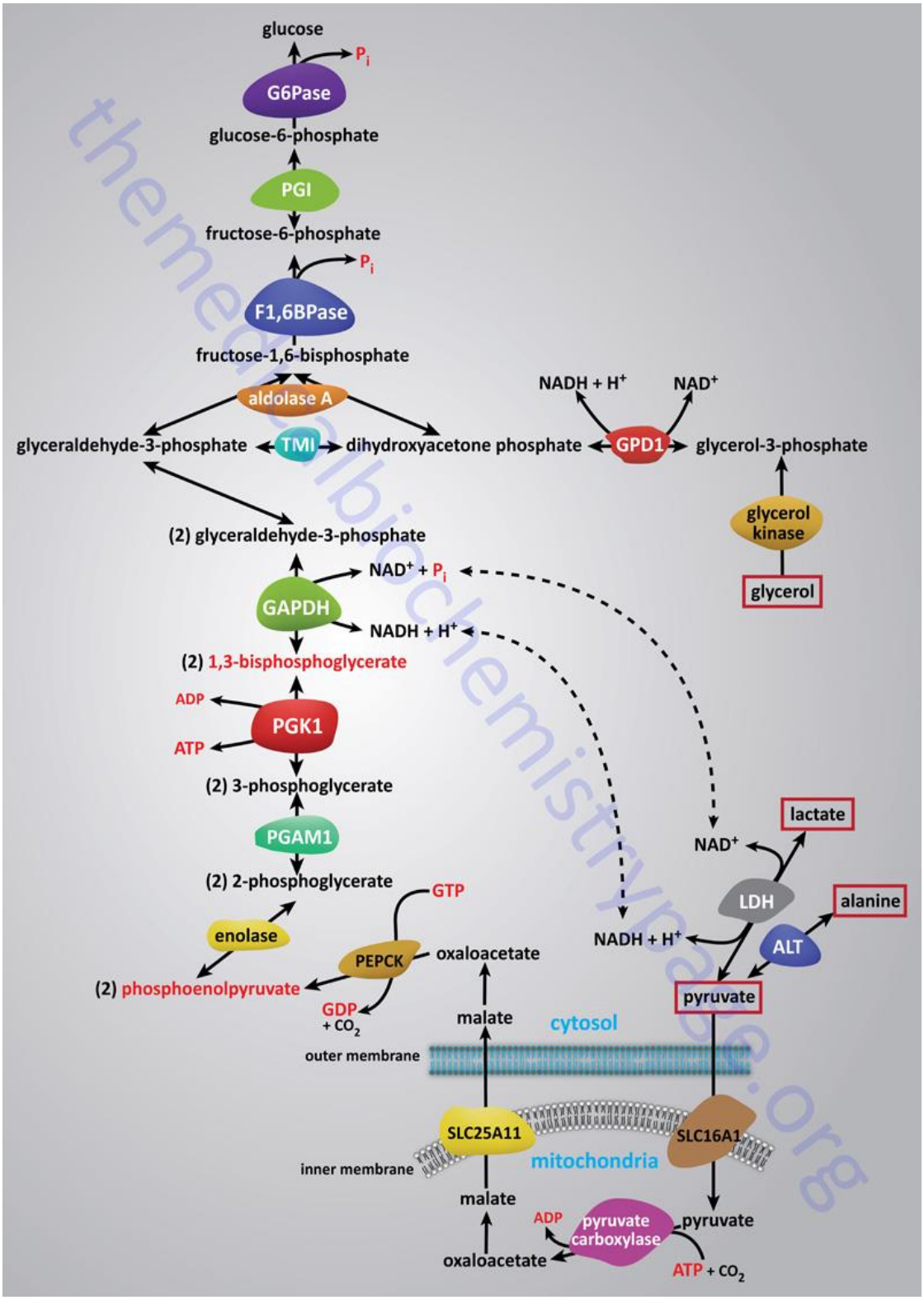
Whether even-chain fatty acids can be converted into glucose in animals has been a longstanding question in biochemistry. It is known that odd-chain fatty acids can be oxidized to yield propionyl CoA, a precursor for succinyl CoA, which can be converted to pyruvate and enter into gluconeogenesis. In plants, specifically seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis.

The existence of glyoxylate cycles in humans has not been established, and it is widely held that fatty acids cannot be converted to glucose in humans directly. However, carbon-14 has been shown to end up in glucose when it is supplied in fatty acids. Despite these findings, it is considered unlikely that the 2-carbon acetyl-CoA derived from the oxidation of fatty acids would produce a net yield of glucose via the citric acid cycle - however, acetyl-CoA can be converted into pyruvate and lactate through the ketogenic pathway.Put simply, acetic acid (in the form of acetyl-CoA) is used to partially produce glucose; acetyl groups can only form part of the glucose molecules (not the 5th carbon atom) and require extra substrates (such as pyruvate) in order to form the rest of the glucose molecule. But a roundabout pathway does lead from acetyl-coA to pyruvate, via acetoacetate, acetone, acetol and then either propylene glycol or methylglyoxal.

Location

In mammals, gluconeogenesis is restricted to the liver, the kidney and possibly the intestine. However these organs use somewhat different gluconeogenic precursors. The liver uses primarily lactate, alanine and glycerol while the kidney uses lactate, glutamine and glycerol. Propionate is the principal substrate for gluconeogenesis in the ruminant liver, and the ruminant liver may make increased use of gluconeogenic amino acids, e.g. alanine, when glucose demand is increased. The capacity of liver cells to use lactate for gluconeogenesis declines from the preruminant stage to the ruminant stage in calves and lambs. In sheep kidney tissue, very high rates of gluconeogenesis from propionate have been observed. The intestine uses mostly glutamine and glycerol.

In all species, the formation of oxaloacetate from pyruvate and TCA cycle intermediates is restricted to the mitochondrion, and the enzymes that convert Phosphoenolpyruvic acid (PEP) to glucose are found in the cytosol. The location of the enzyme that links these two parts of gluconeogenesis by converting oxaloacetate to PEP, PEP carboxykinase, is variable by species: it can be found entirely within the mitochondria, entirely within the cytosol, or dispersed evenly between the two, as it is in humans. Transport of PEP across the mitochondrial membrane is accomplished by dedicated transport proteins; however no such proteins exist for oxaloacetate. Therefore, in species that lack intra-mitochondrial PEP carboxykinase, oxaloacetate must be converted into malate or aspartate, exported from the mitochondrion, and converted back into oxaloacetate in order to allow gluconeogenesis to continue.



Pathway

Gluconeogenesis is a pathway consisting of a series of eleven enzyme-catalyzed reactions. The pathway may begin in the mitochondria or cytoplasm, this being dependent on the substrate being used. Many of the reactions are the reversible steps found in glycolysis. Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate. This reaction also requires one molecule of ATP, and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in -oxidation in the liver) and inhibited by high levels of ADP and glucose.

Oxaloacetate is reduced to malate using NADH, a step required for its transportation out of the mitochondria. Malate is oxidized to oxaloacetate using NAD^+ in the cytosol, where the remaining steps of gluconeogenesis take place. Oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate using the enzyme phosphoenolpyruvate carboxykinase. A molecule of GTP is hydrolyzed to GDP during this reaction. The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose 6-phosphate, using one water molecule and releasing one phosphate. This is also the rate-limiting step of gluconeogenesis. Glucose-6-phosphate is formed from fructose 6-phosphate by phosphoglucoisomerase. Glucose-6-phosphate can be used in other metabolic pathways or dephosphorylated to free glucose. Whereas free glucose can easily diffuse in and out of the cell, the phosphorylated form (glucose-6-phosphate) is locked in the cell, a mechanism by which intracellular glucose levels are controlled by cells. The final reaction of gluconeogenesis, the formation of glucose, occurs in the lumen of the endoplasmic reticulum, where glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulum's membrane.

RECIPROCAL CONTROL OF GLYCOLYSIS AND GLUCONEOGENESIS

Gluconeogenesis and glycolysis are coordinated so that within a cell one pathway is relatively inactive while the other is highly active. If both sets of reactions were highly active at the same time, the net result would be the hydrolysis of four nucleotide triphosphates (two ATP plus

two GTP) per reaction cycle. Both glycolysis and gluconeogenesis are highly exergonic under cellular conditions, and so there is no thermodynamic barrier to such simultaneous activity. However, the *amounts* and *activities* of the distinctive enzymes of each pathway are controlled so that both pathways are not highly active at the same time. The rate of glycolysis is also determined by the concentration of glucose, and the rate of gluconeogenesis by the concentrations of lactate and other precursors of glucose.

The interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate is stringently controlled (Figure 16.30). As discussed in Section 16.2.1, AMP stimulates phosphofructokinase, whereas ATP and citrate inhibit it. Fructose 1,6-bisphosphatase, on the other hand, is inhibited by AMP and activated by citrate. A high level of AMP indicates that the energy charge is low and signals the need for ATP generation. Conversely, high levels of ATP and citrate indicate that the energy charge is high and that biosynthetic intermediates are abundant. Under these conditions, glycolysis is nearly switched off and gluconeogenesis is promoted.

hosphofructokinase and fructose 1,6-bisphosphatase are also reciprocally controlled by *fructose 2,6-bisphosphate in the liver* (Section 16.2.2). The level of F-2,6-BP is low during starvation and high in the fed state, because of the antagonistic effects of glucagon and insulin on the production and degradation of this signal molecule. *Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase*. Hence, glycolysis is accelerated and gluconeogenesis is diminished in the fed state. During starvation, gluconeogenesis predominates because the level of F-2,6-BP is very low. Glucose formed by the liver under these conditions is essential for the viability of brain and muscle.

The interconversion of phosphoenolpyruvate and pyruvate also is precisely regulated. Recall that pyruvate kinase is controlled by allosteric effectors and by phosphorylation (Section 16.2.3). High levels of ATP and alanine, which signal that the energy charge is high and that building blocks are abundant, inhibit the enzyme in liver. Conversely, pyruvate carboxylase, which catalyzes the first step in gluconeogenesis from pyruvate, is activated by acetyl CoA and inhibited by ADP. Likewise, ADP inhibits phosphoenolpyruvate carboxykinase. Hence, gluconeogenesis is favored when the cell is rich in biosynthetic precursors and ATP.

The amounts and the activities of these essential enzymes also are regulated. The regulators in this case are hormones. Hormones affect gene expression primarily by changing the rate of transcription, as well as by regulating the degradation of mRNA. Insulin, which rises subsequent to eating, stimulates the expression of phosphofructokinase, pyruvate kinase, and the bifunctional enzyme that makes and degrades F-2,6-BP. Glucagon, which rises during starvation, inhibits the expression of these enzymes and stimulates instead the production of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase. Transcriptional control in eukaryotes is much slower than allosteric control; it takes hours or days in contrast with seconds to minutes. The richness and complexity of hormonal control are graphically displayed by the promoter of the phosphoenolpyruvate carboxykinase gene, which contains regulatory sequences that respond to insulin, glucagon, glucocorticoids, and thyroid hormone

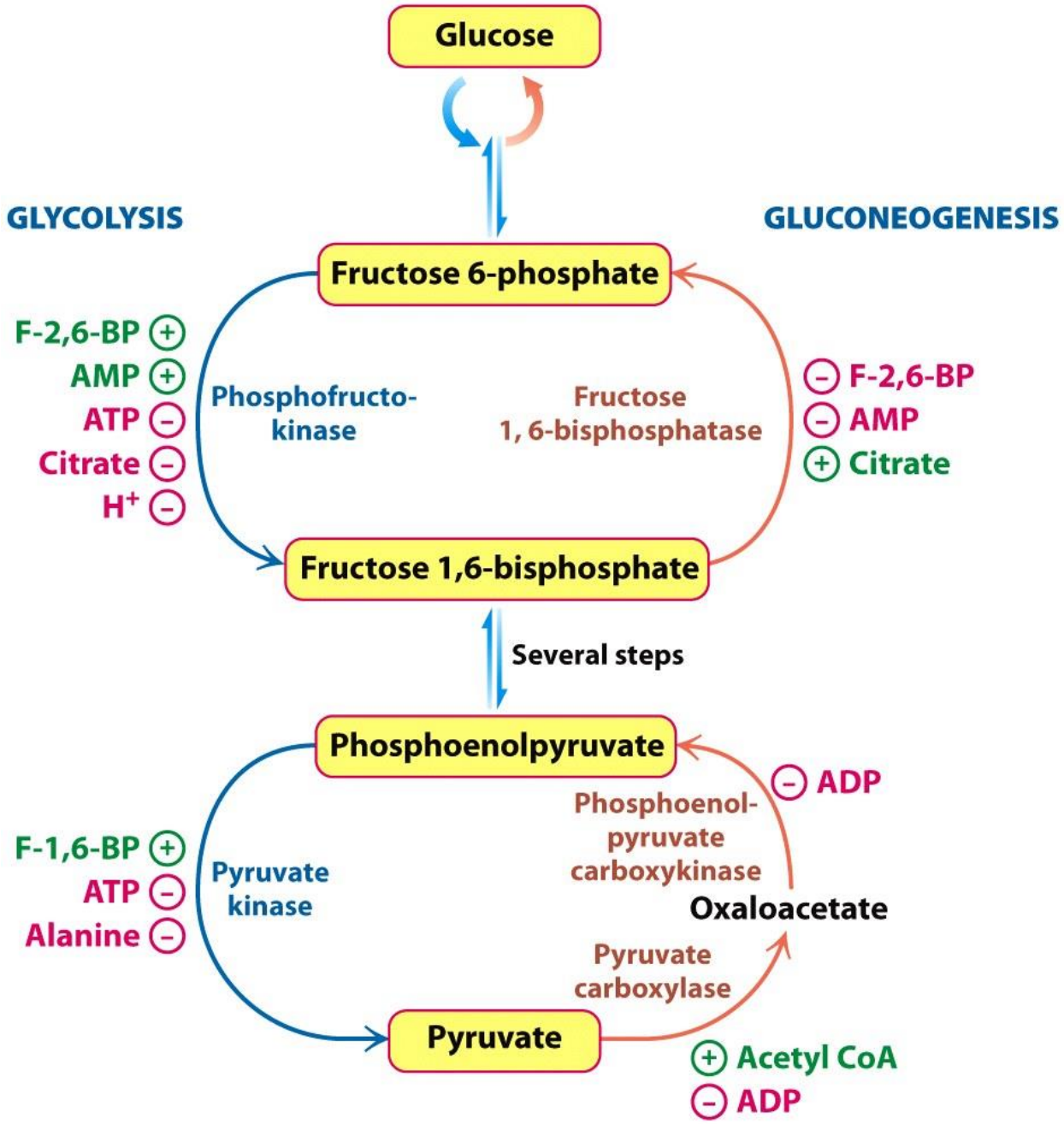


Figure 16.30
***Biochemistry*, Seventh Edition**

PENTOSE PHOSPHATE PATHWAY (HMP SHUNT)

The pentose phosphate pathway (also called the phosphogluconate pathway and the

hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars). While it does involve oxidation of glucose, its primary role is anabolic rather than catabolic. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non-oxidative synthesis of 5-carbon sugars. For most organisms, the pentose phosphate pathway takes place in the cytosol; in plants, most steps take place in plastids.

Similar to glycolysis, the pentose phosphate pathway appears to have a very ancient evolutionary origin. The reactions of this pathway are (mostly) enzyme catalysed in modern cells. They also occur however non-enzymatically under conditions that replicate those of the Archean ocean, and are then catalyzed by metal ions, ferrous iron Fe (II) in particular. The origins of the pathway could thus date back to the prebiotic world.

Pathway

The generation of reducing equivalents, in the form of NADPH, used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis). Production of ribose-5-phosphate (R5P), used in the synthesis of nucleotides and nucleic acids. Production of erythrose-4-phosphate (E4P), used in the synthesis of aromatic amino acids. Aromatic amino acids, in turn, are precursors for many biosynthetic pathways, including the lignin in wood.

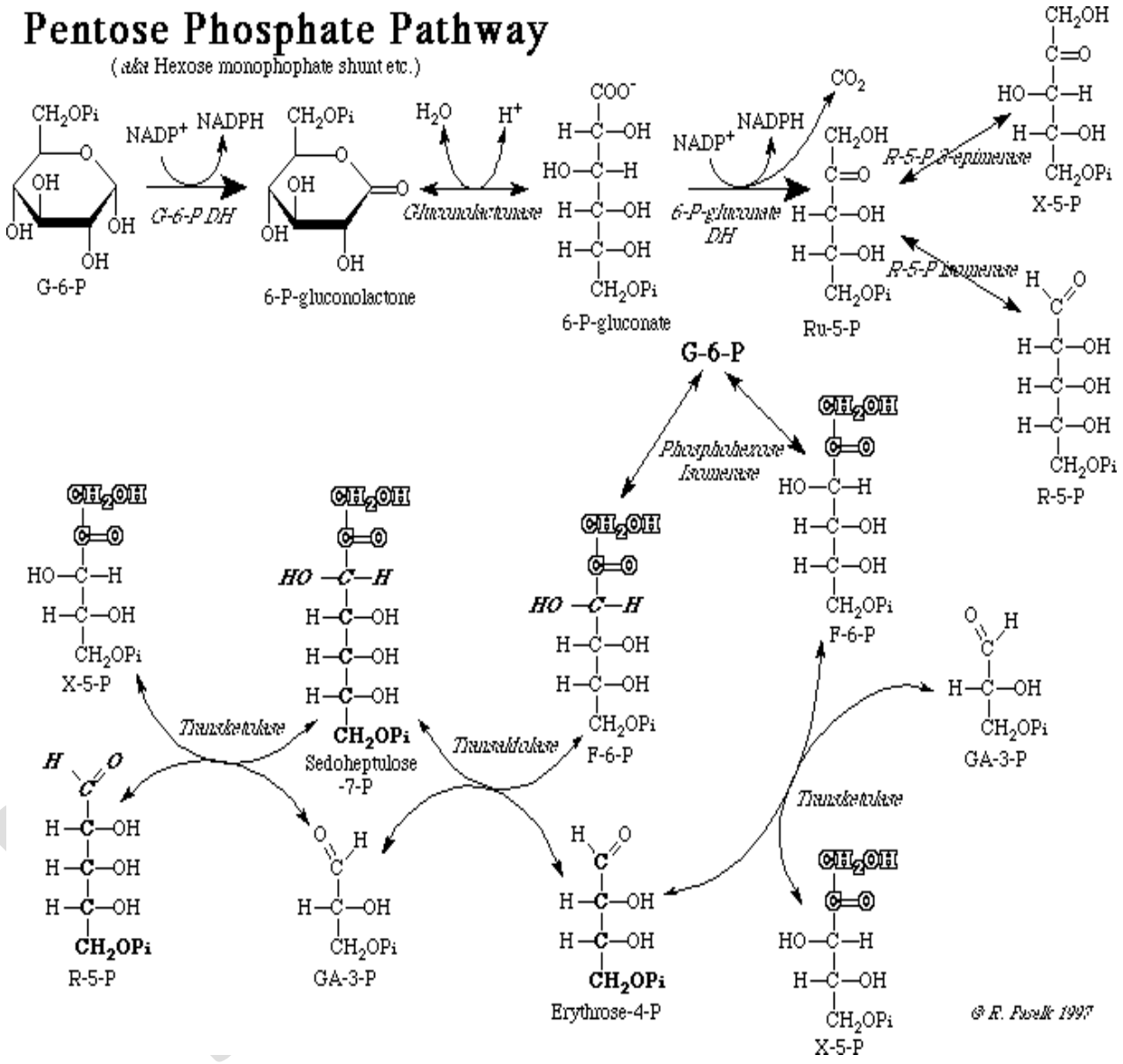
Dietary pentose sugars derived from the digestion of nucleic acids may be metabolized through the pentose phosphate pathway, and the carbon skeletons of dietary carbohydrates may be converted into glycolytic/gluconeogenic intermediates. In mammals, the PPP occurs exclusively in the cytoplasm, and is found to be most active in the liver, mammary gland and adrenal cortex in the human. The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans.

One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces glutathione via glutathione reductase, which converts reactive H2O2 into H2O by glutathione peroxidase. If absent, the H_2O_2 would be converted to hydroxyl free radicals by Fenton chemistry, which can attack the cell. Erythrocytes, for example, generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.Hydrogen peroxide is also generated for phagocytes in a process often referred to as a respiratory burst.

Phases

Oxidative phase

In this phase, two molecules of NADP+ are reduced to NADPH, utilizing the energy from the conversion of glucose-6-phosphate into ribulose 5-phosphate.



Regulation

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme of this pathway. It is allosterically stimulated by $NADP^+$. The ratio of NADPH: $NADP^+$ is normally about 100:1 in liver cytosol[citation needed]. This makes the cytosol a highly-reducing environment. An NADPH-utilizing pathway forms $NADP^+$, which stimulates Glucose-6-phosphate ehydrogenase

to produce more NADPH. This step is also inhibited by acetyl CoA.

Erythrocytes and the pentose phosphate pathway

Several deficiencies in the level of activity of glucose-6-phosphate dehydrogenase have been observed to be associated with resistance to the malarial parasite Plasmodium falciparum among individuals of Mediterranean and African descent. The basis for this resistance may be a weakening of the red cell membrane (the erythrocyte is the host cell for the parasite) such that it cannot sustain the parasitic life cycle long enough for productive growth.

POSSIBLE QUESTIONS
UNIT I

PART A (1 mark)
Question number 1-20 (Online examination)

PART B (2 Marks)

1. Write a note on autotrophs
2. Brief about metabolic pathways
3. What do you mean by reducing power of the cell
4. Write a note on HMP
5. Write a notes on galactosemia
6. Explain the overview of intermediary metabolism?
7. Give an account on catabolism and anabolism?
8. What do you mean by amphibolic?
9. what are the feeder pathways for glycolysis
10. List the sources of gluconeogenesis

PART C (6 Marks)

11. Briefly explain the metabolism of glycolysis
12. Describe the fermentation process
13. Explain in detail about galactosemia.
14. Importance of reciprocal regulation of glycolysis
15. Explain the gluconeogenesis
16. Explain about pentose phosphate pathway and its importance.
17. Explain about reducing power of the cell
18. Explain reciprocal regulation of gluconeogenesis
19. Derive the ATP produced by complete oxidation of glucose
20. Explain the importance and reactions of HMP shunt

Karpagam Academy of Higher Education
Department of Biochemistry
Metabolism of Carbohydrates and Lipids (17BCU301) MCQ
UNIT I

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	1	Catabolism is	Breakdown of biological molecules	Synthesis of biological molecules	Conversion of biological molecules	Utilizing of biological molecules	Breakdown of biological molecules
2	1	Amphibolic is	Catabolism	Anabolism	Metabolism	Both catabolism and anabolism	Both catabolism and anabolism
3	1						
4	1	Primary role of pentose phosphate pathway is	Catabolic	Anabolic	Both A and B	Amphibolic	Anabolic
5	1						
6	1	Pentose phosphate pathway is parallel to	Glycolysis	Gluconeogenesis	Fermentation	Respiration	Glycolysis
7	1	Pentose phosphate pathway is also termed as	Glycolysis	Gluconeogenesis	Phosphogluconate pathway	Glycogenolysis	Phosphogluconate pathway
8	1						
9	1	Uncoupling of mitochondrial oxidative phosphorylation	Allows continued mitochondrial ATP for	Halts all mitochondrial metabolism	Halts mitochondrial ATP formation, but alc	Slows the conversion of glucose to pyruva	Halts mitochondrial ATP formation, but allows continued O2 consumption
10	1	Which is not a metabolic intermediate used in amphibolic pathways?	Glyceraldehyde-3-phosphate		Acetyl CoA	Oxaloacetic acid	Fructose-1,6-bisphosphate
11	1	Complete oxidative breakdown of glucose results in _____ ATP molecules	32	36	32	39	36
12	1						
13	1	Which one of the following is a rate limiting enzyme of gluconeogenesis?	Hexokinase	Phsophofructokinase	Pyruvate carboxylase	Pyruvate kinase	Pyruvate carboxylase
14	1	Different enzymes that catalyze same reaction are called	isoenzymes	coenzymes	cofactors	isofactors	isoenzymes
15	1						
16	1	Gluconeogenesis occurs in	Adipose tissue	Muscles	Kidneys	Brain	Kidneys
17	1	In glycolysis a net gain of two ATPs are generated by what process?	Chemiosmosis	ADP processing	Substrate level phosphorylation	Electron transport chain	Substrate level phosphorylation
18	1	The preparatory reaction breaks	Glucose into pyruvates	Pyruvates into glucose	Pyruvates into acetyl-coa and carbon dioxi	Pyruvates into acetyl-coa and water	Pyruvates into acetyl-coa and carbon dioxide
19	1	Embden-Meyerhof pathway referred as	Gluconeogenesis	Glycolysis	Citric acid	Glycogenesis	Glycolysis
20	1	In gluconeogenesis, glucose is generated by	Non carbohydrate carbon substrates	Carbohydrate carbon substrates	Sucrose	Yeast	Non carbohydrate carbon substrates
21	1						
22	1	Gluconeogenesis is often associated with	Ketosis	Hexoses	Pentoses	Aldolase	Ketosis
23	1						
24	1	In vertebrates, gluconeogenesis mainly takes place in	Stomach	Liver	Heart	Intestine	Liver
25	1						
26	1	The first two intermediates in the process of glycolysis are, respectively	Glucose 6-phosphate and glucose 1-pho	Glucose 1-phosphate and glucose 6-phospha	Glucose 6-phosphate and fructose 6-phosp	Glucose 1-phosphate and fructose 1-phos	Glucose 6-phosphate and fructose 6-phosphate
27	1	The name of the process in which glucose 6-phosphate is converted to glycogen is	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis	Glycogenesis
28	1	Which of the following is a reactant in the first step of gluconeogenesis?	Carbon dioxide	GTP	Glucose	Phosphoenol pyruvate	Carbon dioxide
29	1	Which of the following metabolite integrates glucose and fatty acid metabolism?	Acetyl CoA	Pyruvate	Citrate	Lactate	Acetyl CoA
30	1	Gluconeogenesis is decreased by	Glucagon	Epinephrine	Glucocorticoids	Insulin	Insulin

UNIT-II
SYLLABUS

Glycogen metabolism: Glycogenesis and glycogenolysis, regulation of glycogen metabolism, glycogen storage diseases.
Citric acid cycle : Production of acetyl CoA, reactions of citric acid cycle, anaplerotic reactions, amphibolic role, regulation of citric acid cycle, glyoxalate pathway, coordinated regulation of glyoxalate and citric acid pathways.

INTRODUCTION

Glycogen is a *readily mobilized storage form of glucose.* It is a very large, branched polymer of glucose residues that can be broken down to yield glucose molecules when energy is needed. Most of the glucoseresidues in glycogen are linked by α-1,4-glycosidic bonds. Branches at about every tenth residue are created by α-1,6-glycosidic bonds.

Glycogen is an important fuel reserve for several reasons. The controlled breakdown of glycogen and release of glucose increase the amount of glucose that is available between meals. Hence, glycogen serves as a buffer to maintain blood-glucose levels. Glycogen's role in maintaining blood-glucose levels is especially important because glucose is virtually the only fuel used by the brain, except during prolonged starvation. Moreover, the glucose from glycogen is readily mobilized and is therefore a good source of energy for sudden, strenuous activity. Unlike fatty acids, the released glucose can provide energy in the absence of oxygen and can thus supply energy for anaerobic activity.

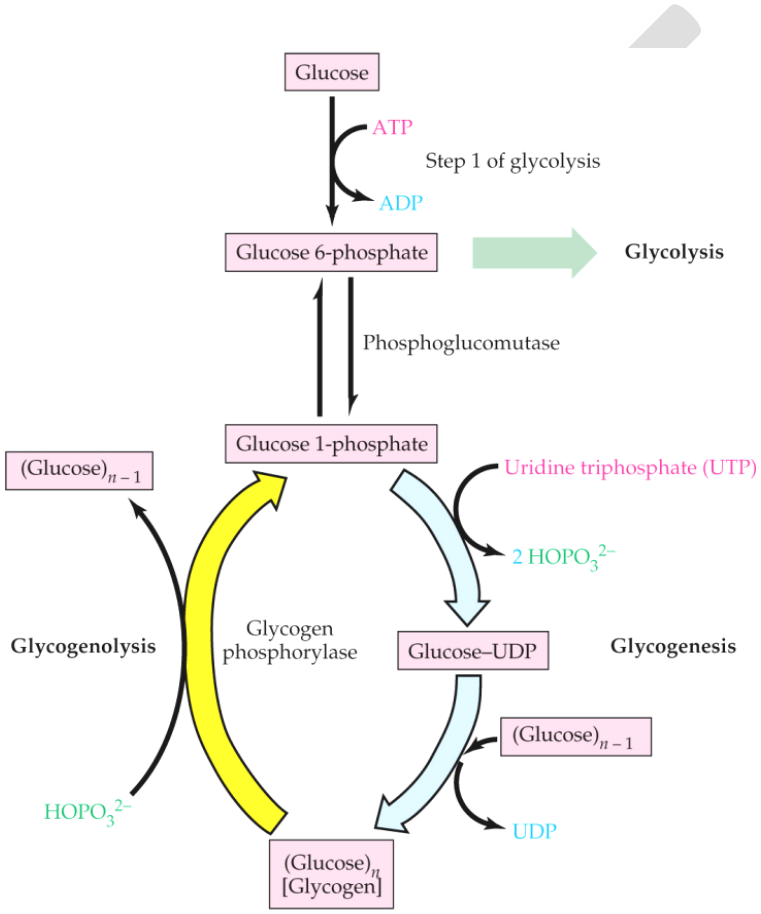
GLYCOGENESIS

Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage. This process is activated during rest periods following the Cori cycle, in the liver, and also activated by insulin in response to high glucose levels, for example after a carbohydrate-containing meal.

Steps

Glucose is converted into glucose-6-phosphate by the action of glucokinase or Hexokinase.

Glucose-6-phosphate is converted into glucose-1-phosphate by the action of Phosphoglucomutase, passing through an obligatory intermediate step of glucose-1,6-bisphosphate. Glucose-1-phosphate is converted into UDP-glucose by the action of Uridyl Transferase (also called UDP-glucose pyrophosphorylase) and pyrophosphate is formed, which is hydrolysed by pyrophosphatase into 2 molecules of Pi.



Glucose molecules are assembled in a chain by glycogen synthase, which must act on a pre-existing glycogen primer or glycogenin (small protein that forms the primer). The mechanism for joining glucose units is that glycogen synthase binds to UDPG, causing it to break down into an oxonium ion, also formed in glycogenolysis. This oxonium ion can readily add to the 4-hydroxyl group of a glucosyl residue on the 4 end of the glycogen chain.

Control and regulations

Glycogenesis responds to hormonal control.

One of the main forms of control is the varied phosphorylation of glycogen synthase and glycogen phosphorylase. This is regulated by enzymes under the control of hormonal activity, which is in turn regulated by many factors. As such, there are many different possible effectors when compared to allosteric systems of regulation.

Epinephrine

Glycogen phosphorylase is activated by phosphorylation, whereas glycogen synthase is inhibited. Glycogen phosphorylase is converted from its less active "b" form to an active "a" form by the enzyme phosphorylase kinase. This latter enzyme is itself activated by protein kinase A and deactivated by phosphoprotein phosphatase-1.

Protein kinase A itself is activated by the hormone adrenaline. Epinephrine binds to a receptor protein that activates adenylate cyclase. The latter enzyme causes the formation of cyclic AMP from ATP; two molecules of cyclic AMP bind to the regulatory subunit of protein kinase A, which activates it allowing the catalytic subunit of protein kinase A to dissociate from the assembly and to phosphorylate other proteins. Returning to glycogen phosphorylase, the less active "b" form can itself be activated without the conformational change. 5'AMP acts as an allosteric activator, whereas ATP is an inhibitor, as already seen with phosphofructokinase control, helping to change the rate of flux in response to energy demand.

Epinephrine not only activates glycogen phosphorylase but also inhibits glycogen synthase. This amplifies the effect of activating glycogen phosphorylase. This inhibition is achieved by a similar mechanism, as protein kinase A acts to phosphorylate the enzyme, which lowers activity. This is known as co-ordinate reciprocal control. Refer to glycolysis for further information of the regulation of glycogenesis.

Insulin

Insulin has an antagonistic effect to epinephrine signaling via the beta-adrenergic receptor (G-Protein coupled receptor). When insulin binds to its receptor (insulin receptor), it results in the activation (phosphorylation) of Akt which in turn activates Phosphodiesterase (PDE). PDE then will inhibit cyclic AMP (cAMP) action and cause inactivation of PKA which will cause

Hormone Sensitive Lipase (HSL) to be dephosphorylated and inactive so that lipolysis and lipogenesis is not occurring simultaneously.

Calcium ions

Calcium ions or cyclic AMP (cAMP) act as secondary messengers. This is an example of negative control. The calcium ions activate phosphorylase kinase. This activates glycogen phosphorylase and inhibits glycogen synthase.

GLYCOGENOLYSIS

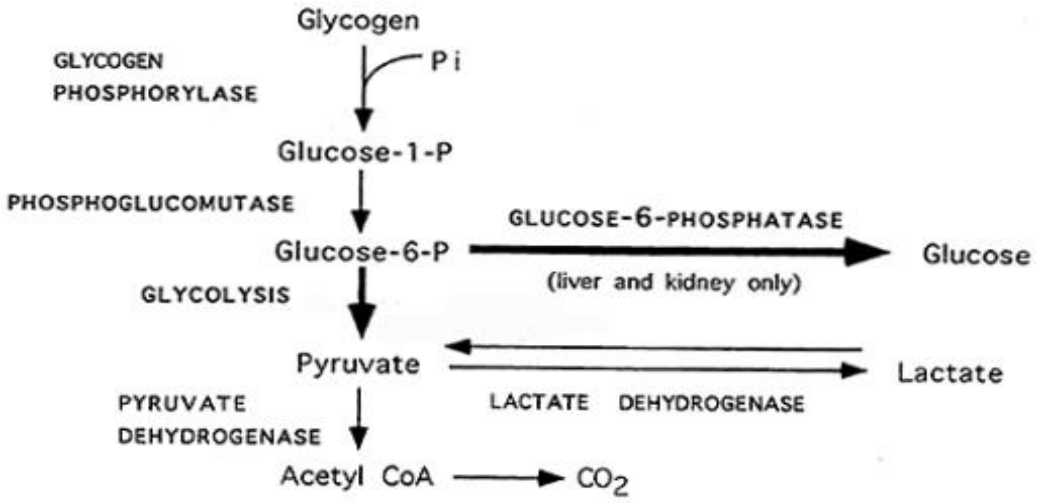
Glycogenolysis is the breakdown of glycogen (n) to glucose-1-phosphate and glycogen (n-1). Glycogen branches are catabolized by the sequential removal of glucose monomers via phosphorolysis, by the enzyme glycogen phosphorylase

Mechanism

The overall reaction for the breakdown of glycogen to glucose-1-phosphate is:

Glycogen (n residues) + Pi is in equilibrium with glycogen(n-1 residues) + glucose-1-phosphate

Here, glycogen phosphorylase cleaves the bond linking a terminal glucose residue to a glycogen branch by substitution of a phosphoryl group for the linkage. Glucose-1-phosphate is converted to glucose-6-phosphate by the enzyme phosphoglucomutase. Glucose residues are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with a linkage. Glycogen debranching enzyme then transfers three of the remaining four glucose units to the end of another glycogen branch. This exposes the branching point, which is hydrolysed by glucosidase, removing the final glucose residue of the branch as a molecule of glucose and eliminating the branch. This is the only case in which a glycogen metabolite is not glucose-1-phosphate. The glucose is subsequently phosphorylated to glucose-6-phosphate by hexokinase.



Function

Glycogenolysis takes place in the cells of the muscle and liver tissues in response to hormonal and neural signals. In particular, glycogenolysis plays an important role in the fight-or-flight response and the regulation of glucose levels in the blood. In myocytes (muscle cells), glycogen degradation serves to provide an immediate source of glucose-6-phosphate for glycolysis, to provide energy for muscle contraction.

In hepatocytes (liver cells), the main purpose of the breakdown of glycogen is for the release of glucose into the bloodstream for uptake by other cells. The phosphate group of glucose-6-phosphate is removed by the enzyme glucose-6-phosphatase, which is not present in myocytes, and the free glucose exits the cell via GLUT2 facilitated diffusion channels in the hepatocyte cell membrane.

REGULATION OF GLYCOGEN METABOLISM

Mechanisms of regulation of glycogenesis and glycogenolysis:

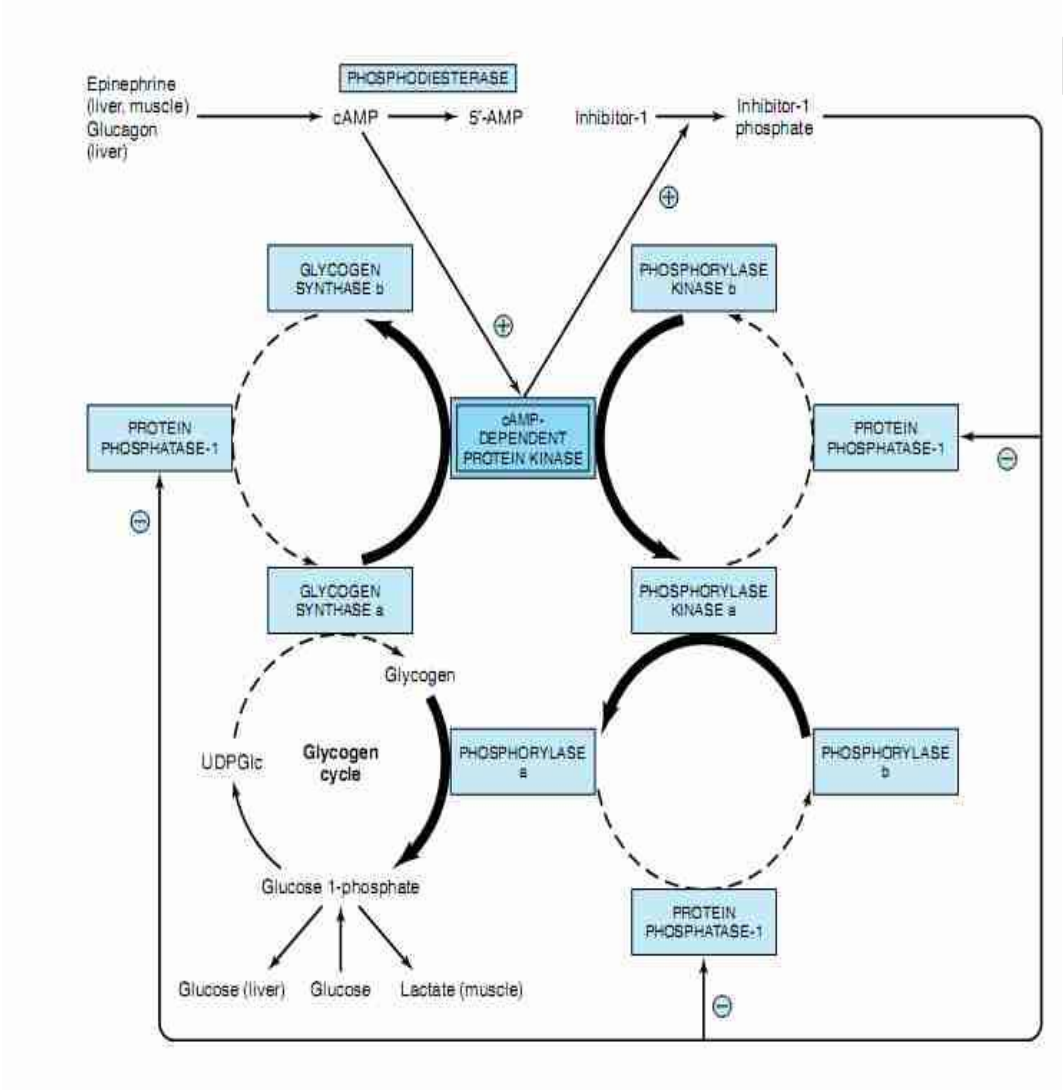
1. Allosteric mechanism
2. Hormonal mechanism
3. Influence of calcium

1. Allosteric regulation:

- The control is carried out in such a way that glycogen synthesis is increased when substrate and energy levels are high. On the other hand, glycogen breakdown is enhanced when glucose concentration and energy levels are low.

2. Hormonal regulation:

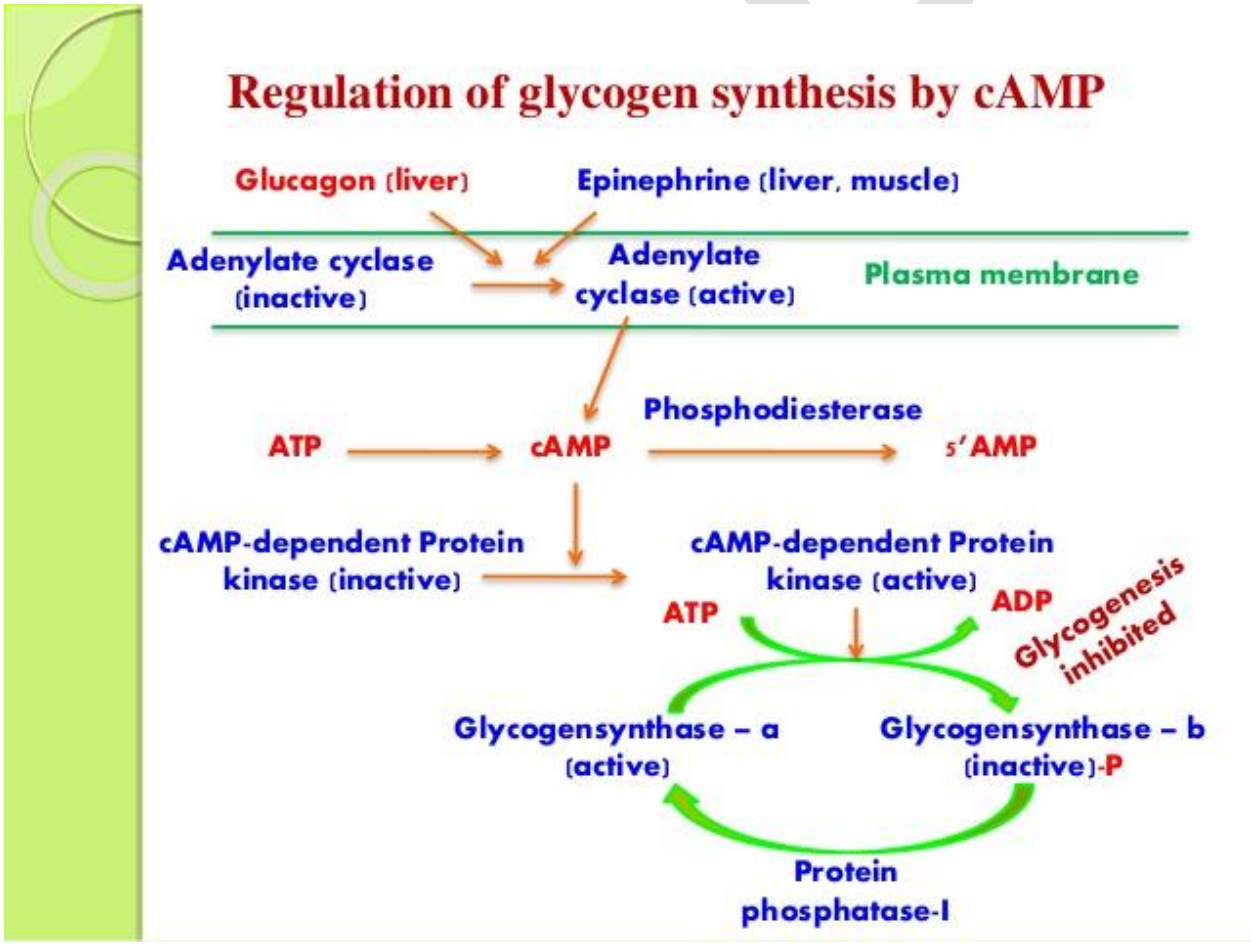
- The hormones, through a complex series of reactions bring, about covalent modification : phosphorylation and dephosphorylation of enzyme proteins.
- cAMP acts as a second messenger for hormones.



3. Influence of calcium:

- Calcium ions are released from sarcoplasmic reticulum during muscle contraction
- Calcium binds to calmodulin (calcium modulating protein) and directly activates phosphorylase kinase without the involvement of cAPM-dependent protein kinase.
- Muscle phosphorylase kinase activates glycogen phosphorylase.

Increased glucagon or epinephrine level = increased glycogenolysis
Increased insulin = increased glycogenesis



Glycogenolysis is regulated hormonally in response to blood sugar levels by glucagon and insulin, and stimulated by epinephrine during the fight-or-flight response. In myocytes, glycogen degradation may also be stimulated by neural signals.

Clinical significance

Parenteral (intravenous) administration of glucagon is a common human medical intervention in diabetic emergencies when sugar cannot be given orally. It can also be administered intramuscularly.

GLYCOGEN STORAGE DISEASE

Glycogen storage disease (GSD, also glycogenosis and dextrinosis) is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types.

GSD has two classes of cause: genetic and acquired. Genetic GSD is caused by any inborn error of metabolism (genetically defective enzymes) involved in these processes

Glucose is a large energy source for the body. It is stored by the body in the form of glycogen and released into the blood as needed with the help of special proteins called enzymes.

There are different types of GSD but all people who have GSD are born with the disease. When a person has GSD:

- The liver cannot control the use of glycogen and glucose because certain enzymes are missing that control the change of sugar (glucose) into its storage form (glycogen) or release of glucose from glycogen.
- An abnormal amount of glycogen is stored in the liver.
- Not enough glucose is in the blood (also called hypoglycemia).

Many sugars (including glucose) are found in foods and are used by the body as a source of energy. After a meal, blood glucose levels rise. The body stores the extra glucose that is not needed right away as glycogen in the liver and muscles. Later, as the blood glucose levels in the body begin to drop, the body uses this stored energy.

These sugars, stored in the form of glycogen, need to be processed by enzymes in the body before they can carry out their functions. If the enzymes needed to process them are missing, the glycogen or one of its related starches can build up in the liver, causing problems.

Glycogen storage disorders are classified according to which protein (enzyme) is lacking or not

working normally and also which part of the body is affected by the disease. Glycogen storage disorders mostly tend to affect your liver and muscles. However, some glycogen storage disorders can affect other parts of the body such as the kidney, heart, blood vessels, nervous system and bowel (see below). The different types of glycogen storage disorder include:

- Type Ia (von Gierke's disease), type Ib.
- Type II (Pompe's disease).
- Type III (Forbes-Cori disease).
- Type IV (Andersen's disease).
- Type V (McArdle's disease).
- Type VI (Hers' disease).
- Type VII (Tarui's disease).
- Type IX (liver phosphorylase kinase deficiency).
- Type XI (Fanconi-Bickel syndrome).
- Type 0 (Lewis' disease).

Table 21.1 Glycogen-storage diseases

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II Pompe	α-1,4-Glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure.	Cardiorespiratory failure causes death, usually before age 2.
III Cori	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV Andersen	Branching enzyme (α-1,4 → α-1,6)	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V McArdle	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI Hers	Phosphorylase	Liver	Increased amount.	Like type I, but milder course.
VII	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.

Note: Types I through VII are inherited as autosomal recessives. Type VIII is sex linked.

Type I glycogen storage disorder is the most common. About one quarter of people who have glycogen storage disorder have type I. It is due to a lack of the enzyme known as glucose-6-phosphatase. Type VIII and type X are now classified with type VI.

CITRIC ACID CYCLE

Tricarboxylic acid cycle, (TCA cycle), also called Krebs cycle and citric acid cycle, the second stage of cellular respiration, the three-stage process by which living cells break down organic fuel molecules in the presence of oxygen to harvest the energy they need to grow and divide. This metabolic process occurs in most plants, animals, fungi, and many bacteria. In all organisms except bacteria the TCA cycle is carried out in the matrix of intracellular structures called mitochondria. The TCA cycle plays a central role in the breakdown, or catabolism, of organic fuel molecules—i.e., glucose and some other sugars, fatty acids, and some amino acids.

Before these rather large molecules can enter the TCA cycle they must be degraded into a two-carbon compound called acetyl coenzyme A (acetyl CoA). Once fed into the TCA cycle, acetyl CoA is converted into carbon dioxide and energy.

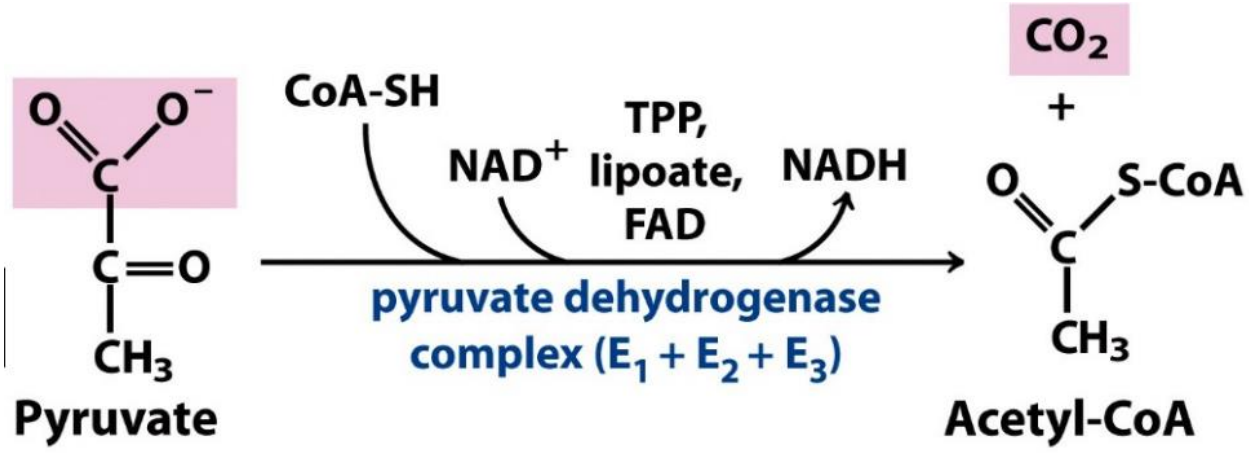
Production of acetyl CoA

In order for pyruvate, the product of glycolysis, to enter the next pathway, it must undergo several changes to become acetyl Coenzyme A (acetyl CoA). Acetyl CoA is a molecule that is further converted to oxaloacetate, which enters the citric acid cycle (Krebs cycle). The conversion of pyruvate to acetyl CoA is a three-step process.

Step 1. A carboxyl group is removed from pyruvate, releasing a molecule of carbon dioxide into the surrounding medium. (Note: carbon dioxide is one carbon attached to two oxygen atoms and is one of the major end products of cellular respiration.) The result of this step is a two-carbon hydroxyethyl group bound to the enzyme pyruvate dehydrogenase; the lost carbon dioxide is the first of the six carbons from the original glucose molecule to be removed. This step proceeds twice for every molecule of glucose metabolized (remember: there are two pyruvate molecules produced at the end of glycolysis); thus, two of the six carbons will have been removed at the end of both of these steps.

Step 2. The hydroxyethyl group is oxidized to an acetyl group, and the electrons are picked up by NAD^+, forming NADH (the reduced form of NAD+). The high- energy electrons from NADH will be used later by the cell to generate ATP for energy.

Step 3. The enzyme-bound acetyl group is transferred to CoA, producing a molecule of acetyl CoA. This molecule of acetyl CoA is then further converted to be used in the next pathway of metabolism, the citric acid cycle.



TCA cycle (Citric acid cycle, Krebs cycle)

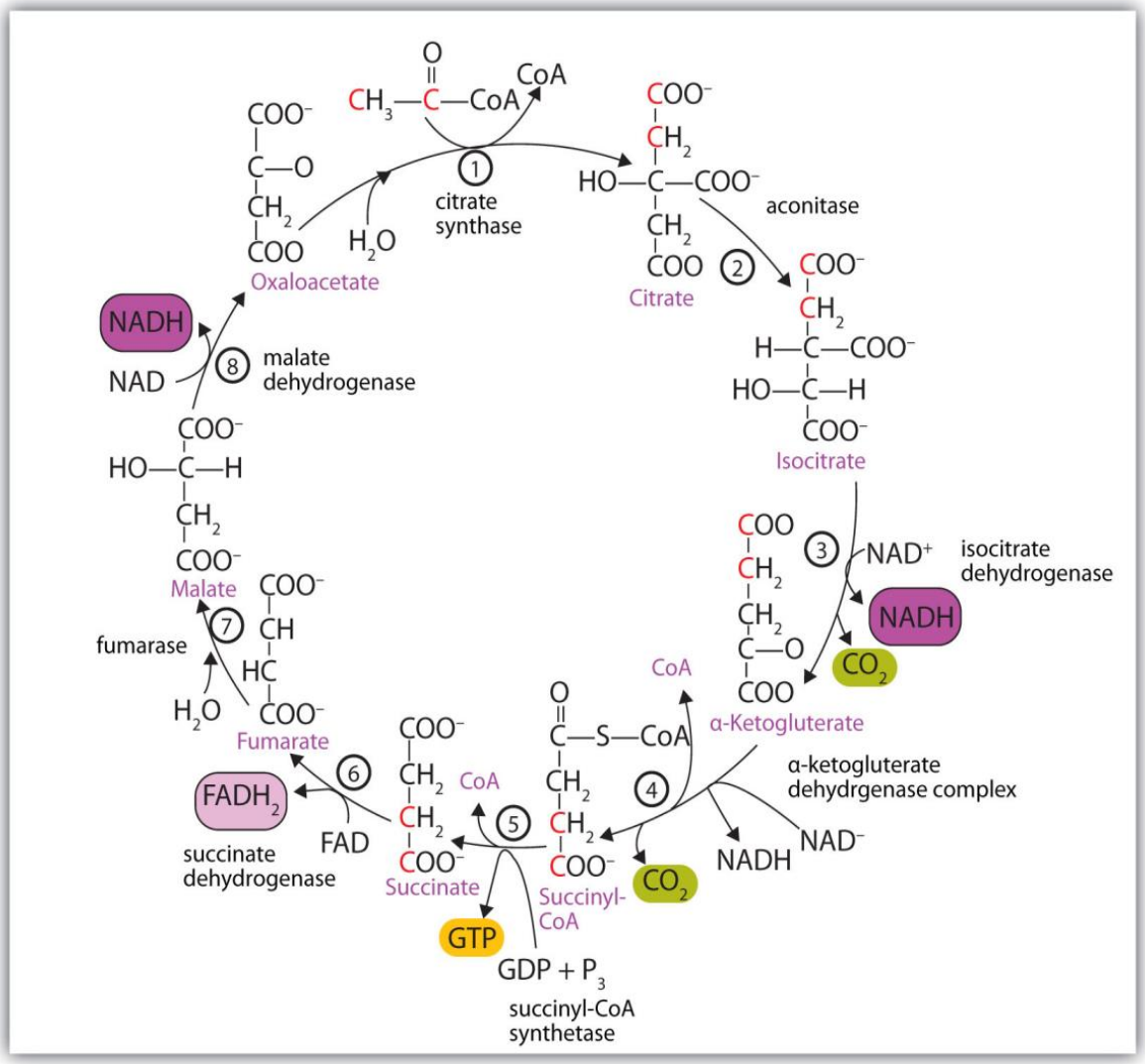
The citric acid cycle (CAC) – also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle – is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP). In addition, the cycle provides precursors of certain amino acids, as well as the reducing agentNADH, that are used in numerous other biochemical reactions. Its central importance to many biochemical pathways suggests that it was one of the earliest established components of cellular metabolism

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. In prokaryotic cells, such as bacteria which lack mitochondria, the citric acid cycle reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion

Reactions of TCA cycle

The TCA cycle consists of eight steps catalyzed by eight different enzymes . The cycle is initiated (1) when acetyl CoA reacts with the compound oxaloacetate to form citrate and to release coenzyme A (CoA-SH). Then, in a succession of reactions, (2) citrate is rearranged to form isocitrate; (3) isocitrate loses a molecule of carbon dioxide and then undergoes oxidation to form alpha-ketoglutarate; (4) alpha-ketoglutarate loses a molecule of carbon dioxide and is oxidized to form succinyl CoA; (5) succinyl CoA is enzymatically converted to succinate; (6)

succinate is oxidized to fumarate; (7) fumarate is hydrated to produce malate; and, to end the cycle, (8) malate is oxidized to oxaloacetate. Each complete turn of the cycle results in the regeneration of oxaloacetate and the formation of two molecules of carbon dioxide.



Energy is produced in a number of steps in this cycle of reactions. In step 5, one molecule of adenosine triphosphate (ATP), the molecule that powers most cellular functions, is produced. Most of the energy obtained from the TCA cycle, however, is captured by the compounds nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) and converted later to ATP. Energy transfers occur through the relay of electrons from one substance to another, a process carried out through the chemical reactions known as oxidation and reduction,

or redox reactions. (Oxidation involves the loss of electrons from a substance and reduction the addition of electrons.) For each turn of the TCA cycle, three molecules of NAD^+ are reduced to NADH and one molecule of FAD is reduced to $FADH_2$. These molecules then transfer their energy to the electron transport chain, a pathway that is part of the third stage of cellular respiration. The electron transport chain in turn releases energy so that it can be converted to ATP through the process of oxidative phosphorylation.

Steps

Two carbon atoms are oxidized to CO_2, the energy from these reactions being transferred to other metabolic processes by GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the TCA cycle may later donate its electrons in oxidative phosphorylation to drive ATP synthesis; $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme functioning both in the TCA cycle and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$, therefore, facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the Succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

Major metabolic pathways converging on the TCA cycle

Several catabolic pathways converge on the TCA cycle. Reactions that form intermediates of the TCA cycle in order to replenish them (especially during the scarcity of the intermediates) are called anaplerotic reactions. The citric acid cycle is the third step in carbohydrate catabolism (the breakdown of sugars). Glycolysis breaks glucose (a six-carbon-molecule) down into pyruvate (a three-carbon molecule). In eukaryotes, pyruvate moves into the mitochondria. It is converted into acetyl-CoA by decarboxylation and enters the citric acid cycle.

In protein catabolism, proteins are broken down by proteases into their constituent amino acids. The carbon backbone of these amino acids can become a source of energy by being converted to acetyl-CoA and entering into the citric acid cycle. In fat catabolism, triglycerides are hydrolyzed to break them into fatty acids and glycerol. In the liver the glycerol can be converted into glucose via dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by way of gluconeogenesis. In many tissues, especially heart tissue, fatty acids are broken down through a process known as beta oxidation, which results in acetyl-CoA, which can be used in the citric

acid cycle. Beta oxidation of fatty acids with an odd number of methylene bridges produces propionyl CoA, which is then converted into succinyl-CoA and fed into the citric acid cycle. The total energy gained from the complete breakdown of one molecule of glucose by glycolysis, the citric acid cycle, and oxidative phosphorylation equals about 30 ATP molecules, in eukaryotes. The citric acid cycle is called an amphibolic pathway because it participates in both catabolism and anabolism.

ANAPLEROTIC REACTION

naplerotic reactions (from the Greek ἀνά= 'up' and πληρόω= 'to fill') are chemical reactions that form intermediates of a metabolic pathway. Examples of such are found in the citric acid cycle (TCA cycle). In normal function of this cycle for respiration, concentrations of TCA intermediates remain constant; however, many biosynthetic reactions also use these molecules as a substrate. Anaplerosis is the act of replenishing TCA cycle intermediates that have been extracted for biosynthesis

he TCA cycle is a hub of metabolism, with central importance in both energy production and biosynthesis. Therefore, it is crucial for the cell to regulate concentrations of TCA cycle metabolites in the mitochondria. Anaplerotic flux must balance cataplerotic flux in order to retain homeostasis of cellular metabolism

There are 4 major reactions classed as anaplerotic, and it is estimated that the production of oxaloacetate from pyruvate has the most physiologic importance.

From	To	Reaction	Notes
Pyruvate	oxaloacetate	pyruvate + HCO_3^- + ATP oxaloacetate + ADP + P_i + H_2O	This reaction is catalysed by pyruvate carboxylase, an enzyme activated by acetyl-CoA, indicating a lack of oxaloacetate. It occurs in

			animal mitochondria. Most important anaplerotic reaction; depending on severity, deficiency causes lactic acidosis, severe psychomotor deficiency or death in infancy [1] Pyruvate can also be converted to L-malate, another intermediate, in a similar way.
Aspartate	oxaloacetate	-	This is a reversible reaction forming oxaloacetate from aspartate in a transamination reaction, via aspartate transaminase.
Glutamate	α-ketoglutarate	glutamate + NAD^+ + H_2O NH_4^+ + α-ketoglutarate + NADH + H^+.	This reaction is catalysed by glutamate-dehydrogenase.
β-Oxidation of fatty acids	succinyl-CoA	-	When odd-chain fatty acids are oxidized, one molecule of succinyl-CoA is formed per fatty acid. The final enzyme is methylmalonyl-CoA mutase. Triheptanoin (fat with three heptanoic (C7:0) fatty acids) may be used to treat pyruvate

			carboxylase deficiency
adenylosuccinate	fumarate	adenylosuccinate AMP + fumarate	This reaction is catalysed by adenylosuccinate lyase and occurs in purine synthesis and purine nucleotide cycle. Defect of this enzyme [2]causes psychomotor retardation.

Table 15–3 Anaplerotic reactions

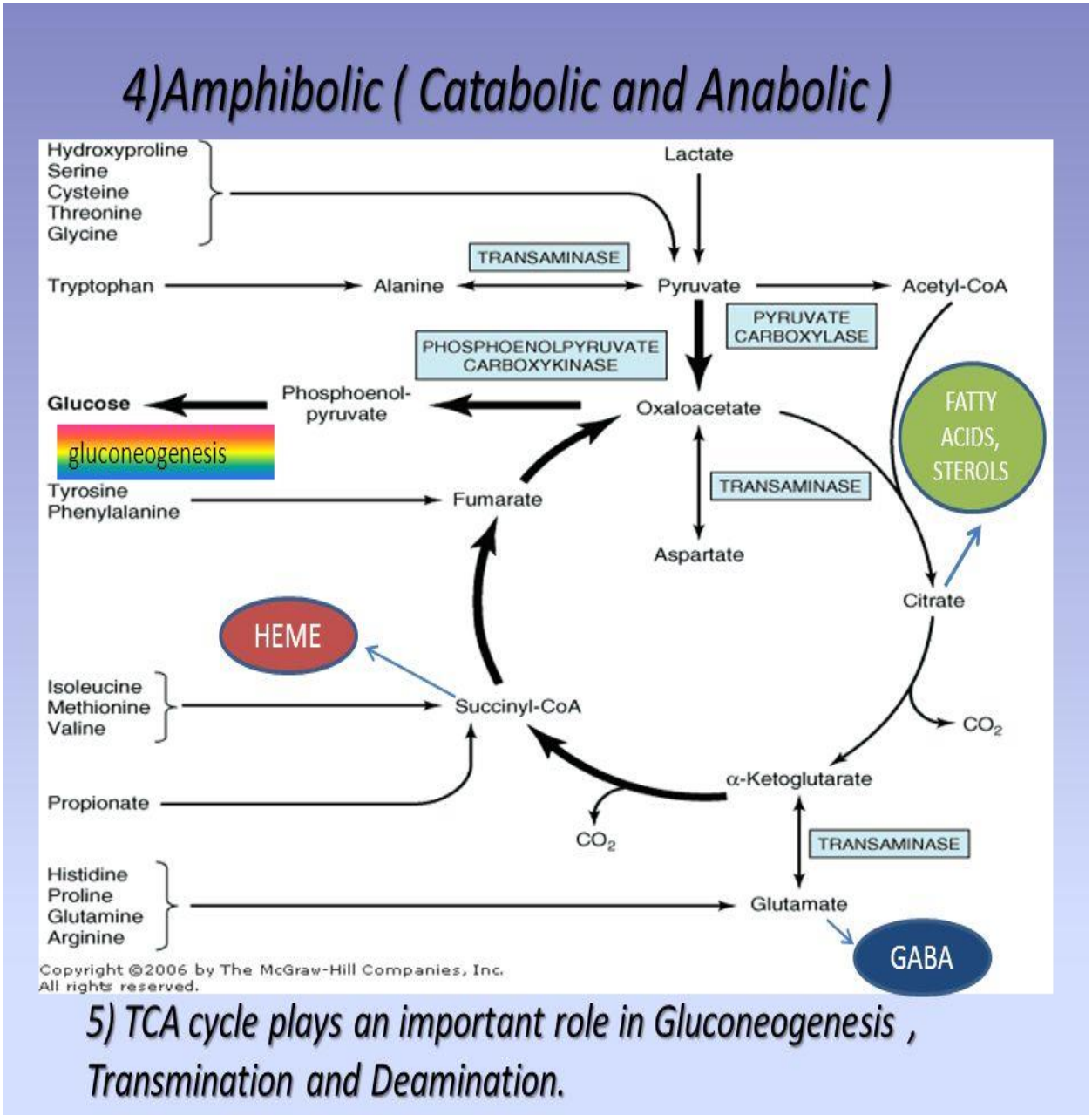
Reaction	Tissue(s)/organism(s)
$Pyruvate + HCO_3^- + ATP \xrightleftharpoons{\text{pyruvate carboxylase}} oxaloacetate + ADP + P_i$	Liver, kidney
$Phosphoenolpyruvate + CO_2 + GDP \xrightleftharpoons{\text{PEP carboxykinase}} oxaloacetate + GTP$	Heart, skeletal muscle
$Phosphoenolpyruvate + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} oxaloacetate + P_i$	Higher plants, yeast, bacteria
$Pyruvate + HCO_3^- + NAD(P)H \xrightleftharpoons{\text{malic enzyme}} malate + NAD(P)^+$	Widely distributed in eukaryotes and prokaryotes

AMPHIBOLIC ROLE

The term amphibolic is used to describe a biochemical pathway that involves both catabolism and anabolism

The citric acid cycle (The Krebs Cycle) is a good example of *amphibolic pathway*. The first reaction of the cycle, in which oxaloacetate (a four carbon compound) condenses with acetate (a two carbon compound) to form citrate (a six carbon compound) is typically anabolic. The next

few reactions, which are intramolecular rearrangements, produce isocitrate. The following two reactions are typically catabolic. COO is lost in each step and succinate (a four carbon compound) is produced.

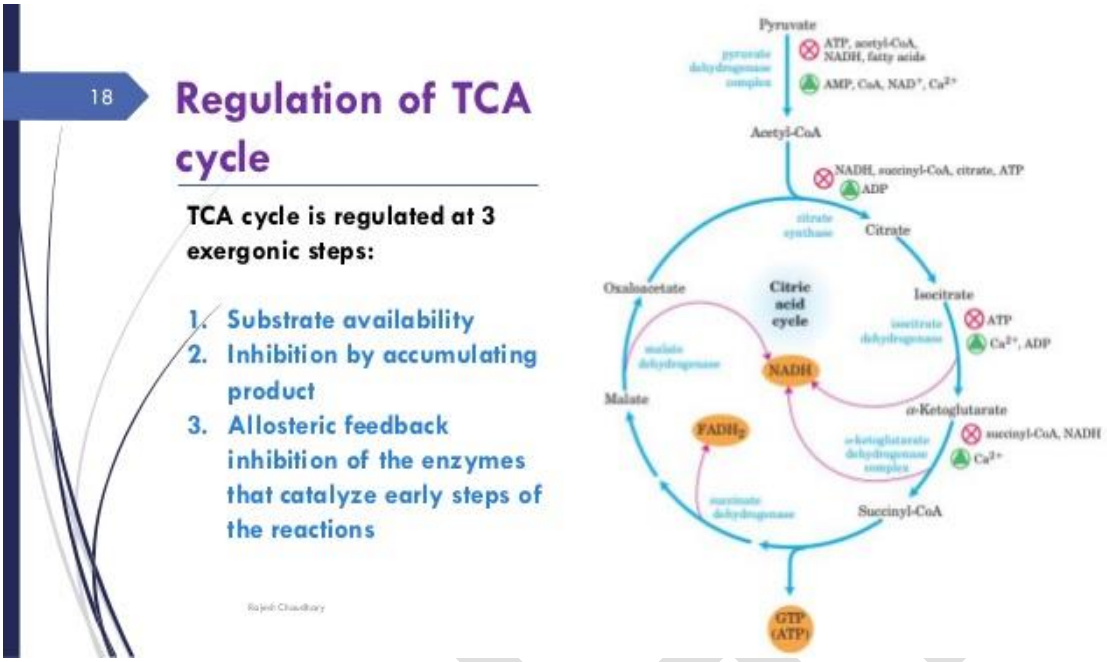


REGULATION OF CITRIC ACID CYCLE

The regulation of the citric acid cycle is largely determined by product inhibition and substrate availability. If the cycle were permitted to run unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzyme such as NADH and ATP. The major eventual substrate of the cycle is ADP which gets converted to ATP. A reduced amount of ADP causes accumulation of precursor NADH which in turn can inhibit a number of enzymes. NADH, a product of all dehydrogenases in the citric acid cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits alpha-ketoglutarate dehydrogenase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

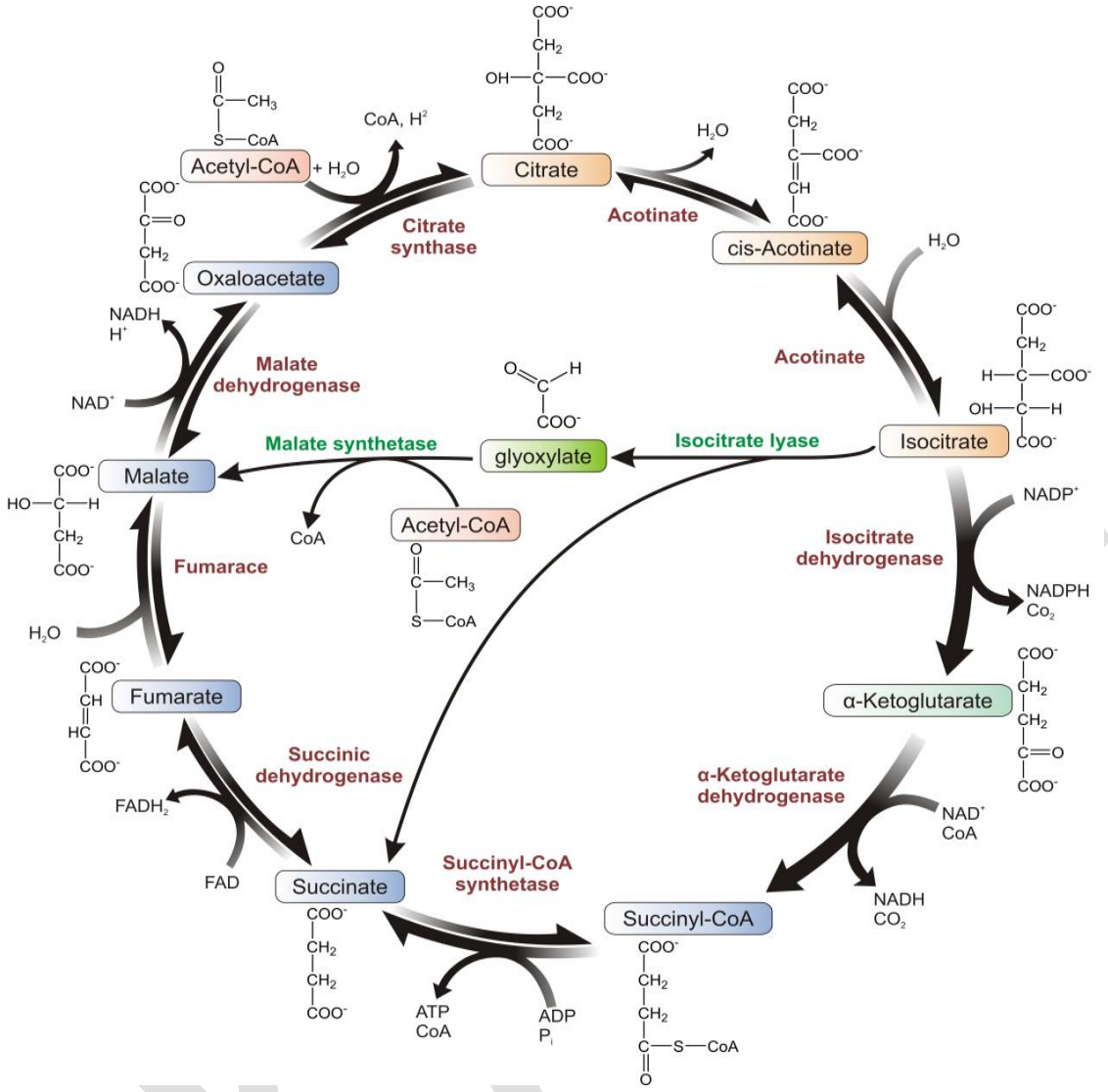
Calcium is also used as a regulator in the citric acid cycle. Calcium levels in the mitochondrial matrix can reach up to the tens of micromolar levels during cellular activation. It activates pyruvate dehydrogenase phosphatase which in turn activates the pyruvate dehydrogenase complex. Calcium also activates isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate, a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.



GLYOXYLATE PATHWAY

The glyoxylate cycle, a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi. The glyoxylate cycle centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates. In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis. In recent years, however, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals and suggests that animals encode alternative enzymes of the cycle that differ in function from known MS and ICL in non-metazoan species.



Similarities with TCA cycle

The glyoxylate cycle utilizes three of the five enzymes associated with the tricarboxylic acid cycle and shares many of its intermediate steps. The two cycles vary when, in the glyoxylate cycle, ICL converts isocitrate into glyoxylate and succinate instead of -ketoglutarate as seen in the TCA cycle. This bypasses the decarboxylation steps that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose. The glyoxylate cycle then continues on, using glyoxylate and acetyl-CoA to produce malate.

Role in gluconeogenesis

Fatty acids from lipids are commonly used as an energy source by vertebrates as fatty acids are degraded through beta oxidation into acetate molecules. This acetate, bound to the active thiol group of coenzyme A, enters the citric acid cycle (TCA cycle) where it is fully oxidized to carbon dioxide. This pathway thus allows cells to obtain energy from fat. To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.

Cell-wall containing organisms, such as plants, fungi, and bacteria, require very large amounts of carbohydrates during growth for the biosynthesis of complex structural polysaccharides, such as cellulose, glucans, and chitin. In these organisms, in the absence of available carbohydrates (for example, in certain microbial environments or during seed germination in plants), the glyoxylate cycle permits the synthesis of glucose from lipids via acetate generated in fatty acid -oxidation.

The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO_2. The two initial steps of the glyoxylate cycle are identical to those in the citric acid cycle: acetate citrate isocitrate. In the next step, catalyzed by the first glyoxylate cycle enzyme, isocitrate lyase, isocitrate undergoes cleavage into succinate and glyoxylate. Glyoxylate condenses with acetyl-CoA, yielding malate. Both malate and oxaloacetate can be converted into phosphoenolpyruvate, which is the product of phosphoenolpyruvate carboxykinase, the first enzyme in gluconeogenesis. The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids. Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

Function in organisms

Plants

In plants the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. The seed cannot produce biomass using photosynthesis because of lack of an organ to perform this function. The lipid stores of germinating seeds are used for the formation of the carbohydrates that fuel the growth and development of the organism.

The glyoxylate cycle can also provide plants with another aspect of metabolic diversity. This

cycle allows plants to take in acetate both as a carbon source and as a source of energy. Acetate is converted to Acetyl CoA (similar to the TCA cycle). This Acetyl CoA can proceed through the glyoxylate cycle, and some succinate is released during the cycle. The four carbon succinate molecule can be transformed into a variety of carbohydrates through combinations of other metabolic processes; the plant can synthesize molecules using acetate as a source for carbon. The Acetyl CoA can also react with glyoxylate to produce some NADPH from $NADP^+$, which is used to drive energy synthesis in the form of ATP later in the Electron Transport Chain.

Pathogenic fungi

The glyoxylate cycle may serve an entirely different purpose in some species of pathogenic fungi. The levels of the main enzymes of the glyoxylate cycle, ICL and MS, are greatly increased upon contact with a human host. Mutants of a particular species of fungi that lacked ICL were also significantly less virulent in studies with mice compared to the wild type. The exact link between these two observations is still being explored, but it can be concluded that the glyoxylate cycle is a significant factor in the pathogenesis of these microbes.

Vertebrates

Vertebrates were once thought to be unable to perform this cycle because there was no evidence of its two key enzymes, isocitrate lyase and malate synthase. However, some research suggests that this pathway may exist in some, if not all, vertebrates. Specifically, some studies show evidence of components of the glyoxylate cycle existing in significant amounts in the liver tissue of chickens. Data such as these support the idea that the cycle could theoretically occur in even the most complex vertebrates. Other experiments have also provided evidence that the cycle is present among certain insect and marine invertebrate species, as well as strong evidence of the cycle's presence in nematode species. However, other experiments refute this claim Some publications conflict on the presence of the cycle in mammals: for example, one paper has stated that the glyoxalate cycle is active in hibernating bears, but this report was disputed in a later paper. On the other hand, no functional genes related to known forms of malate synthase or isocitrate lyase have been identified in placental mammal genomes, while malate synthase appears to be functional in some non-placental mammals and other vertebrates. Vitamin D may regulate this pathway in vertebrates.

COORDINATED REGULATION OF GLYOXYLAT AND CITRIC ACID PATHWAYS

The pathway is essentially a modified version of the citric acid cycle. The glyoxylate cycle make use of five enzymes used in the citric acid cycle. They are citrate synthase, aconitase, succinate dehydrogenase, fumarase, and malate dehydrogenase. The glyoxylate cycle side step the two oxidative decarboxylation steps of the TCA cycle. It diverts the isocitrate through the isocitrate lyase and malate synthase reactions. This bypass allows simple carbon compounds to be used as a sole source of carbon as well as the biosynthesis of macromolecules.

Coordinate regulation of glyoxylate cycle and TCA cycle The enzymes of the glyoxylate cycle are repressed by the presence of glucose or another more rapidly utilized substrate.

In anaerobic condition, anaerobic respiratory control system suppresses the citric acid cycle and the glyoxylate cycle under anaerobic conditions. Glyoxylate and Citric Acid Cycles shares some common intermediates which necessitate that these pathways are regulated and coordinated together. One of the crucial intermediate is Isocitrate, found at branching point amongst citric acid and glyoxylate cycles. Covalent type of modification of a protein kinase phosphorylates regulates the activity of Isocitrate dehydrogenase and ultimately the dehydrogenase gets inactivated. Inactivated isocitrate dehydrogenase leads to the diversion of isocitrate into the glyoxylate cycle and from there it is diverted towards the glucose biosynthesis. The phosphate group is removed by phosphoprotein phosphatase from isocitrate dehydrogenase, which reactivates the enzyme with sending more isocitrate through the TCA cycle. Lowered concentration of regulators of these cycles, results into signalling enough flux through the TCA cycle and isocitrate dehydrogenase is inactivated by the protein kinase

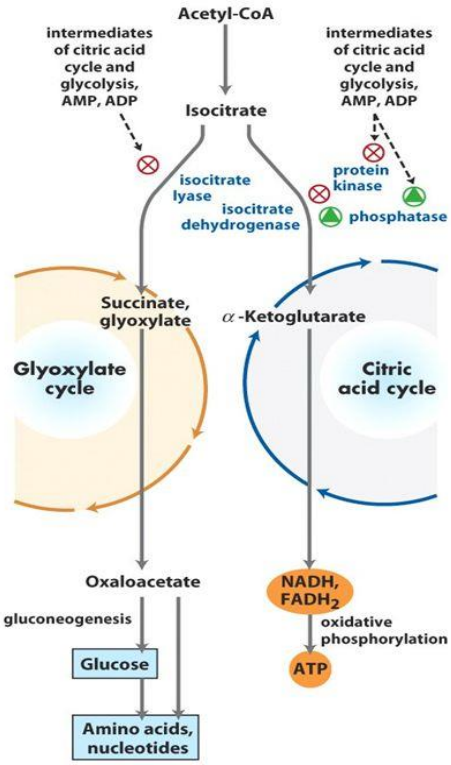
Table 1 List of allosteric effectors of kinase and phosphatase

Intermediates of the Glycolytic pathway	Intermediates of the Citric acid cycle	Energy depletion indicating cofactors
Phosphoenolpyruvate*	Citrate	AMP*
Pyruvate*	Isocitrate*	ADP*
3-Phosphoglycerate*	α-Ketoglutarate*	$NADP^+$
Fructose-6-phosphate	Oxaloacetate*	

The kinase activity inhibited by all compounds shown in above table.

** stimulates the phosphatase activity.*

The intermediates that acts as an activator of isocitrate dehydrogenase also act as an allosteric inhibitors of isocitrate lyase Under the condition where the metabolisms yield is fast and provides sufficient• energy to keep the concentrations of anaplerotic molecule low, leads to the inactivation of isocitrate dehydrogenase When isocitrate lyase is gets free from inhibition and isocitrate enters into the• glyoxylate pathway, there it is used for synthesis of amino acids, carbohydrates and other cellular materials.



Coordinated regulation of glyoxylate and citric acid cycles

POSSIBLE QUESTIONS
UNIT II

PART A (1 Mark)
Question number 1 – 20 (Online examination)

PART B (2Marks)

1. Define glycogenesis
2. Define glycogenolysis
3. Write note on PDH complex
4. Note on glyoxalate pathway
5. Derive the energetic of TCA cycle
6. List the glycogen storage diseases
7. What do you mean by glycogenesis
8. Define the term analphloretic reaction
9. Which hormone control the glycogenesis and glycogenolysis

PART C (6 Marks)

1. Give a note on glycogen storage disease
2. Write a note on anaplerotic reactions
3. Describe the glyoxalate pathway
4. Discuss coordinated regulation of glyoxalate pathway
5. Discuss in detail about the regulation of citric acid cycle
6. Explain the metabolism of Citric acid cycle
7. Discuss about Glycogenesis
8. Write notes on glycogenolysis
9. Describe the citric acid cycle and its significance
10. Give an account of glyoxalate pathway
11. Explain about the regulation of glycogen metabolism
12. Briefly explain the glyoxylate cycle

Karpagam Academy of Higher Education
Department of Biochemistry
Metabolism of Carbohydrates and Lipids (17BCU301) MCQ
UNIT II

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	2	The formation of citrate from oxalo acetate and acetyl CoA is	Oxidation	Reduction	Condensation	Hydrolysis	Condensation
2	2	The carrier of the citric acid cycle is	Succinate	Fumarate	Malate	Oxaloacetate	Oxaloacetate
3	2	The Key enzymes in glycolysis are	Glucokinase	Glucokinase and Phosphofructokinase	Glucokinase, Phosphofructokinase and pyruvate kinase	Glucokinase, Phosphofructokinase and fructose -1- phosphatase	Glucokinase, Phosphofructokinase and pyruvate kinase
4	2	The complete oxidation of glucose occurs in	Glycolysis	HMP shunt	Glycolysis and TCA cycle	TCA cycle	Glycolysis and TCA cycle
5	2	TCA Cycle takes place in	Cytosol	Ribosomes	Mitochondria	Nucleus	Mitochondria
6	2	TCA Cycle is called as amphibolic pathway because it	Produces energy	Is catabolic and anabolic	Produces CO_2 and H_2O	Occurs in mitochondria	Is catabolic and anabolic
7	2	Von Gierke's disease is due to deficiency of enzyme	Glucose-6-phosphatase	Glucose -1-phosphatase	Fructose-6-phosphatase	Fructose-1-phosphatase	Glucose-6-phosphatase
8	2	Pentose provided by HMP shunt is used for	Energy production	Fatty acid production	Nucleic acid synthesis	Steroid synthesis	Nucleic acid synthesis
9	2	Essential pentosuria is due to deficiency of enzyme	Xylulose reductase	Xylitol dehydrogenase	Xylitol synthetase	Xylitol decarboxylase	Xylitol dehydrogenase
10	2	Rate limiting enzyme in glycogenolysis is	Phosphorylase	Phosphoglucomutase	Glucose 6 phosphatase	Fructose 1,6 phosphatase	Phosphorylase
11	2	Rate limiting enzyme in glycogenesis is	Glucokinase	Phophoglucomutase	UDPG phosphorylase	Glycogen synthetase	Glycogen synthetase
12	2	Tricarboxylic acid cycle to be continuous requires the regeneration of	Pyruvic acid	Oxaloacetic acid	α-oxoglutaric acid	Malic acid	Oxaloacetic acid
13	2	Where does the TCA cycle take place in bacteria?	Mitochondrial matrix	Cytoplasm	Mitochondrial membrane	Cytoplasmic membrane	Cytoplasm
14	2	In aerobic respiration when is the first molecule of carbon dioxide released?	During chemiosmosis	When pyruvic acid is reduced to lactic acid	During the conversion step when pyruvic acid is converted to acetyl-CoA	When glucose is phosphorylated in glycolysis	During the conversion step when pyruvic acid is converted to acetyl-CoA
15	2	What pathway is a significant intermediate source of pentoses for nucleic acid synthesis?	Glycolysis	TCA cycle	Electron transport chain	Hexose monophosphate shunt	Hexose monophosphate shunt
16	2	One turn of the citric acid cycle produces	2 NADH, 2 $FADH_2$, 2 ATP	3 NADH, 1 $FADH_2$, 1 ATP	3 NADH, 2 $FADH_2$, 1 ATP	3 NADH, 1 $FADH_2$, 2 ATP	3 NADH, 1 $FADH_2$, 1 ATP
17	2	An enzymatic reaction that is most similar to the conversion of pyruvate to acetyl CoA is catalyzed by	Citrate synthase	Alpha-ketoglutarate dehydrogenase	Succinyl-coa synthetase	Isocitrate dehydrogenase	Alpha-ketoglutarate dehydrogenase
18	2	Intermediates of the citric acid cycle are replenished by a reaction converting pyruvate to	Oxaloacetate	Citrate	Alpha-ketoglutarate	Succinyl-coa	Oxaloacetate
19	2	End product of TCA cycle is	Citric acid	Pyruvic acid	Lactic acid	CO_2 and water	CO_2 and water
20	2	_____ is the precursor for the	L-gulonate	L-alanine	Methionine	L-aspartic acid	L-gulonate
21	2	synthesis of ascorbic acid					
22	2	In the glyoxylate cycle, the sequential action of citrate lyase and isocitrate lyase converts acetyl CoA into glyoxylate and oxaloacetate into:	Malate	Aspartate	Pyruvate	Succinate	Succinate
23	2	ATP and NADH inhibit	Isocitrate hydrogenase	Isocitrate dehydrogenase	Pyruvate dehydrogenase	Pyruvate hydrogenase	Isocitrate dehydrogenase
24	2						
25	2	In hydration, fumarate is converted by fumarase to	L-Malate	D-Malate	A-Malate	C-Malate	L-Malate
26	2						
27	2	High levels of NADH will lower concentration of	Acetate	Dehydrogenase	Oxaloacetate	Carbonate	Oxaloacetate
28	2						
29	2	Number of enzyme catalyzed reactions in gluconeogenesis are	12	13	11	10	11
30	2						
31	2	Which of the following processes requires UTP molecules?	Formation of glycogen from glucose 6-phosphate	Degradation of glycogen to glucose 6-phosphate	Formation of glucose 1-phosphate from glucose 6-phosphate	Degradation of glucose 1-phosphate from glucose 6-phosphate	Formation of glycogen from glucose 6-phosphate
32	2	Glycogen is converted to glucose in which of the following processes?	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis	Glycogenolysis
33	2	Which of the following intermediates is not involved in glycolysis but is in gluconeogenesis?	Fructose 6-phosphate	Pyruvate	Oxaloacetate	Acetyl Coa	Oxaloacetate
34	2	In which of the following processes is glucose 6-phosphate the end product?	Glycogenesis	Glycogenolysis	Glycolysis	Citric acid cycle	Glycogenolysis
35	2	The compound glucose 1-phosphate is encountered in which of the following processes?	Glycogenesis and glycogenolysis	Glycolysis and gluconeogenesis	Glycogenesis and gluconeogenesis	Glycolysis and glycogenolysis	Glycogenesis and glycogenolysis
36	2	The compound oxaloacetate is an intermediate in the conversion of	Glycogen to glucose	Pyruvate to glucose	Pyruvate to acetyl coa	Glycogen to pyruvate	Pyruvate to glucose
37	2	In the human body, under aerobic conditions and anaerobic conditions, respectively, pyruvate is converted to	Lactate and ethanol	Lactate and acetyl Coa	Ethanol and lactate	Acetyl Coa and lactate	Acetyl Coa and lactate
38	2	Which of the following enzyme is not involved in HMP shunt?	Glyceraldehyde-3 phosphate-dehydrogenase	Glucose-6-phosphatedehydrogenase	Transketolase	Phosphogluconate dehydrogenase	Glyceraldehyde-3-phosphatedehydrogenase
39	2	Which of the following is a substrate for aldolase activity in Glycolytic pathway?	Glyceraldehyde-3-phosphate	Glucose-6-phosphate	Fructose-6-phosphate	Fructose1, 6-bisphosphate	Fructose1, 6-bisphosphate
40	2	An allosteric enzyme responsible for controlling the rate of T.C.A cycle is	Malate dehydrogenase	Isocitrate dehydrogenase	Fumarase	Aconitase	Isocitrate dehydrogenase
41	2	Our body can get pentoses from	Glycolytic pathway	Uronic acid pathway	TCA cycle	HMP shunt	HMP shunt
42	2	The number of molecules of ATP produced by the total oxidation of acetyl CoA in TCA cycle is	6	8	10	12	12

UNIT-III

Synthesis of carbohydrates : Calvin cycle, regulation of calvin cycle, regulated synthesis of starch and sucrose, photorespiration, C4 and CAM pathways, synthesis of cell wall polysaccharides, integration of carbohydrate metabolism in plant cell.

INTRODUCTION

The Light Independent Reactions-Dark reactions

The light-independent reactions, or dark reactions,[1] of photosynthesis are chemical reactions that convert carbon dioxide and other compounds into glucose. These reactions occur in the stroma, the fluid-filled area of a chloroplast outside of the thylakoid membranes. These reactions take the products (ATP and NADPH) of light-dependent reactions and perform further chemical processes on them. There are three phases to the light-independent reactions, collectively called the Calvin cycle: carbon fixation, reduction reactions, and ribulose 1,5-bisphosphate (RuBP) regeneration.

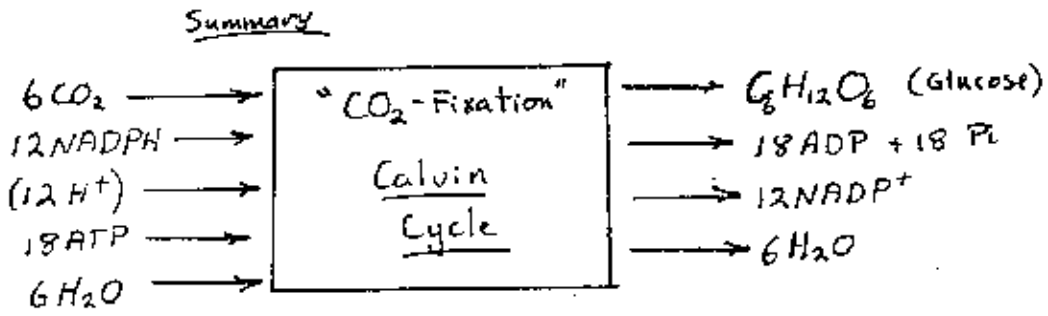
This process occurs only when light is available. Plants do not carry out the Calvin cycle during nighttime. They instead release sucrose into the phloem from their starch reserves. This process happens when light is available independent of the kind of photosynthesis (C3 carbon fixation, C4 carbon fixation, and Crassulacean acid metabolism); CAM plants store malic acid in their vacuoles every night and release it by day in order to make this process work. They are also known as dark reactions.]

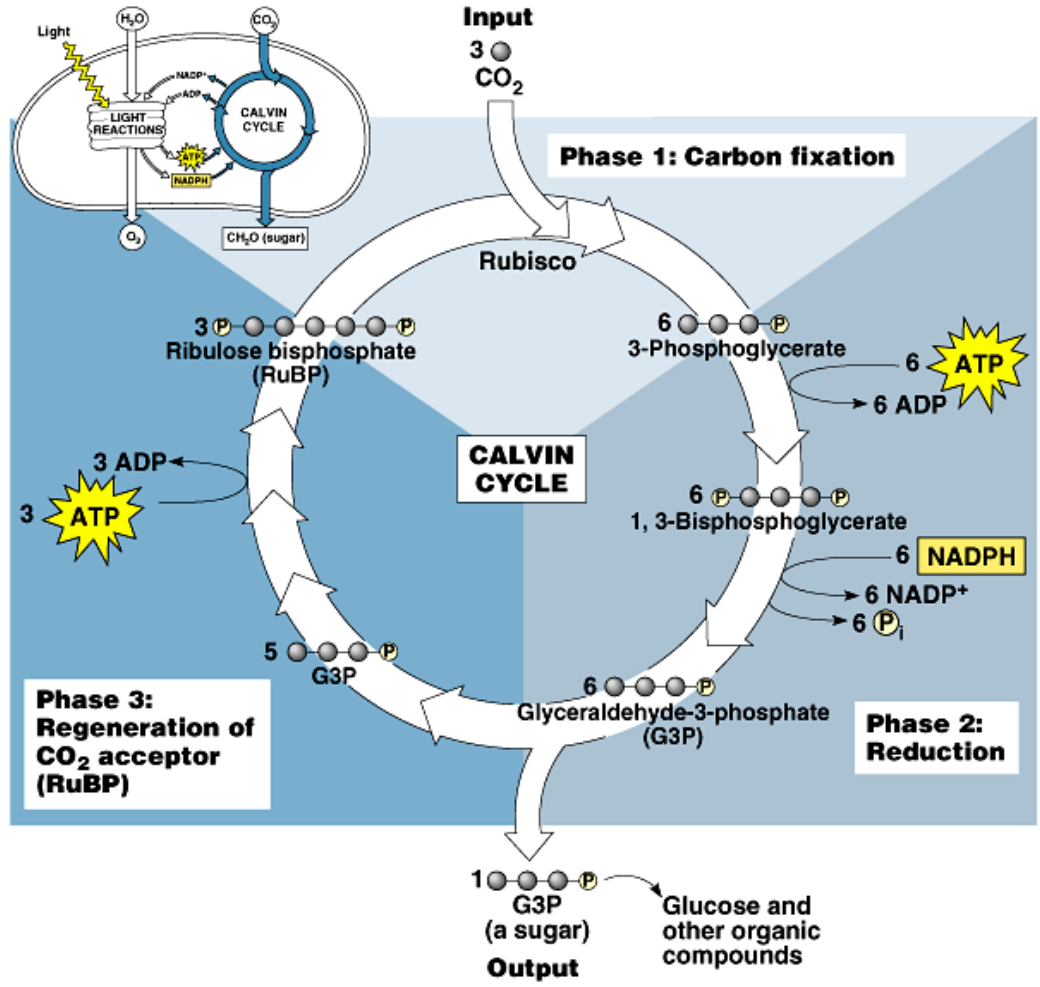
the Calvin cycle, Calvin–Benson–Bassham (CBB) cycle, reductive pentose phosphate cycle or C3 cycle is a series of biochemical redox reactions that take place in the stroma of chloroplast in photosyntheticorganisms.

The cycle was discovered by Melvin Calvin, James Bassham, and Andrew Benson at the University of California, Berkeley by using the radioactive isotope carbon-14.

Photosynthesis occurs in two stages in a cell. In the first stage, light-dependent reactions capture the energy of light and use it to make the energy-storage and transport molecules ATP and NADPH. The Calvin cycle uses the energy from short-lived electronically excited carriers to convert carbon dioxide and water into organic compounds that can be used by the organism (and by animals that feed on it). This set of reactions is also called *carbon fixation*. The key enzyme of the cycle is called RuBisCO. In the following biochemical equations, the chemical species (phosphates and carboxylic acids) exist in equilibria among their various ionized states as governed by the pH.

The Calvin Cycle(C3 plants):





This part of photosynthesis occurs in the stroma of the chloroplasts called carbon dioxide fixation.

The fixation of the CO_2 is carried out by a giant enzyme ribulose biphosphate carboxylase/oxidase (RUBISCO) which is the most abundant enzyme on earth. This enzyme is very sluggish it works much slower than most other enzymes. (i.e. ~ 3 molecules of substrate per sec. compared with ~1000/sec for others). Therefore, there are many copies of this enzyme in the stroma ~ 50% of chloroplast protein.

The first fixation reaction of the cycle uses a five carbon sugar ribulose 1-5 biphosphate and adds to it a CO_2 molecule to form 2 (3 carbon) molecules of 3 - phosphoglycerate. These are

rearranged through a series of energy requiring reactions, using up ATP and NADPH to generate 2 molecules of glyceraldehyde 3 - phosphate. (If this were done six (6) times we now would have 12 molecules of glyceraldehyde - 3 - phosphate (G3P). Two (2) of the G3Ps are removed to make one glucose while the rest 10 G3Ps go back into the cycle to regenerate six (6) of the five (5) carbon sugars ribulose 1-5 biphosphate.

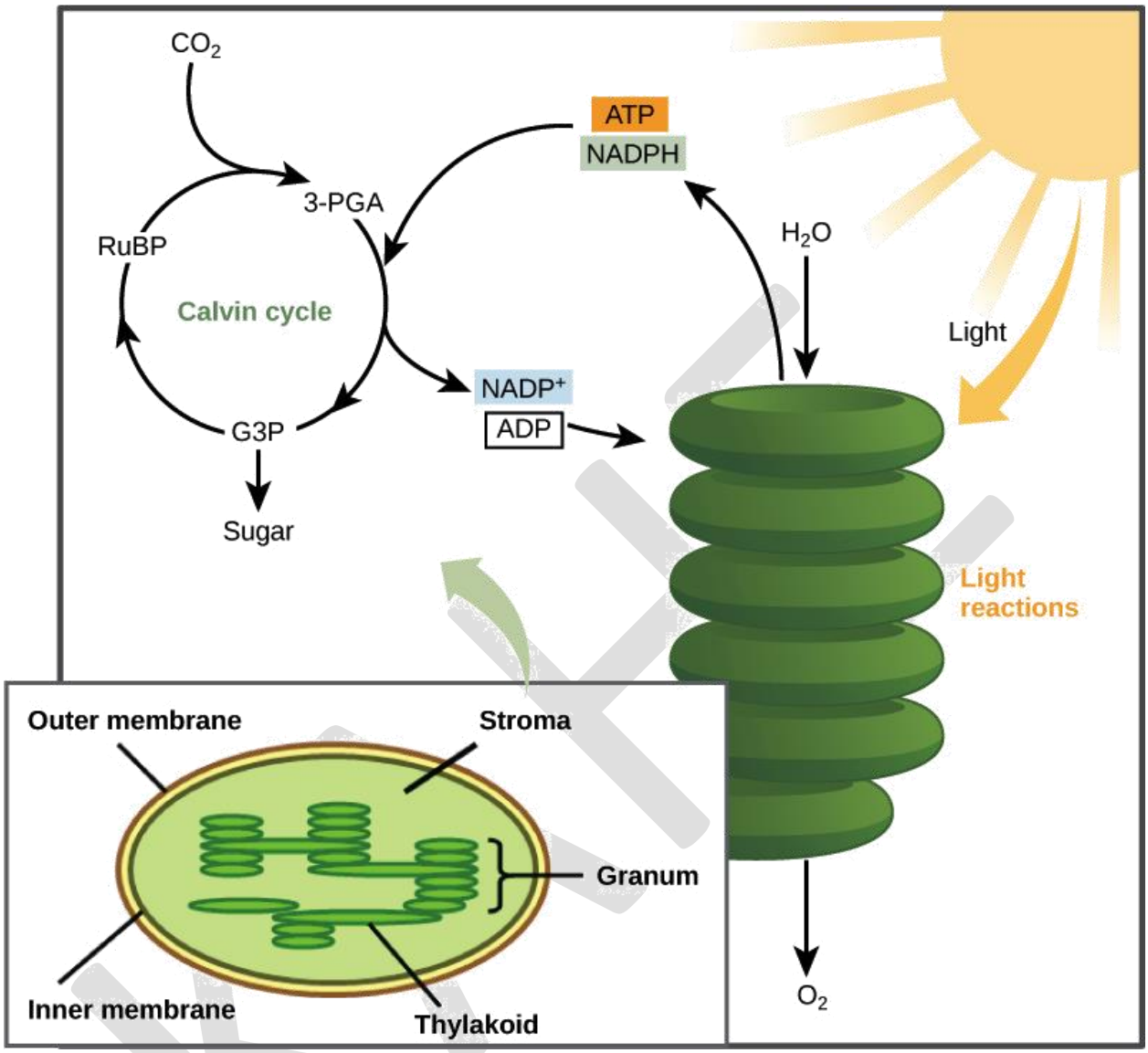
The sum of reactions in the Calvin cycle is the following:

$$3\,CO_2 + 6\,NADPH + 6\,H^+ + 9\,ATP \rightarrow \text{glyceraldehyde-3-phosphate (G3P)} + 6\,NADP^+ + 9\,ADP + 3\,H_2O + 8\,P_i \quad (P_i = \text{inorganic phosphate})$$

REGULATION OF CALVIN CYCLE

n plants, carbon dioxide (\text{CO}_2C, O, start subscript, 2, end subscript) enters the interior of a leaf via pores called stomata and diffuses into the stroma of the chloroplast—the site of the Calvin cycle reactions, where sugar is synthesized. These reactions are also called the light-independent reactions because they are not directly driven by light.

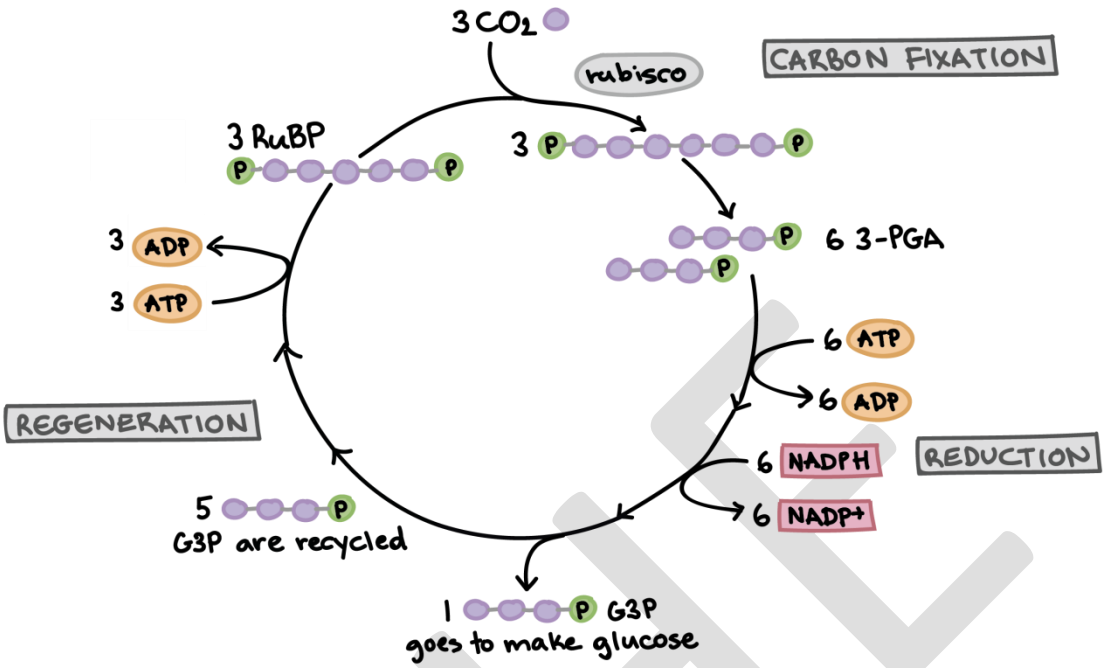
In the Calvin cycle, carbon atoms from \text {CO}_2C, O, start subscript, 2, end subscript are fixed (incorporated into organic molecules) and used to build three-carbon sugars. This process is fueled by, and dependent on, ATP and NADPH from the light reactions. Unlike the light reactions, which take place in the thylakoid membrane, the reactions of the Calvin cycle take place in the stroma (the inner space of chloroplasts).



Reactions of the Calvin cycle

The Calvin cycle reactions can be divided into three main stages: carbon fixation, reduction, and regeneration of the starting molecule.

Here is a general diagram of the cycle:



1.Carbon fixation. A \text {CO}_2CO2C, O, start subscript, 2, end subscript molecule combines with a five-carbon acceptor molecule, ribulose-1,5-bisphosphate (**RuBP**). This step makes a six-carbon compound that splits into two molecules of a three-carbon compound, 3-phosphoglyceric acid (3-PGA). This reaction is catalyzed by the enzyme RuBP carboxylase/oxygenase, or **rubisco**.

2. **Reduction.** In the second stage, ATP and NADPH are used to convert the 3-PGA molecules into molecules of a three-carbon sugar, glyceraldehyde-3-phosphate (**G3P**). This stage gets its name because NADPH donates electrons to, or **reduces**, a three-carbon intermediate to make G3P.

3. **Regeneration.** Some G3P molecules go to make glucose, while others must be recycled to regenerate the RuBP acceptor.

The regeneration stage can be broken down into steps.

1. Triose phosphate isomerase converts all of the G3P reversibly into dihydroxyacetone phosphate (DHAP), also a 3-carbon molecule.
2. Aldolase and fructose-1,6-bisphosphatase convert a G3P and a DHAP into fructose 6-phosphate (6C). A phosphate ion is lost into solution.
3. Then fixation of another CO 2 generates two more G3P.
4. F6P has two carbons removed by transketolase, giving erythrose-4-phosphate. The two carbons on transketolase are added to a G3P, giving the ketose xylulose-5-phosphate (Xu5P).
5. E4P and a DHAP (formed from one of the G3P from the second CO 2 fixation) are converted into sedoheptulose-1,7-bisphosphate (7C) by aldolase enzyme.
6. Sedoheptulose-1,7-bisphosphatase (one of only three enzymes of the Calvin cycle that are unique to plants) cleaves sedoheptulose-1,7-bisphosphate into sedoheptulose-7-phosphate, releasing an inorganic phosphate ion into solution.
7. Fixation of a third CO 2 generates two more G3P. The ketose S7P has two carbons removed by transketolase, giving ribose-5-phosphate (R5P), and the two carbons remaining on transketolase are transferred to one of the G3P, giving another Xu5P. This leaves one G3P as the product of fixation of 3 CO 2, with generation of three pentoses that can be converted to Ru5P.
8. R5P is converted into ribulose-5-phosphate (Ru5P, RuP) by phosphopentose isomerase. Xu5P is converted into RuP by phosphopentose epimerase.
9. Finally, phosphoribulokinase (another plant-unique enzyme of the pathway) phosphorylates RuP into RuBP, ribulose-1,5-bisphosphate, completing the Calvin cycle. This requires the input of one ATP.

Three turns of the Calvin cycle are needed to make one G3P molecule that can exit the cycle and go towards making glucose. G3P molecule contains three fixed carbon atoms, so it takes two G3Ps to build a six-carbon glucose molecule. It would take six turns of the cycle, or 6CO2 18 ATP, and 12 NADPH, to produce one molecule of glucose.

Regulation

It is demonstrated that not only the reactions of non-equilibrium enzymes, as the carboxylation of ribulose 1,5-bisphosphate, but reactions that operate close to a thermodynamic equilibrium, especially the reduction of 3-phosphoglycerate and the transketolase reaction can significantly influence the total turnover period in the Calvin cycle. The role of compensating mechanisms in the maintenance of the photosynthesis rate upon changes of environmental conditions and of enzyme contents is analyzed for the Calvin cycle. It is shown that the change of the total quantity of the metabolites is one of the main self-regulated mechanisms in the Calvin cycle. A change of the ATP/ADP ratio can be used by the cell to maintain the CO2 assimilation rate, when the total quantity of the metabolites is changed.

SUCROSE, STARCH BIOSYNTHESIS AND REGULATION

The major export product from photosynthesis is glyceraldehyde-3-phosphate (G3P), a triose phosphate carbohydrate, which can enter either the starch or sucrose biosynthesis pathway depending on conditions in the cell. During the daytime, much of the carbon that is fixed by photosynthesis remains in the chloroplast and enters the starch biosynthesis pathway. At night, carbon stored in the form of starch is mobilized by conversion to sucrose, which is synthesized in the cytoplasm.

Starch Biosynthesis

Starch, formally known as α-amylose, is a long-chain polysaccharide made of α 1→4 linked glucose, where the chain length numbers in the hundreds or thousands. α-amylose forms a single helix structure because of its regular repeating pattern, and this secondary structure readily crystallizes. The first step in the synthesis of α-amylose is the formation of hexose phosphates, including fructose 6-phosphate, glucose 6-phosphate, and glucose 1-phosphate. Glucose 1-phosphate is further ‘activated’ by reacting with the sugar nucleoside ATP to produce ADP-glucose. This form of glucose is highly reactive and readily joins an elongating chain of α-amylose at the 4-carbon position to give the characteristic α 1→4 linkage.

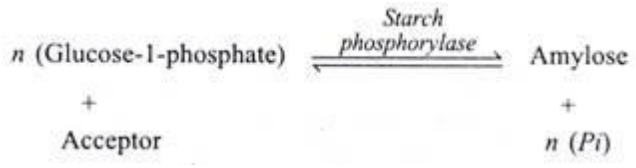
While α-amylose represents about 30% of the total starch in most plants, the rest of the starch is in a highly branched form called amylopectin. Rather than forming straight chain helices that readily crystallize, amylopectin does not crystallize. Amylopectins are formed by starch branching enzymes that form branches among short α-amylose chains that are α 1→6 glycosidic bonds.

Synthesis of starch involves the simultaneous synthesis of amylose (with α-(1: 4) glycosidic linkages) and amylopectin (with α-(1: 6) glycosidic linkages), the two important constituents of starch.

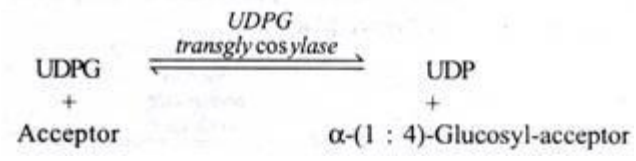
(A) Synthesis of Amylose (Or α-(1: 4) Glycosidic Linkages):

Synthesis of amylose may take place by any of the following ways:-

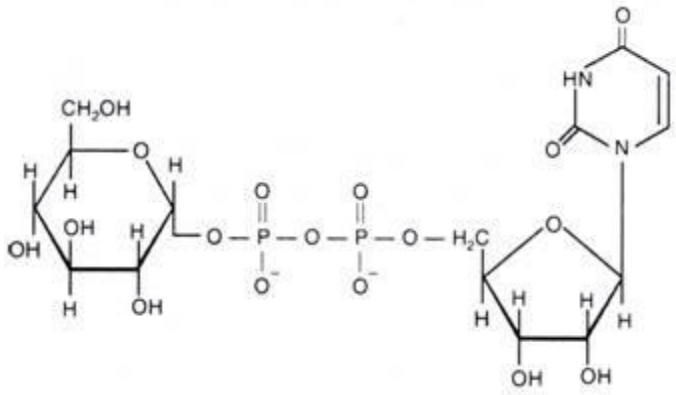
(1) According to Hanes (1940) amylose can be synthesised in the presence of the enzyme starch phosphorylase from glucose-1-phosphate and an acceptor molecule consisting of about 3 to 20 glucose units joined together by α-(1: 4) glycosidic linkages.



(2) Formation of α-(1 : 4) glycosidic linkages may also take place in the presence of the enzyme UDPG-transglycosylase (amylose synthetase) by the transfer of glucose from UDPG (Uridine Di Phosphate Glucose) to an acceptor molecule consisting of 2 to 4 or more glucose units joined together by α-(1 : 4) glycosidic linkages or even a starch molecule.



The structure of UDPG is given below:



UDPG (Uridine Diphosphate Glucose)

(3) According to Akazawa et al (1964) glucose molecule obtained as a result of the hydrolysis of sucrose in the presence of enzyme sucrase is transferred to UDP (Uridine Di Phosphate) molecule to form UDPG. Form UDPG the glucose molecule is transferred to starch (Fig. 13.2)

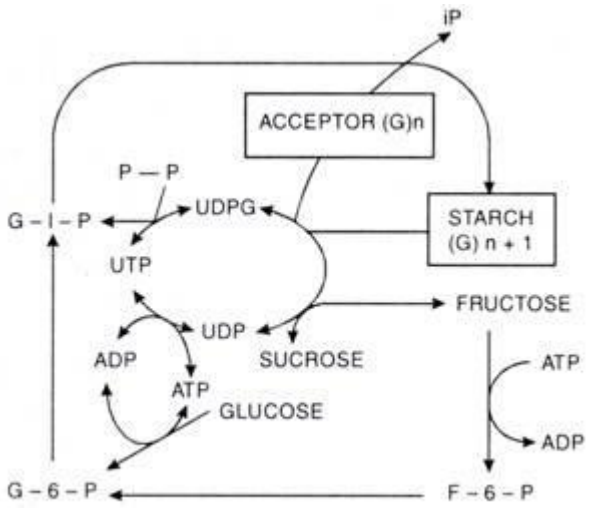


Fig. 13.2 Diagrammatic representation of starch synthesis.

(4) Formation of α-(1: 4) glycosidic linkages leading to the synthesis of; amylose may also take place in the presence of D-Enzyme by the transfer of two or more glucose units from maltodextrins (consisting of more than two glucose units) to a variety of acceptors such as maltotroise, maltotetrose molecules.

(B) Synthesis of Amylopectin (Or α-(1: 6) Glycosidic Linkages):

It takes place in the presence of Q-Enzyme by the transfer of small chains of glucose units joined together by α-(1: 4) glycosidic linkages to an acceptor molecule consisting of at least four α (1:4) linked glucose units. The α-(1: 6) glycosidic bond is established between C-1 of the terminal glucose unit of donor molecule and C-6 of one of the glucose units of the acceptor molecule (Fig. 13.3).

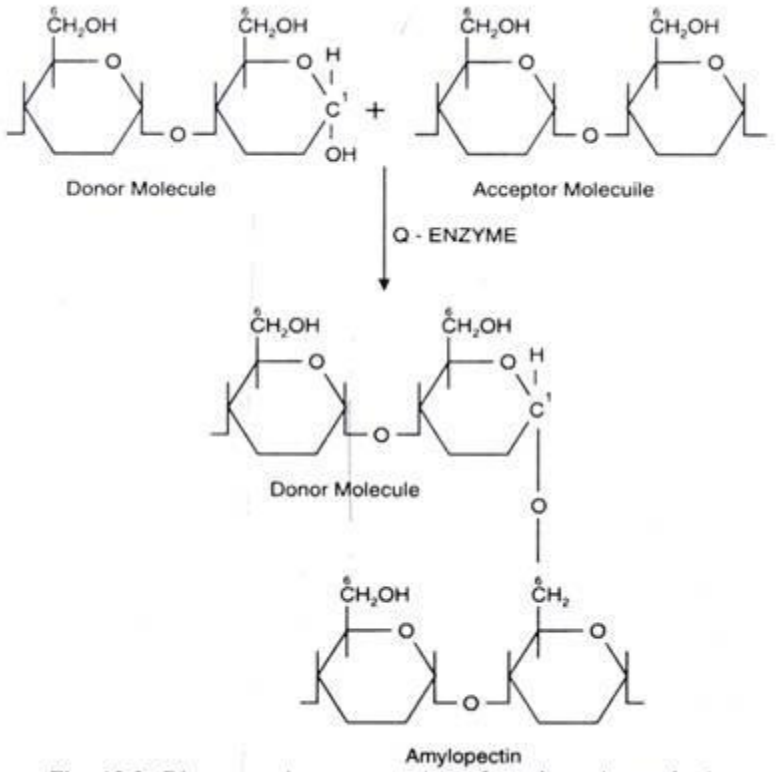


Fig. 13.3. Diagrammatic representation of amylopectin synthesis

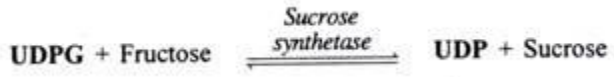
SUCROSE BIOSYNTHESIS

When triose phosphates are exported from the chloroplast, they enter the sucrose biosynthetic pathway in a similar manner as the start of the starch pathway — by condensation to form a pool of hexose phosphates. Also like starch biosynthesis, glucose 1-phosphate reacts with a sugar nucleoside, in this case UTP instead of ATP, to form UDP-glucose. Sucrose is the result of the

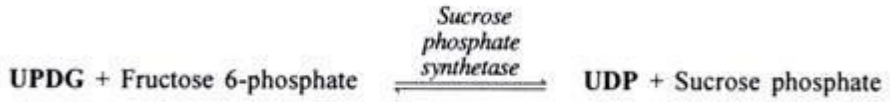
condensation reaction between this UDP-glucose and fructose 6-phosphate. This sucrose serves as the major form of transportable carbohydrate within the plant.

1) From Glucose-1-Phosphate and Fructose in the presence of the enzyme sucrose phosphorylase e.g., in bacteria.

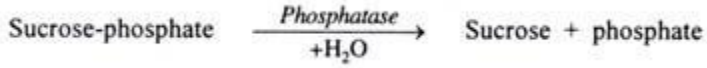
(2) From UDPG (Uridine Di-Phosphate Glucose) and Fructose in the presence of the enzyme sucrose synthetase e.g., in higher plants.



(3) From UDPG and Fructose-6-phosphate in the presence of the enzyme sucrose phosphate synthetase e.g., in higher plants.



Sucrose-phosphate thus produced is hydrolysed in the presence of the enzyme phosphatase to yield sucrose.



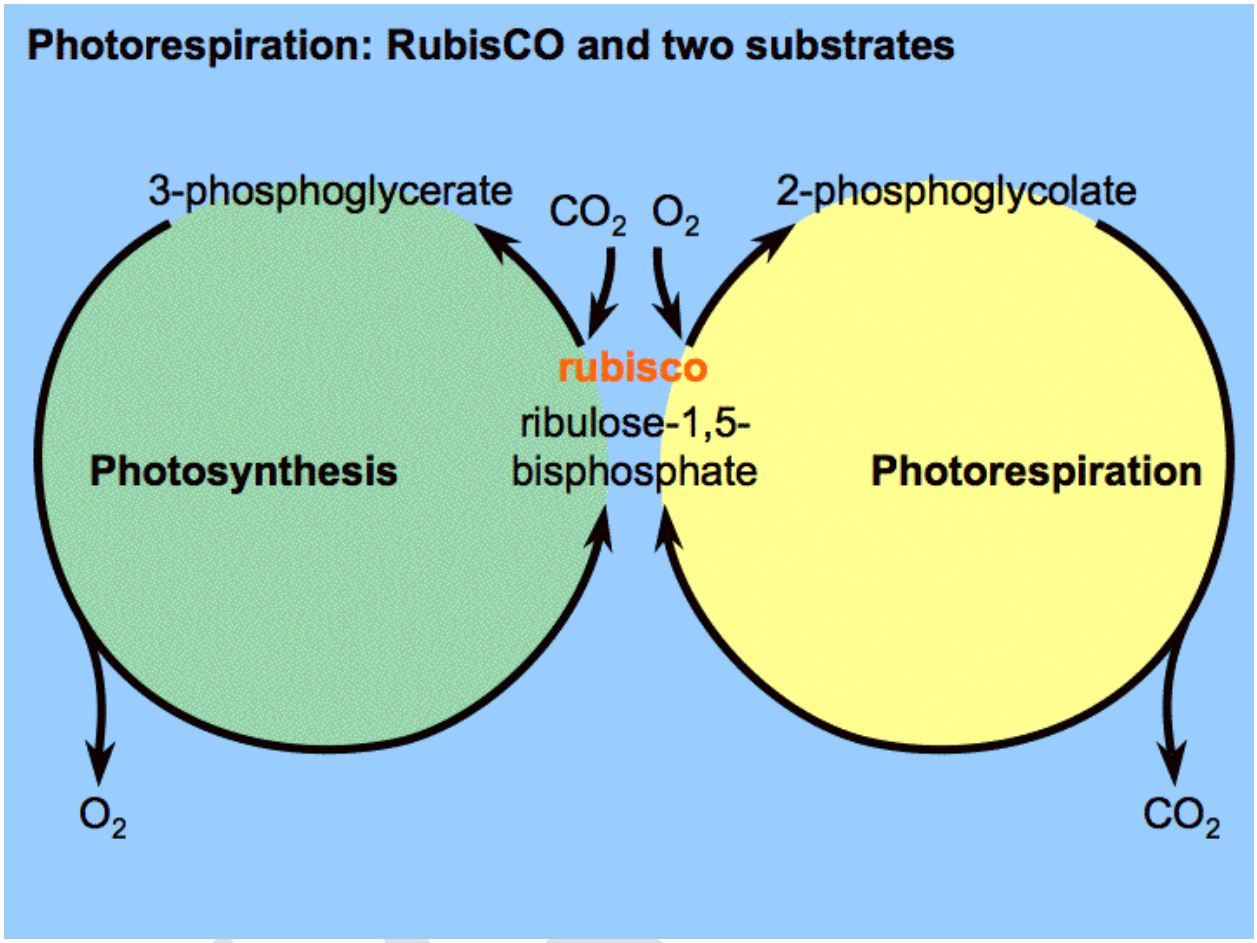
REGULATION

What determines whether the triose phosphates formed by photosynthesis enter the starch or sucrose pathways is the activity of a chloroplast envelope transporter. This transporter, called the triose phosphate-Pi antiporter, exchanges triose phosphates for Pi (inorganic phosphate) between the stroma and cytoplasm. When concentrations of Pi are high in the cyctoplasm, the antiporter is activated and exports triose phosphates in exchange for the uptake of Pi, with the cytoplasmic triose phosphate entering sucrose synthesis. On the other hand, when cytoplasmic Pi is low, no

exchange happens and triose phosphates remain in the chloroplast to enter the starch synthesis pathway.

PHOTORESPIRATION

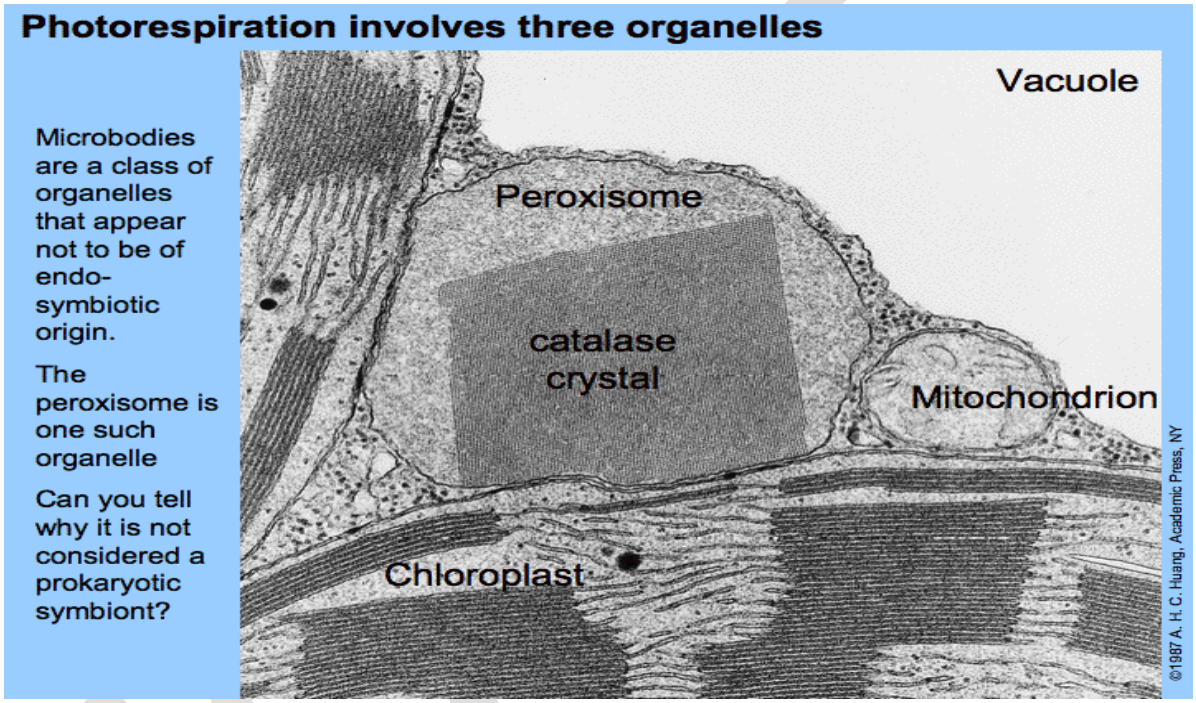
Photorespiration (also known as the oxidative photosynthetic carbon cycle, or C_2 photosynthesis) refers to a process in plant metabolism where the enzyme RuBisCOoxygenates RuBP, causing some of the energy produced by photosynthesis to be wasted. The desired reaction is the addition of carbon dioxide to RuBP (carboxylation), a key step in the Calvin–Benson cycle, however approximately 25% of reactions by RuBisCO instead add oxygen to RuBP (oxygenation), creating a product that cannot be used within the Calvin–Benson cycle. This process reduces the efficiency of photosynthesis, potentially reducing photosynthetic output by 25% in C_3 plants.[1] Photorespiration involves a complex network of enzyme reactions that exchange metabolites between chloroplasts, leaf peroxisomes and mitochondria.



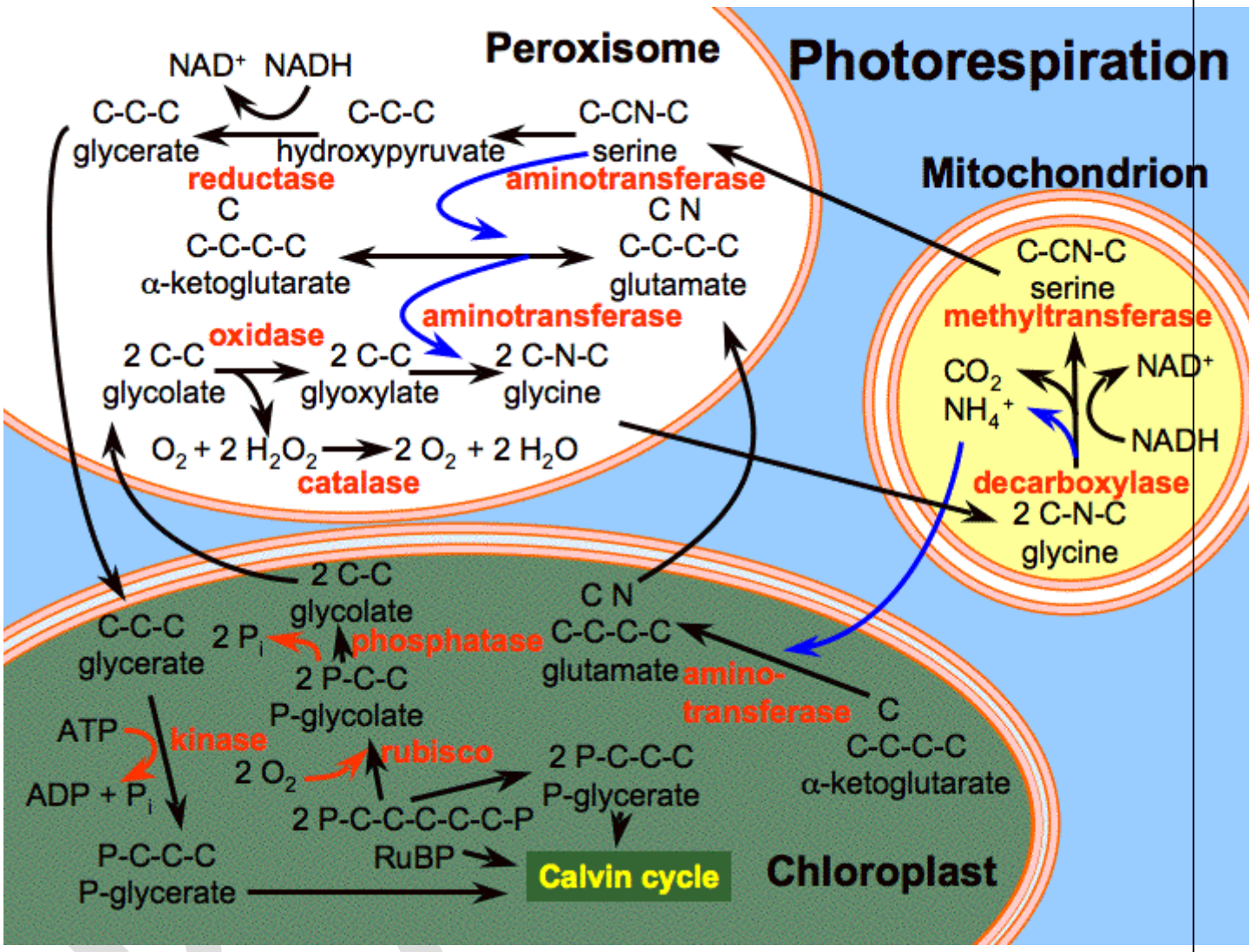
The enzyme, rubisco, not only initiates carbon fixation in the Calvin cycle; it also combines with oxygen to initiate photorespiration. As its name suggests (rubsiCO) the enzyme is both a carboxylase and an oxygenase. The active site of rubisco cannot distinguish the two similar substrates: O=C=O and O=O. As we shall see, the two reactions catalyzed by the same enzyme are diametrically oppposed to each other.

Each reaction pathway undoes the other, and both reactions can operate in a cell simultaneously depending upon the environmental conditions. As both substrates combine with the active site of rubisco, they are competitive inhibitors of each other's reactions. One might recall our earlier discussions about competitive inhibition. The relative concentration of the two substrates and the differential affinity of the enzyme for each substrate will determine which of the reactions (Calvin cycle or Photorespiration) predominate. Fortunately for plants (and for us indirectly!)

rubisco has an affinity for carbon dioxide that is 80 times higher than its affinity for oxygen. However, the relatively low ratio of CO_2 to O_2 of mesophyll fluids in contact with air (0.04) means that, in a typical plant, the Calvin cycle only occurs about three times faster than photorespiration. Temperature also influences the relative rates of photorespiration and the Calvin cycle. Because increased temperature more efficiently removes carbon-dioxide from solution than it does oxygen, high temperatures favor photorespiration.



The photorespiration pathway is an enzymatic one that is not coupled to any electron transfer system. It does not generate ATP. It does use oxygen and it does produce carbon dioxide, and it uses a sugar-phosphate as its primary fuel. The complete pathway is depicted here.



It is worthy to note that this diagram, as others of its type, show the organelles tightly appressed to each other. Indeed there are some famous electron micrographs (example above) that show this, but other micrographs do not show them this way. I say this just to comment that this positioning may be more an efficient design for communication to students than a realistic portrayal of life in a typical cell.

In the chloroplast, rubisco, combines with ribulose-1,5-bisphosphate (RuBP) and oxygen. The five-carbon RuBP is split into the two-carbon 2-phosphoglycolate and the three-carbon 3-phosphoglycerate (PGA). The enzymes of this pathway are enumerated in the diagram above.

The 2-phosphoglycolate is converted to glycolate by phosphoglycolate phosphatase in the chloroplast. The phosphate liberated is returned to the local phosphate pool. The glycolate is transported from the chloroplast into a nearby peroxisome.

In the peroxisome, the glycolate is oxidized by oxygen gas to glyoxylate and hydrogen peroxide by glycolate oxidase. The peroxide is converted to water and oxygen gas by catalase. So the consumption of oxygen in the oxidation is replaced by catalase activity in the peroxisome.

The glyoxylate is converted to the amino acid glycine in the peroxisome. The amino group is transferred to the glyoxylate from glutamate (another amino acid) by glyoxylate:glutamate aminotransferase. The glutamate is converted to α-ketoglutarate (we will remember and come back to that later!). The glycine is transported to the mitochondrion.

In the mitochondrion, glycine decarboxylase carves off carbon dioxide gas from the glycine. This requires NAD^+ to park the hydrogen atom. It also cleaves off the amino group. If you are paying attention to the chemical structures, you realize that the two-carbon amino acid has had both its amino and acid groups removed! There is only one carbon left! This methylene group is parked on a folate molecule in the mitochondrion.

When a second glycine arrives into the mitochondrion from the peroxisome, it combines with the methylene-folate to release the three-carbon amino acid serine through the action of serine hydroxymethyltransferase. Also released for re-use by this enzyme reaction is the folate. The serine is transported to the peroxisome.

In the peroxisome, the serine loses its amino group to α-ketoglutarate (remember, we would get back to that!) to regenerate the glutamate required in an earlier step in the pathway. This amino-transfer is accomplished by serine aminotransferase. In this reaction the serine is converted to hydroxypyruvate.

The peroxisome reduces the hydroxypyruvate to glycerate by hydroxypyruvate reductase. The reducing power for this comes from NADH; if you recall this was produced in an earlier step in the mitochondrion. The glycerate is transported to the chloroplast.

In the chloroplast, the glycerate is converted by glycerate kinase to 3-phosphoglycerate. The phosphate comes from ATP. Instead of producing ATP, photorespiration uses ATP. The 3-phosphoglycerate from the beginning and this new one from the end of photorespiration enter the chloroplast pool of PGA that is used to regenerate RuBP.

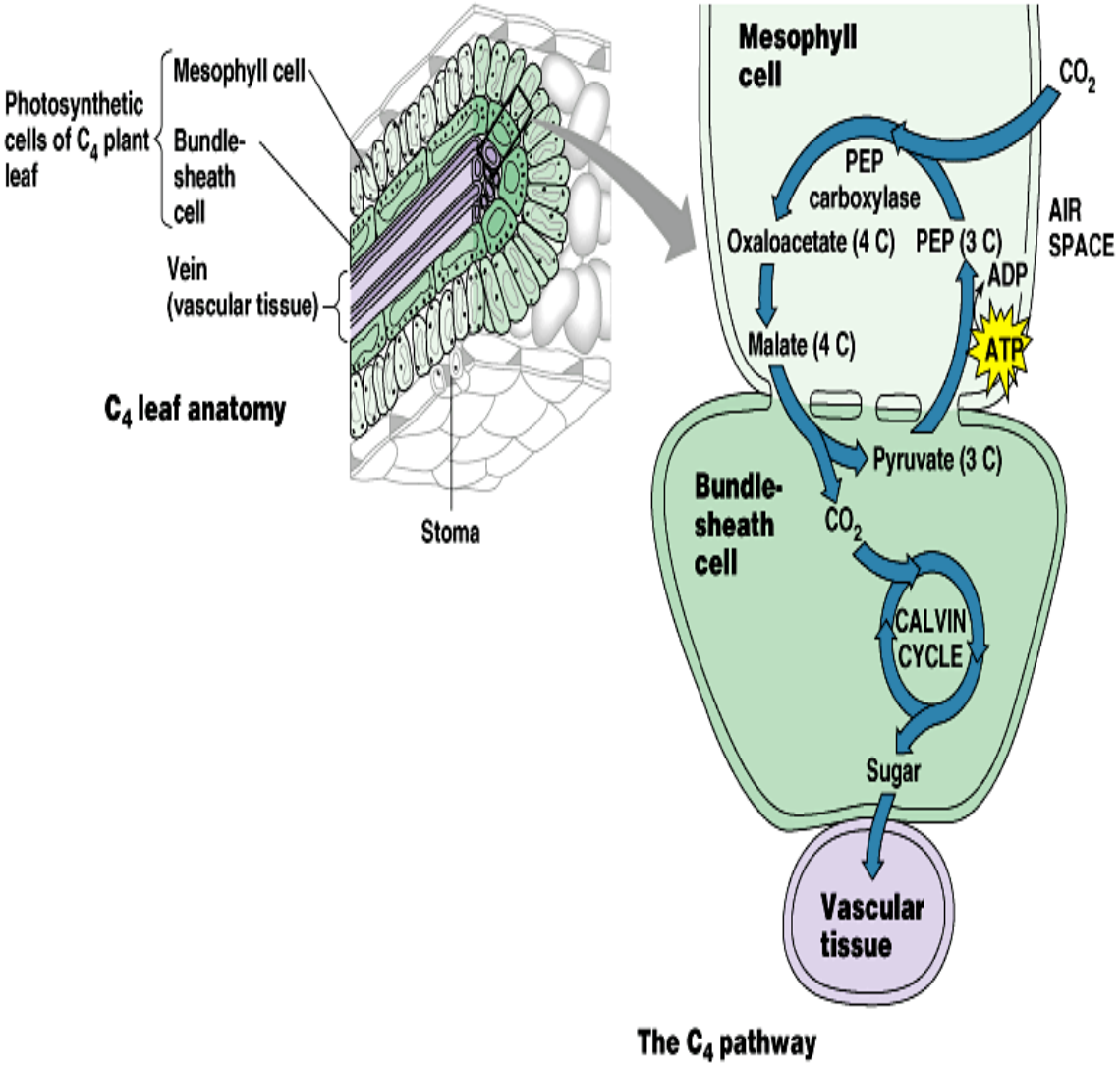
C4 PLANTS

A number of plants display an increased and more efficient net photosynthesis during strong light intensities. A prime example are the Gramineae of warmer regions like maize or sugar-cane.

At the beginning of the sixties observed that the first product of photosynthesis in sugar-cane is not the C_3 unit 3-phosphoglycerate but a unit with four C-atoms. The Australian plant physiologist M. D. HATCH and his English colleague C. R. SLACK confirmed this result and identified the compound as oxaloacetate (OAA). It is produced by the addition of one molecule of carbon dioxide to phosphoenolpyruvate (PEP). The cycle is also known as the HATCH-SLACK-cycle or the C_4 cycle. Plants with this cycle are called C_4-plants (and CAM plants, respectively) in contrast to C_3 plants where the carbon dioxide is directly fed into the CALVIN cycle. The oxaloacetate is usually converted into malate of which the carbon dioxide is split off again with the help of an enzyme.This carbon dioxide is now bound by ribulose-1,5-diphosphate and assimilated via the CALVIN cycle. : Some species use malate instead of aspartate

oxaloacetate + L-glutamate > aspartate + alpha-ketoglutarate.

The reversible binding of carbon dioxide has the function to accumulate and store CO_2. The process consumes energy, so that it could also be spoken of a carbon dioxide pump. It should be mentioned that the HATCH-SLACK cycle requires two molecules of ATP are per fixed carbon dioxide.

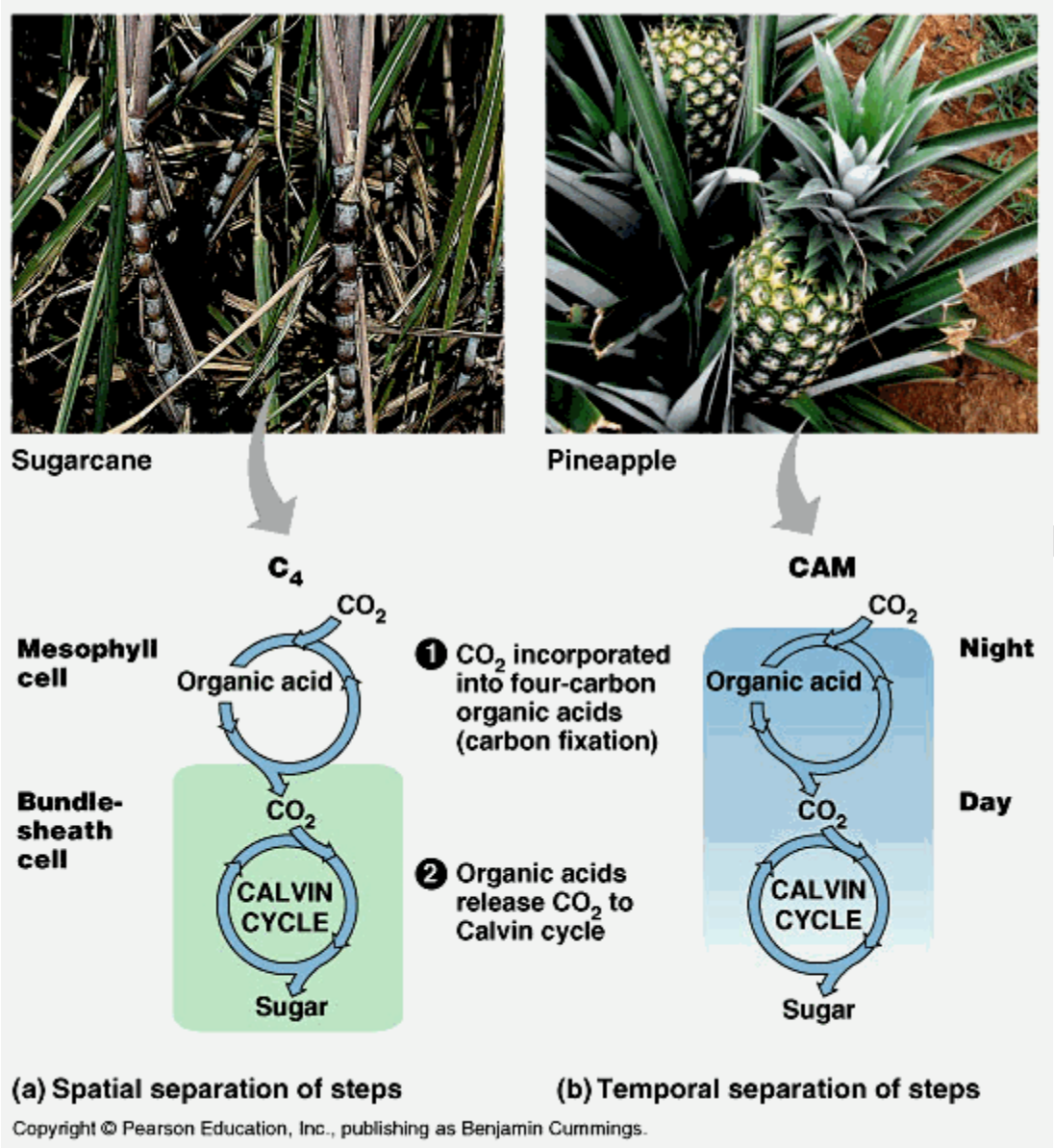


CRASSULACEAN ACID METABOLISM

CAM is the abbreviation of Crassulacean acid metabolism. The name points at the fact that this pathway occurs mainly in Crassulacean species (and other succulent plants). The chemical reaction of the carbon dioxide accumulation is similar to that of C_4 plants but here are carbon

dioxide fixation and its assimilation not separated spatially but in time. CAM plants occur mainly in arid regions. The opening of the stomata to take up carbon dioxide is always connected with large losses of water. To inhibit this loss during intense sun (the transpiration via the cuticle remains intact) has a mechanism developed that allows the uptake of carbon dioxide during the night. The prefixed carbon dioxide is stored in the vacuoles as malate (and isocitrate) and is used during the daytime for photosynthesis.

The ultimate prevention of CO_2 loss is found in desert plants like cactus. CAM In these plants the stomata are open at night. The plant fixes CO_2 into C_4 carbon compounds during the night, then transfers the carbon to the Calvin cycle during the daylight hours while the stomata are completely closed therefore reducing H_2O loss. This is all done in the same cell.



SYNTHESIS OF CELL WALL POLYSACCHARIDES

Synthesis of Cellulose:

Long un-branched chains of cellulose (consisting of β(1→4) linked glucose residues) are synthesized in plants by the enzymes called cellulose synthases. The enzyme cellulose synthase is a multi-submit complex that is situated on plasma membrane and transfers a glucose residue

from a sugar nucleotide donor called uridine diphosphate glucose (UDPG) to an acceptor molecule forming β (1 → 4) glucosyl acceptor.

UDPG + Acceptor → UDP + β (1→4) glucosyl-acceptor

It is believed that sterol-glycosides (i.e., sterols joined to a chain of one or more glucose units) such as β-sitosterol glucoside (Fig. 13.4), probably act as initial acceptors that start the elongation of cellulose chain. The process continues, and after the cellulose chain has attained desired length, the sterol is cut off from the glucan (Cellulose Chain) by the enzyme endoglucanase present in the plasma membrane. The separated cellulose chains are then extruded on the outer side of the plasma membrane (Fig. 13.5).

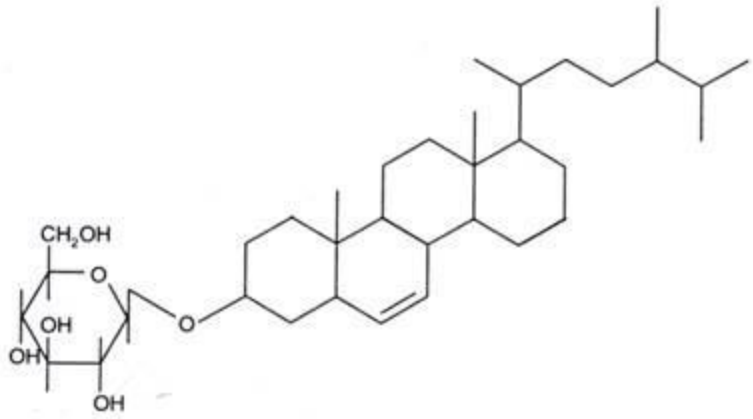


Fig. 13.4. Structure of β-sitosterol glucoside

There are evidences to suggest that glucose in UDPG comes from sucrose, by the action of the reversible enzyme sucrose synthetase (Fig. 13.5). Alternatively, UDP-glucose may be directly obtained from cytoplasm.

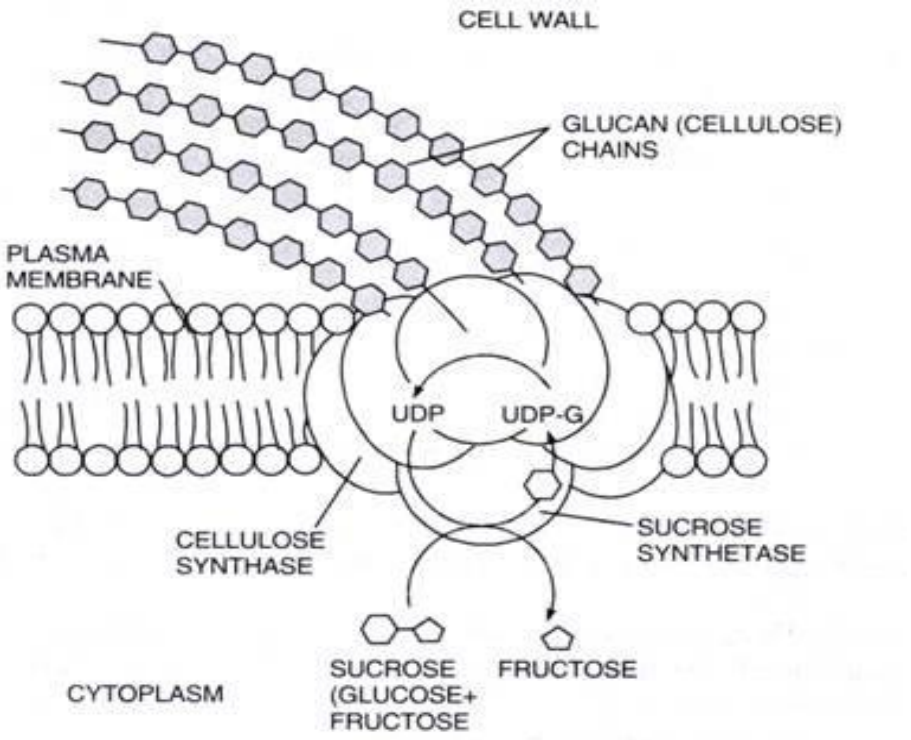
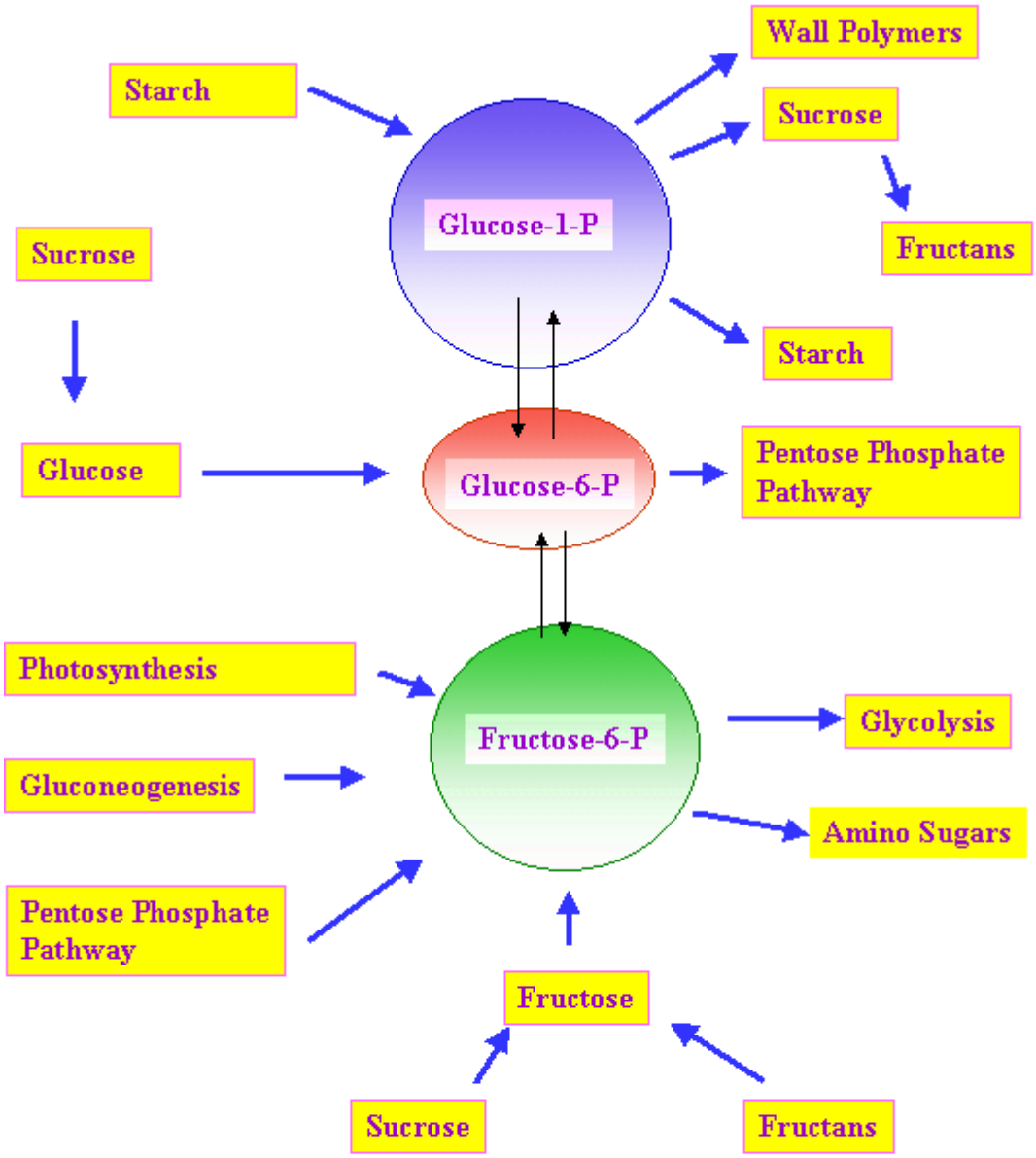


Fig. 13.5. A model showing biosynthesis of cellulose. See text for details.

INTEGRATION OF CARBOHYDRATE METABOLISM IN THE PLANT CELL

Carbohydrate metabolism in a typical plant cell is more complex in several ways than that in a typical animal cell. The plant cell carries out the same processes that generate energy in animal cells (glycolysis, citric acid cycle, and oxidative phosphorylation); it can generate hexoses from three- or four-carbon compounds by glu-coneogenesis; it can oxidize hexose phosphates to pentose phosphates with the generation of NADPH (the oxidative pentose phosphate pathway); and it can produce a polymer of (a1n4)-linked glucose (starch) and degrade it to generate hexoses. But besides these carbohydrate transformations that it shares with animal cells, the photosynthetic plant cell can fix CO2 into organic compounds (the rubisco reaction); use the products of fixation to generate trioses, hexoses, and pentoses (the Calvin cycle); and convert acetyl-CoA generated from fatty acid breakdown to four-carbon compounds (the glyoxylate cycle) and the four-carbon compounds to hexoses (gluconeogenesis). These processes, unique to the plant cell, are segregated in several compartments not found in animal cells: the glyoxylate cycle in gly-oxysomes, the Calvin cycle in chloroplasts, starch synthesis in amyloplasts, and

organic acid storage in vacuoles. The integration of events among these various compartments requires specific transporters in the membranes of each organelle, to move products from one organelle to another or into the cytosol.



POSSIBLE QUESTIONS
UNIT III

PART A (1 Mark)
Question number 1 – 20 (Online examination)

PART B (2 Marks)

1. Write a note on Calvin cycle
2. Write short notes of regulated synthesis of starch
3. Brief about the regulated synthesis of sucrose
4. Write notes on C4 plants
5. Write notes on CAM pathways
6. Give note on cell wall polysaccharide of plant
7. Add note on regulation of calvin cycle
8. Give the importance of calvin cycle

PART C (6 Marks)

1. Explain in detail about the integration of carbohydrate metabolism in plant cell.
2. Write note on synthesis of cell wall polysaccharides
3. Explain in detail about the regulation of calvin cycle
4. Discuss in detail about photorespiration
5. Discuss in detail about the regulated synthesis of starch.
6. Explain in detail about Calvin cycle
7. Discuss about the regulated synthesis of starch
8. Discuss in detail about CAM pathways

Karpagam Academy of Higher Education
Department of Biochemistry
Metabolism of Carbohydrates and Lipids (17BCU301) MCQ
UNIT III

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	3	The general chemical formula of carbohydrate is	$(CH_2O)n$	$(CH_2O)2n$	(CHO)n	CnH_2nO	$(CH_2O)n$
2	3	Which of the following is a debranching enzyme	Glycogen synthetase	Glucose- 6- Phpsphatase	Amylo (1,6) glucosidase	Amylo 1,4- 1,6 transglycosylase	Amylo (1,6) glucosidase
3	3						
4	3	Sucrose is a	Disaccharide	Monosaccharide	Trisaccharide	Polysaccharide	Disaccharide
5	3	Majority of the monosaccharides found in the human body are of …	L-type	D-type	DL-types	LD-types	D-type
6	3	The sugar found in DNA is	Xylose	Ribose	Deoxyribose	Ribulose	Deoxyribose
7	3	The sugar found in RNA is	Ribose	Deoxyribose	Ribulose	Erythrose	Ribose
8	3	Simplest carbohydrate is ….	Dihydroxy acetone	Glycerldehyde	Glucose	Galactose	Glycerldehyde
9	3	In C_3 plants the primary co_2 acceptor is ----------	ribulose 1,5 bis phosphate	Ribulose-5-phosphate	ribulose-1-phosphate	ribose -1-phosphate	ribulose 1,5 bis phosphate
10	3	The sequence of dark reaction in photosynthesis was established by	A.A Bensen	J.Bassham	Melvin Calvin	J.C.Bose	Melvin Calvin
11	3	The Benson – Calvin cycle takes place in	Chloroplasts	etioplasts	Mitochondria	cytoplasm	Chloroplasts
12	3	Sugarcane & cynodon dactylon are	C_4 plants	C_3 Plants	C_2 plants	CAM plants	C_4 plants
13	3	The primary acceptor of CO_2 in C_4 plant is	PEP	Pyruvate	Alanine	oxaloacetate	PEP
14	3	In CAM plants CO_2 assimilation occurs during	Day	Night	Day & Night	Evening	Night
15	3	In C_4 plants Pyruvate, Phosphate dikinase is located mainly in the	mesophyll cells of chloroplast	bundle sheath cells of chloroplast	mitochondria	peroxisomes	mesophyll cells of chloroplast
16	3	Plants in which the Hatch slack pathway takes place are called as	C_2 plants	C_3 plants	C_4 plants	CAM plants	C_4 plants
17	3	In bundle sheath cells, malate is decarboxylated to form	oxaloacetate	citrate	pyruvate	PEP	pyruvate
18	3	The calvin cycle enzymes are present in	Stroma	Thylakoid lumen	Grana	Thylakoid membrane	Stroma
19	3	During photophosphorylation the NADPH and ATP are	Absorbed	Released	Reduced	Oxidised	Released
20	3	Thylakoids of grana possess the	enzymes of calvin cycle	enzymes of photophosphorylation	enzymes for C_3 cycle	enzymes of C_4 cycle	enzymes of photophosphorylation
21	3	Hydrogen peroxide is formed during	Calvin cycle	Hatch-Slack cycle	CAM cycle	photorespiration	photorespiration
22	3	ATP molecules are synthesized in all except	cyclic photophosphorylation	non cyclic photophosphorylation	dark respiration	photorespiration	photorespiration
23	3	The optimum temperature of photoresperation is	10 - 20°C	25 - 35°C	40 - 60°C	35 - 45°C	25 - 35°C
24	3	The nocturnal opening of stomata is the characteristic feature of	water plants	C_4 plants	CAM plants	C_3 plants	CAM plants
25	3	The first stable compound formed in C_3 cycle is	DHAP	phosphoglyceric acid	oxalo acetic acid	glycolic acid	phosphoglyceric acid
26	3	The first stable compound formed in C_4 cycle is	DHAP	phosphoglyceric acid	oxalo acetic acid	glycolic acid	oxalo acetic acid
27	3	The optimum temperature for the growth of C_4 plants is	30 - 45°C	25 - 35°C	0 - 20°C	40 - 60°C	30 - 45°C
28	3	For the synthesis of each molecule of glucose from CO_2 in a photosynthesis how many ATP molecules are required?	18	12	3	2	18
29	3	For the synthesis of each molecule of glucose from CO_2 in a photosynthesis how many NADPH molecules are utilized	18	12	3	2	12
30	3	Number of ATP molecules synthesized in non cyclic photophosphorylation is	2	3	1	4	1
31	3	Number of ATP molecules synthesized in cyclic photophosphorylation is	2	3	1	4	2
32	3	The following statement is true with cyclic photo phosphorylation	PS I is involved	PS II is involved	photo oxidation of water takes place	$NADP^+$ is reduced to NADPH + H^+	PS I is involved
33		Carotenes are usually found in	PS I	PSII	Both PS I and PSII	neither PS I nor PSII	PS I
34	3	Xanthophylls are usually found in	PS I	PSII	Both PS I and PSII	neither PS I nor PSII	PSII
35	3	In C_4 cycle oxalo acetic acid is converted to malic acid by the enzyme	malic dehydrogenase	malate decarboxylase	malic oxidase	transaminase	malic dehydrogenase
36	3	In C_4 plants malic enzyme is present in	mesophyll cells	bundle sheath cells	xylem	phloem	bundle sheath cells
37	3	Photosynthetic yield is more in	C_4 plants	C_3 plants	C_2 plants	CAM plants	C_4 plants
38	3	CAM cycle is observed in all the plants except	cactus	orchid	chlorella	bryophyllum	chlorella
39	3	In C_4 cycle the enzyme involved in conversion of oxalo acetic acid to malic acid is	malic enzyme	malate dehydrogenase	transaminase	malate decarboxylase	malate dehydrogenase
40	3	Only mesophyll cells are involved in	C_3 cycle	C_4 cycle	CAM cycle	C_2 cycle	C_3 cycle
41	3	In CAM plants carbohydrate synthesis takes place during	night time	day time	both night and day time	only in the evening	day time
42	3	Which of the following is formed during photo respiration	H_2O_2	O_2	OH^- ions	O_2	H_2O_2
43	3	In photo respiration glyoxylic acid is converted to glycine by	transaminase	decarboxylase	dehydrogenase	reductase	transaminase
44	3	In photo respiration the enzyme involved in detoxification of H_2O_2 is	catalase	decarboxylase	transaminase	dehydrogenase	catalase
45	3	When the atmospheric CO_2 is less than 1% ribulose di phosphate is converted to	3 phospho glyceric acid	glycolic acid	glyoxylic acid	glycine	glycolic acid

UNIT-IV
SYLLABUS

Fatty acid oxidation : Digestion, mobilisation and transport of cholesterol and triacyl glycerols, fatty acid transport to mitochondria, β oxidation of saturated, unsaturated, odd and even numbered and branched chain fatty acids, regulation of fatty acid oxidation, peroxisomal oxidation, ω oxidation, ketone bodies metabolism, ketoacidosis.
Fatty acid synthesis : Fatty acid synthase complex. Synthesis of saturated, unsaturated, odd and even chain fatty acids and regulation.

METABOLISM OF LIPIDS

Introduction.

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are as diverse as their chemistry. Fats and oils are the principal stored forms of energy in many organisms. Phospholipids and sterols are major structural elements of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light absorbing pigments, hydrophobic anchors for proteins, "chaperones" to help membrane proteins fold, emulsifying agents in the digestive tract, hormones, and intracellular messengers.

Lipids are indispensable for cell structure and function. Due to their hydrophobic and nonpolar nature, lipids differ from rest of the body compounds and are unique in their action

DIGESTION AND ABSORPTION OF LIPID

The bulk of dietary lipid is triglyceride, phospholipids, sterols like cholesterol and many minor lipids, including fat-soluble vitamins.

In order for the triglyceride to be absorbed, two processes must occur:

- Large aggregates of dietary triglyceride, which are virtually insoluble in an aqueous environment, must be broken down physically and held in suspension - a process called emulsification.
- Triglyceride molecules must be enzymatically digested to yield monoglyceride and fatty acids, both of which can efficiently diffuse or be transported into the enterocyte

The key players in these two transformations are bile acids and pancreatic lipase, both of which are mixed with chyme and act in the lumen of the small intestine. Bile acids are also necessary to solubilize other lipids, including cholesterol.

DIGESTION

Most of the fat in the human diet is in the form of triacylglycerol (TAG), which consists of three fatty acids linked to glycerol.

In the digestive tract, TAG is hydrolyzed by the enzyme lipase, to release free fatty acids and monoglycerides

The key issue in the digestion and absorption of fats is one of solubility: lipids are hydrophobic, and thus are poorly soluble in the aqueous environment of the digestive tract. The digestive enzyme, lipase, is water soluble and can only work at the surface of fat globules. Digestion is greatly aided by emulsification, the breaking up of fat globules into much smaller emulsion droplets. Bile salts and phospholipids are amphipathic molecules that are present in the bile.

Minor digestion of lipid occurs in mouth. Where,

TAG —Lingual lipase→ Free fatty acid + Glycerol

In Stomach,

TAG —Gastric lipase→ Free fatty acid + Monoacyl glycerol

In Small intestine,

(I) Emulsification of lipids occurs by three complementary mechanisms

(i) Detergent action of bile salts

Lipids in the presence of bile salts converted into smaller particles. The bile salts also prevent the reaggregation of smaller lipid droplets into larger aggregates

TAG $\xrightarrow{\text{Bile salts}}$ smaller partilcles

(ii) Surfactant action of degraded lipids

Initial digestion products like free fatty acids and MAG promote emulsification. Here the Initial digestion products and phospholipids are called surfactants.

(iii) Mechanical mixing of food

Motility in the small intestine breaks fat globules apart into small droplets that are coated with bile salts and phospholipids, which prevent the emulsion droplets from re-associating.

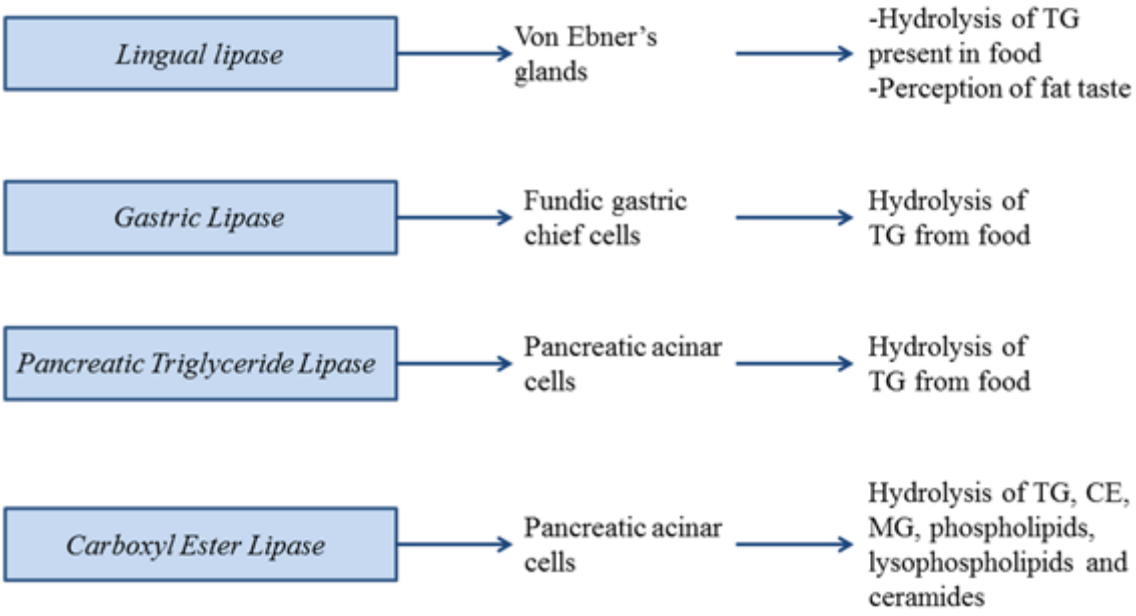
(II) Digestion by pancreatic enzymes

TAG $\xrightarrow{\text{Pancreatic lipase}}$ Free fatty acid + Glycerol

Cholesterol ester / Lipid ester $\xrightarrow{\text{lipid esterase}}$ Cholesterol+ free fatty acid

Phospholipid $\xrightarrow{\text{phospholipase}}$ Glycerol+ free fatty acid+ nitrogenous base

Figure:Emulsification and digestion of lipids



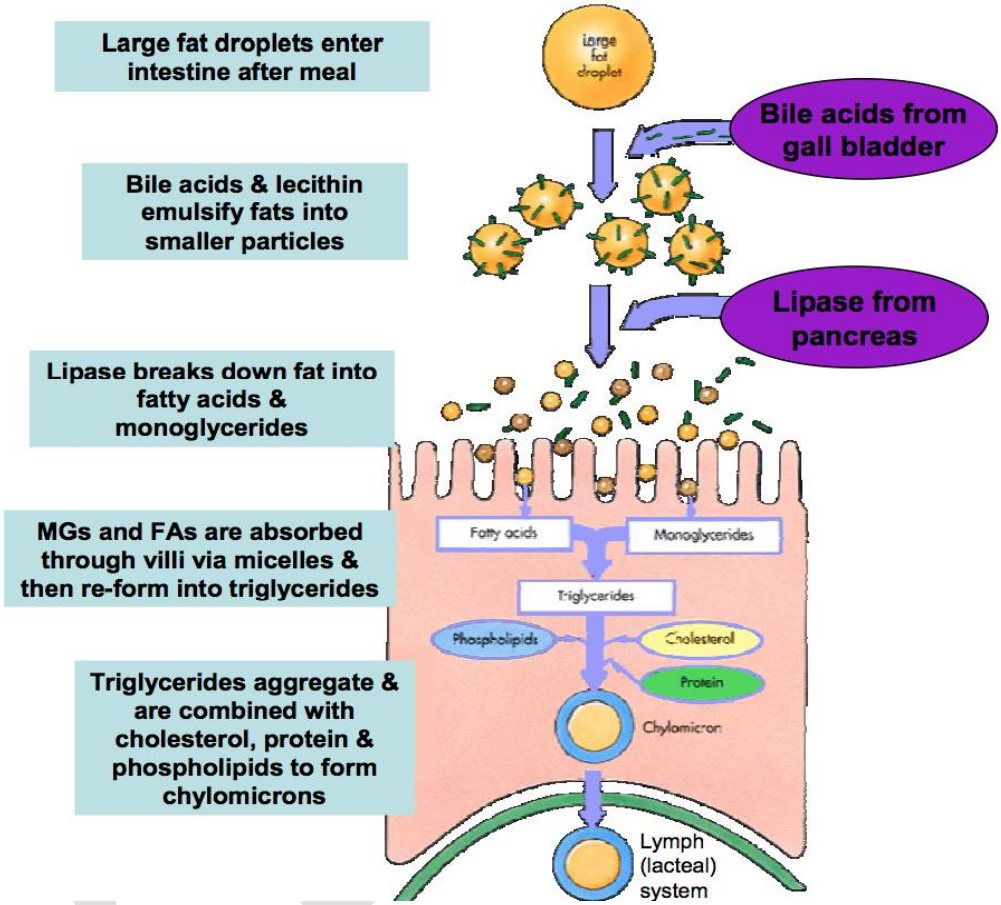
The emulsion droplets are where digestion occurs. Emulsification greatly increases the surface area where water-soluble lipase can work to digest TAG. Another factor that helps is colipase, an amphipathic protein that binds and anchors lipase at the surface of the emulsion droplet.

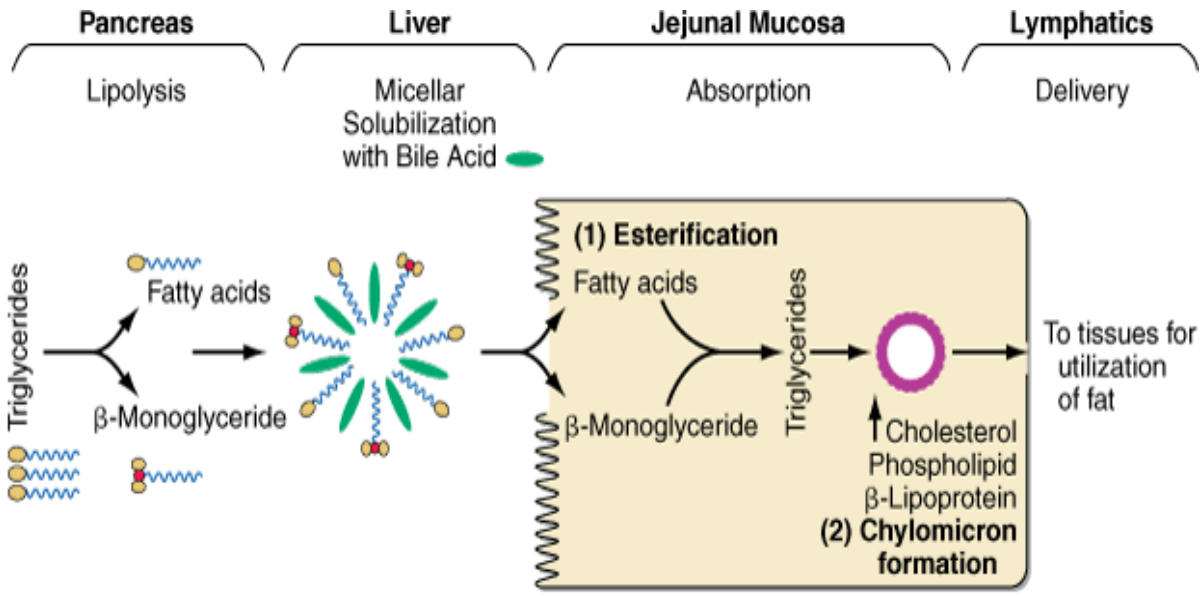
ABSORPTION

Micelles

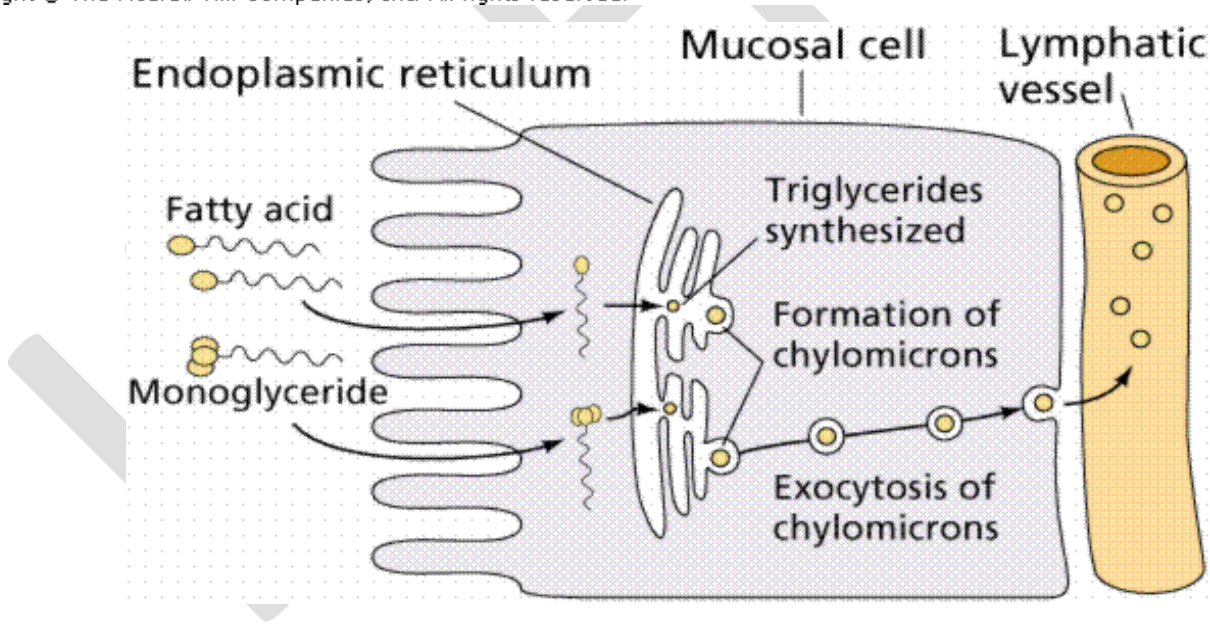
After digestion, monoglycerides and fatty acids associate with bile salts and phopholipids to form micelles. Micelles are about 200 times smaller than emulsion droplets (4-7nm versus 1µm for emulsion droplets). Micelles transport the poorly soluble monoglycerides and fatty acids to the surface of the enterocyte where they can be absorbed. As well, micelles contain fat soluble vitamins and cholesterol. Because of their nonpolar nature, monoglycerides and fatty acids can just diffuse across the plasma membrane of the enterocyte. Some absorption may be facilitated by specific transport proteins.

Figure: Digestion and absorption of lipid





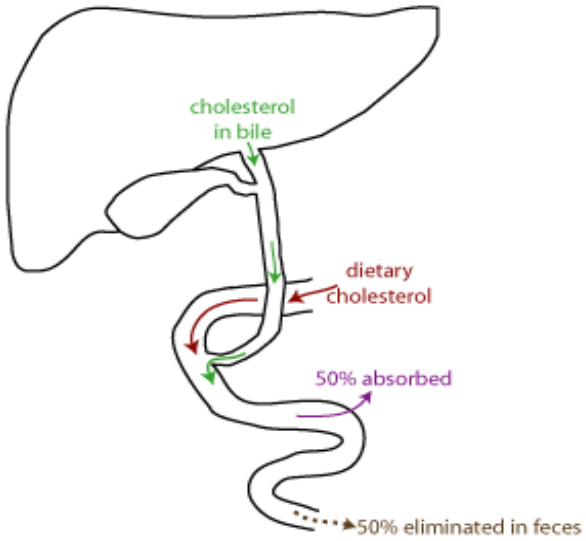
Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com



Cholesterol absorption

As shown in the figure, some of the cholesterol in the small intestine is dietary cholesterol, and some is put there by the liver, arriving via the bile. Of the total cholesterol that passes through the small intestine, only half is typically absorbed, and the rest is eliminated in

the feces. Figure: Digestion and absorption of cholesterol

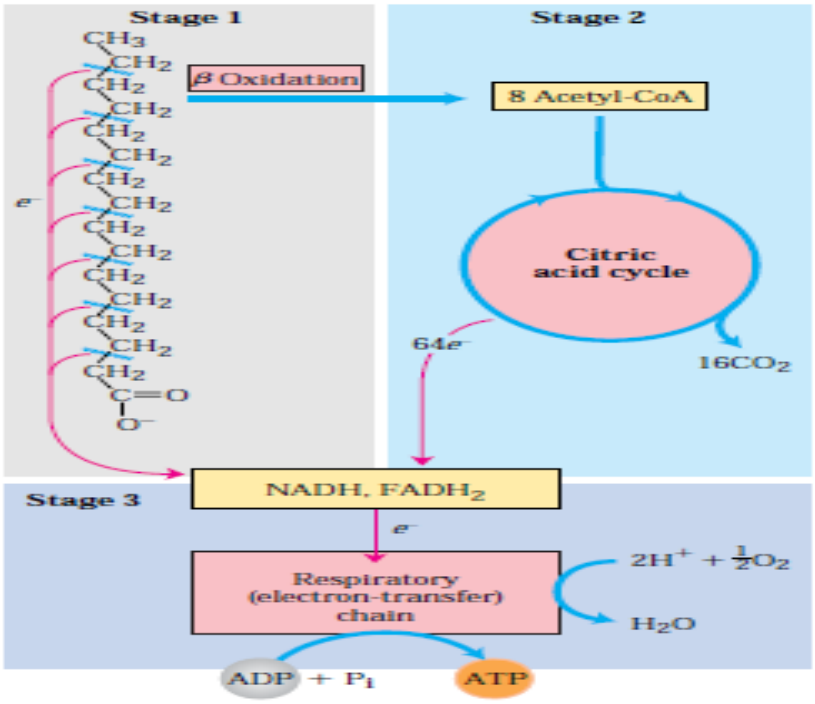


OXIDATION OF FATTY ACID

Mitochondrial oxidation of fatty acids takes place in three stages. In the first stage β-oxidation-fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16-carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two carbons of palmitate (originally C-15 and C-16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H^+) from the fatty acyl moiety by dehydrogenases.

In the second stage of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation. The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and $FADH_2$, which in the third stage

donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP.



The *β- o*xidation of Saturated Fatty Acids

Consist of three steps as follows

a)Fatty acid activation

Fatty acids are oxidized inside the mitochondrial matrix but the fatty acids to be oxidized come from the cytosol. Fatty acids are activated in the cytosol by esterification with Coenzyme A (CoA) to form acyl-CoA (RCO-CoA, where R is the fatty acid acyl group).

Activated medium-chain fatty acids (C8 and C10) freely diffuse into mitochondria to be oxidized but long chain fatty acids do not diffuse into mitochondria so they must be transported in.

b) Trnasport of fattyacid into Mitochondria

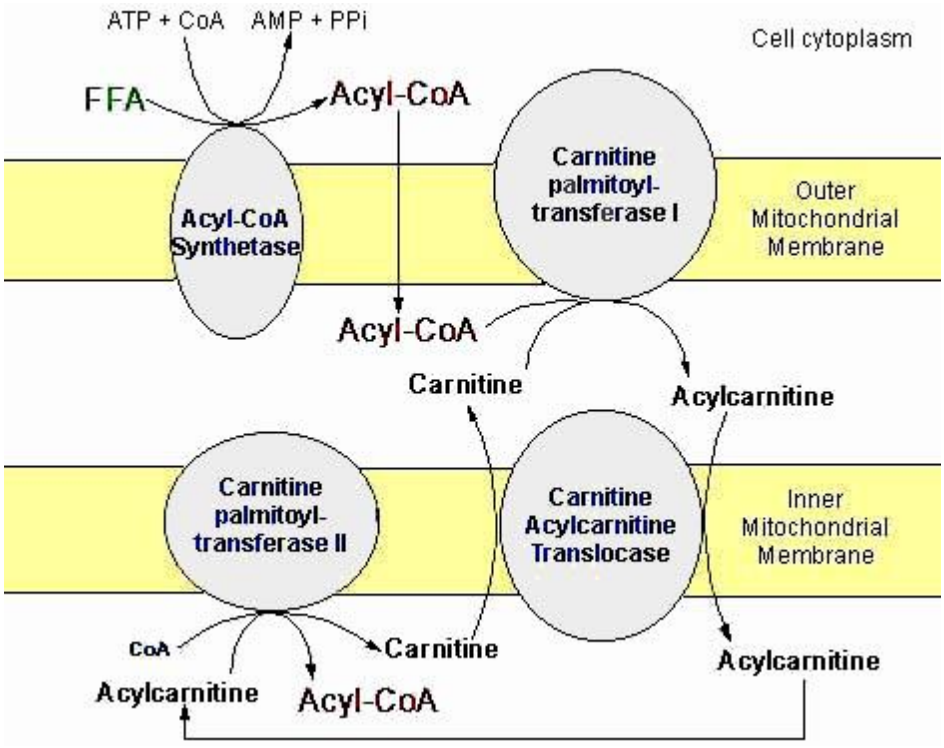
The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of beta-oxidation. The carnitine

shuttle consists of three enzymes (carnitine palmitoyltransferase 1 (CPT1A and CPT1B) , carnitine acylcarnitine translocase (SLC25A20), carnitine palmitoyl-transferase 2 (CPT2)) and a small, soluble molecule, carnitine, to transport fatty acids as their long-chain fatty acylcarnitine esters.

The transport of long chain fatty acids into mitochondria for oxidation is accomplished by the carnitine palmitoyltransferase system (CPTI and CPTII). CPTI exchanges carnitine for the CoA attached to long chain fatty acids to form a fatty acid-carnitine conjugate (RCO-carnitine).

The fatty acid-carnitine is transported into the matrix by a transporter protein in the inner mitochondrial membrane.

Once the fatty acid-carnitine is inside the matrix, CPTII exchanges CoA for carnitine to produce fatty acid-CoA once again, ready to enter fatty acid oxidation in the matrix to produce energy. The free carnitine is transported back out to renew the cytoplasmic pool of carnitine and allow the transfer process to continue.



c) *β* oxidation of saturated fatty acid

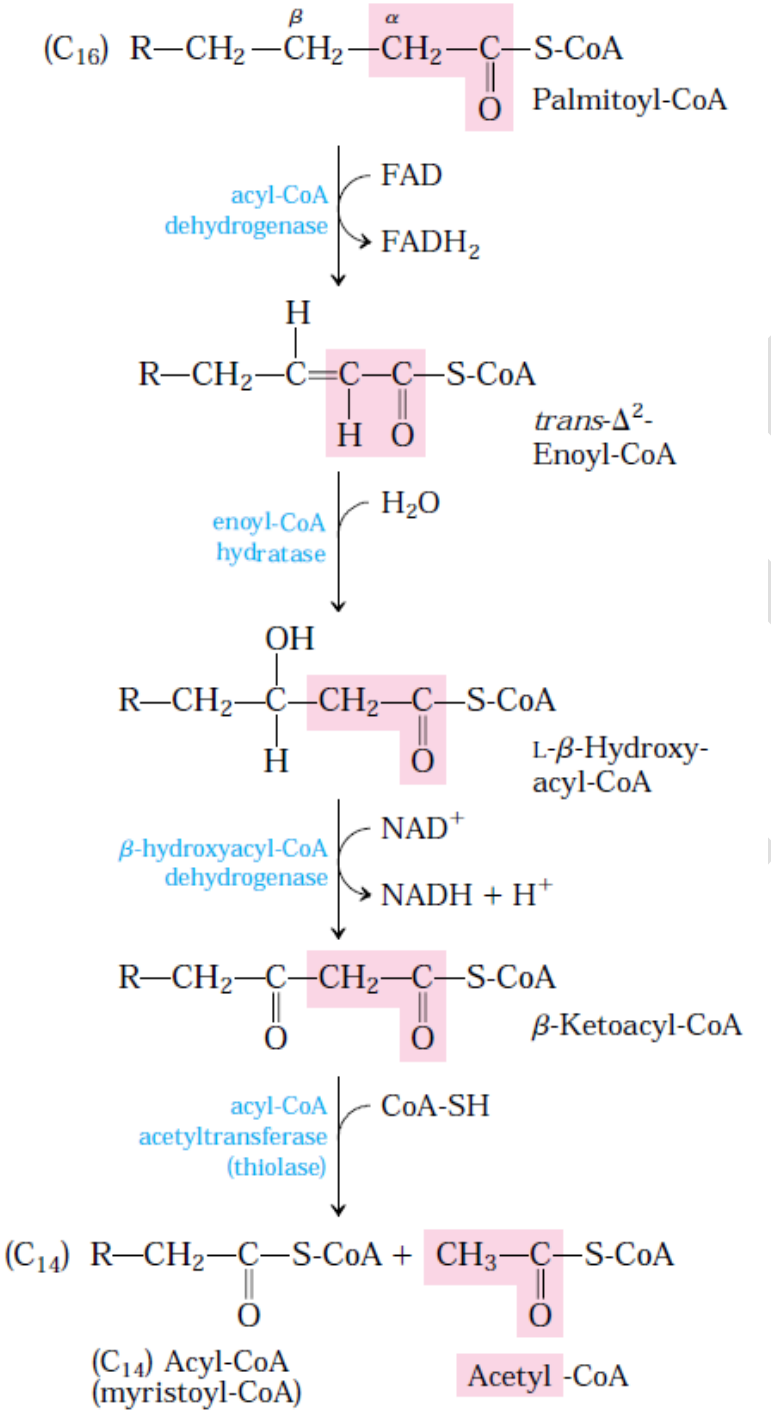
Four enzyme-catalyzed reactions make up the first stage of fatty acid oxidation. First, dehydrogenation of fatty acyl–CoA produces a double bond between the α and β carbon atoms (C-2 and C-3), yielding a *trans*-Δ^2-enoyl-CoA (the symbol Δ^2designates the position of the double bond) the new double bond has the trans configuration, whereas the double bonds in naturally occurring unsaturated fatty acids are normally in the cis configuration.

This first step is catalyzed by three isozymes of acyl-CoA dehydrogenase, each specific for a range of fatty-acyl chain lengths: very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons; medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons; and short-chain (SCAD), acting on fatty acids of 4 to 8 carbons. All three isozymes are flavoproteins with FAD as a prosthetic group. The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the electron-transferring flavoprotein (ETF). The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle; in both reactions the enzyme is bound to the inner membrane, a double bond is introduced into a carboxylic acid between the α and β carbons, FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O_2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

In the second step of the β -oxidation cycle, water is added to the double bond of the *trans*-Δ^2-enoyl-CoA to form the L stereoisomer of β -hydroxyacyl-CoA (3-hydroxyacyl-CoA). This reaction, catalyzed by enoyl-CoA hydratase, is formally analogous to the fumarase reaction in the citric acid cycle, in which H_2O adds across an α -β double bond.

In the third step, L- β -hydroxyacyl-CoA is dehydrogenated to form β -ketoacyl-CoA, by the action of β -hydroxyacyl-CoA dehydrogenase; NAD^+ is the electron acceptor. This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA. The NADH formed in the reaction donates its electrons to NADH dehydrogenase, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O_2. The reaction catalyzed by β -hydroxyacyl-CoA dehydrogenasen is closely analogous to the malate dehydrogenase reaction of the citric acid cycle.

The fourth and last step of the β-oxidation cycle is catalyzed by acyl-CoA acetyltransferase, more commonly called thiolase, which promotes reaction of β - ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms. This reaction is called thiolysis, by analogy with the process of hydrolysis, because the β -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.



The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain. For fatty

acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the trifunctional protein (TFP). TFP is a heterooctamer of $\alpha_4\beta_4$ subunits. Each α subunit contains two activities, the enoyl-CoA hydratase and the β -hydroxyacyl-CoA dehydrogenase; the β -subunits contain the thiolase activity. This tight association of three enzymes may allow efficient substrate channeling from one active site to the next, without diffusion of the intermediates away from the enzyme surface. When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

The β -oxidation sequence is an elegant mechanism for destabilizing and breaking these bonds. The first three reactions of β -oxidation create a much less stable C-C bond, in which the α_carbon (C-2) is bonded to *two* carbonyl carbons (the β -ketoacyl-CoA intermediate). The ketone function on the β- carbon (C-3) makes it a good target for nucleophilic attack by the -SH of coenzyme A, catalyzed by thiolase. The acidity of the α -hydrogen and the resonance stabilization of the carbanion generated by the departure of this hydrogen make the terminal -CH2-CO-S-CoA a good leaving group, facilitating breakage of the α– β bond.

Energetic of β –oxidation

Palmitic acid (16 carbons) undergoes 7 times β-oxidation and produces 8 molecules of acetyl-CoA. Each time, β-oxidation produces 5 ATP.

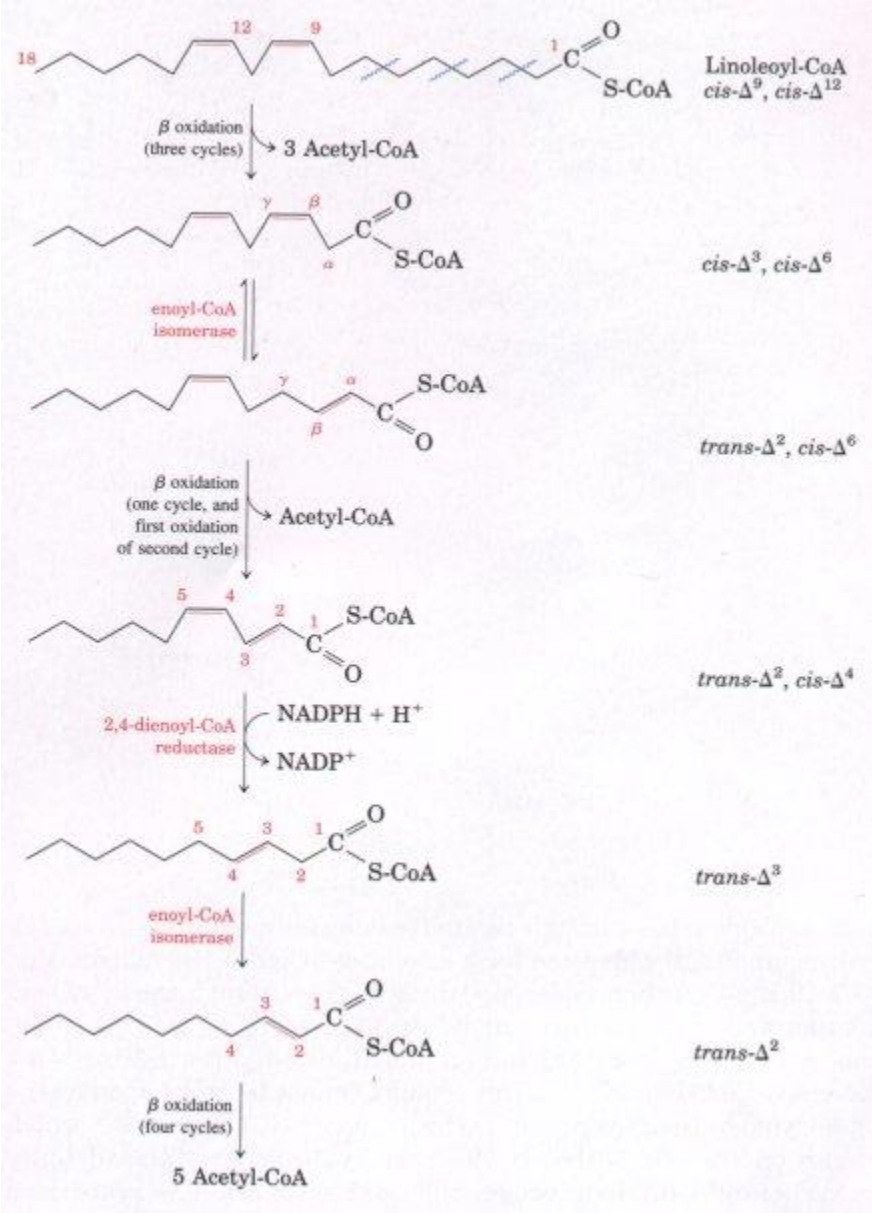
Total number of ATP formed through β-oxidation	7 X5 =35
Total number of ATP formed on oxidation of acetyl-CoA through TCA cycle	8 X12 =96
Total	131
2ATP utilised for initial activation of Fatty acid	-2
Net Total yield	129 ATP

***β* oxidation of Unsaturated fatty acid**

β-Oxidation of unsaturated fatty acids poses a problem since the location of a cis bond can prevent the formation of a trans-Δ^2 bond. These situations are handled by an additional two enzymes, Enoyl CoA isomerase or 2,4 Dienoyl CoA reductase.

Whatever the conformation of the hydrocarbon chain, β-oxidation occurs normally until the acyl CoA (because of the presence of a double bond) is not an appropriate substrate for acyl CoA dehydrogenase, or enoyl CoA hydratase:

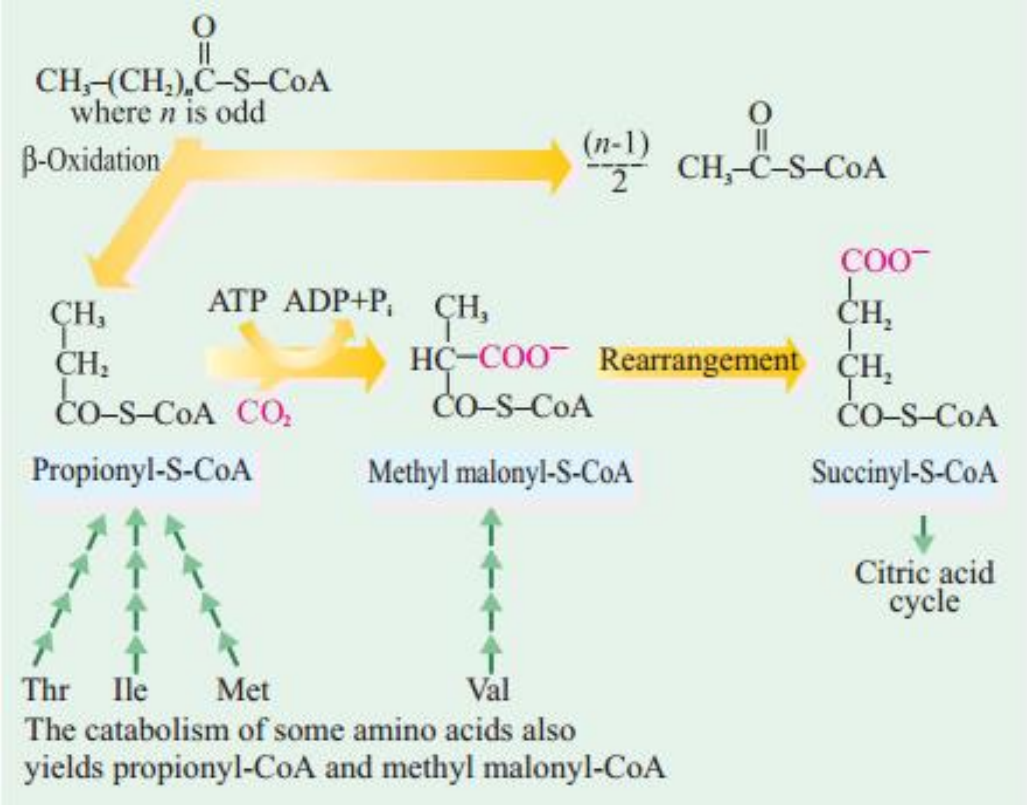
- If the acyl CoA contains a *cis-Δ^3 bond*, then *cis-Δ^3*-Enoyl CoA isomerase will convert the bond to a *trans-Δ^2* bond, which is a regular substrate.
- If the acyl CoA contains a *cis-Δ^4 double bond*, then its dehydrogenation yields a 2,4-dienoyl intermediate, which is not a substrate for enoyl CoA hydratase. However, the enzyme 2,4 Dienoyl CoA reductase reduces the intermediate, using NADPH, into *trans-Δ^3*-enoyl CoA. As in the above case, this compound is converted into a suitable intermediate by 3,2-Enoyl CoA isomerase.



Oxidation of Odd-chain Fatty Acids

Most naturally-occurring lipids contain fatty acids with an even number of carbon atoms, yet fatty acids with an odd number of carbon atoms are found in significant amounts in the lipids of many plants and some marine animals. Small quantities of C-3 propionate are added as a mould inhibitor to some breads and cereals, and thus propionate enters the human diet. Besides, cattle and other ruminants form large amounts of propionate during fermentation of

carbohydrates in the rumen. The propionate so formed is absorbed into the blood and oxidized by the liver and other tissues.



The odd-carbon long-chain fatty acids are oxidized by the same pathway as the even-carbon fattty acids, starting at the carboxyl end of the chain. However, the substrate for the last pass through the β oxidation cycle is a fatty acyl-CoA, in which the fatty acid has 5 carbon atoms. When this is oxidized and finally cleaved, the products are acetyl-CoA and propionyl CoA, rather than 2 moles of acetyl-CoA produced in the normal β oxidation cycle. The acetyl-CoA is, of course, oxidized via the citric acid cycle but the oxidation of propionylCoA presents an interesting problem, since at first glance the propionic acid (or propionylCoA) appears to be a substrate unsuitable for β oxidation. However, the substrate is held by two strikingly dissimilar pathways: methylmalonate pathway and β-hydroxy-propionate pathway

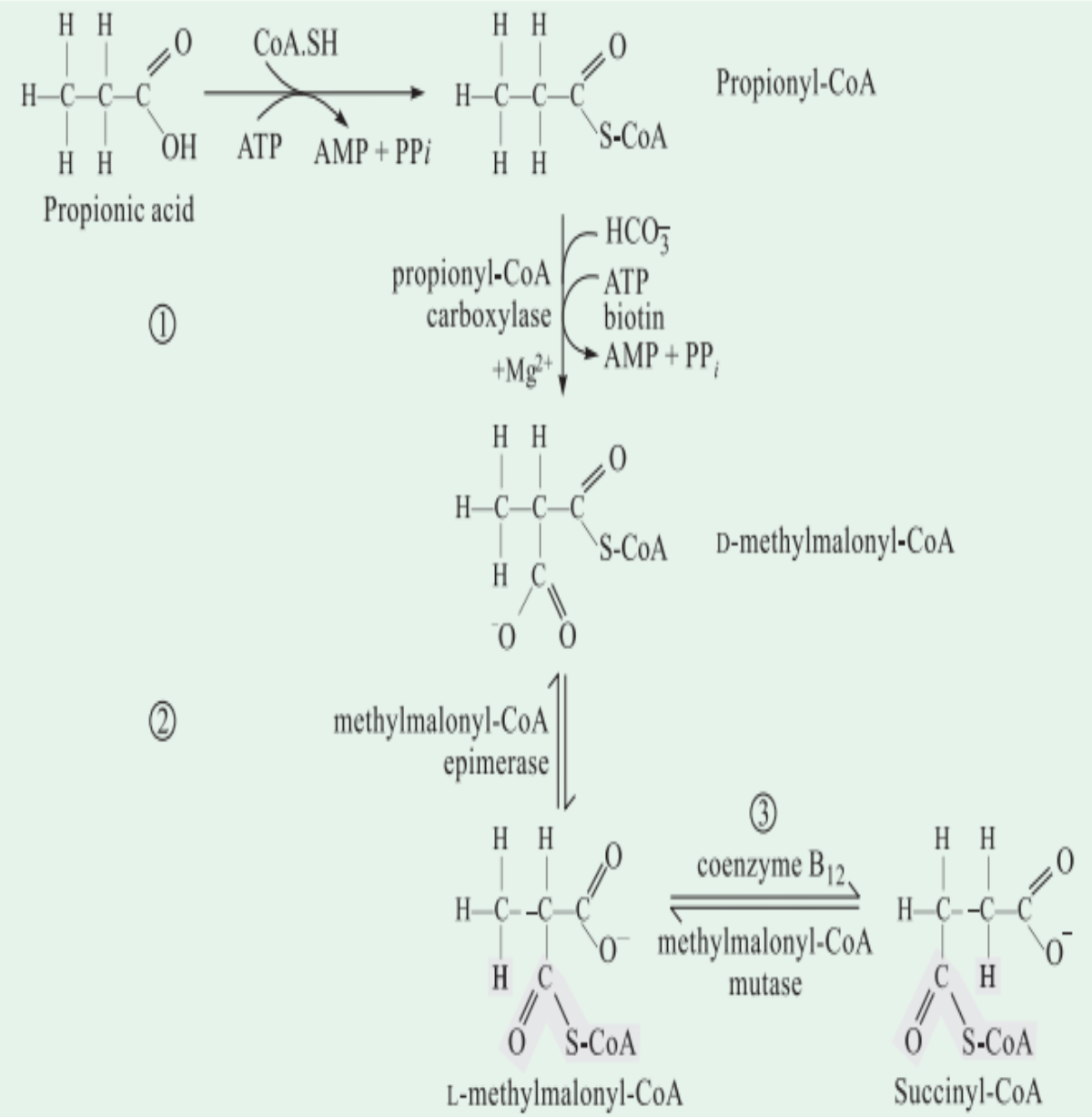
(a) Methylmalonate Pathway

This pathway is found only in animals and occurs in the mitochondria of liver, cardiac and skeletal muscles, kidney and other tissues. Propionate (or propionyl-CoA) is also produced

by the oxidation of isoleucine, valine, methionine and threonine. Propionate is catalyzed by acetylCoA synthetase to produce propionyl-CoA. The propionyl-CoA is carboxylated to form the D stereoisomer of methylmalonyl-CoA by an enzyme propionyl-CoA carboxylase, which contains the cofactor biotin. In this reaction, as in pyruvate carboxylase reaction, the CO_2 (or its hydrated ion, HCO_3^-) is activated by attachment to biotin before its transfer to the propionate moiety. The formation of the carboxybiotin intermediate requires energy, which is provided by the cleavage of ATP to AMP and PPi. The dmethylmalonyl-CoA, thus formed, is enzymatically epimerized to L-methylmalonyl-CoA, by the action of methylmalonyl-CoA epimerase (The epimerase labilizes the α-hydrogen atom, followed by uptake of a proton from the medium, thus catalyzing interconversion of D- and L-methylmalonylCoA). The L-methylmalonyl-CoA undergoes an intramolecular rearrangement to form succinylCoA by the enzyme methylmalonyl-CoA mutase, which requires as its coenzyme deoxyadenosyl-cobalamin or coenzyme B12. When [2–^{14}C] methyl-malonyl-CoA was converted by the mutase enzyme, the label (marked by an asterisk, below) was found in the 3 position of succinyl-CoA, thus indicating an intramolecular transfer of the entire thioester group, –CO–S– CoA, rather than migration of the carboxyl carbon.

The role of the coenzyme B12 is to remove a hydrogen from one carbon atom by transferring it directly to an adjacent carbon atom, simultaneously effecting the exchange of a second (R) substituent. The H and R are not released into solution.

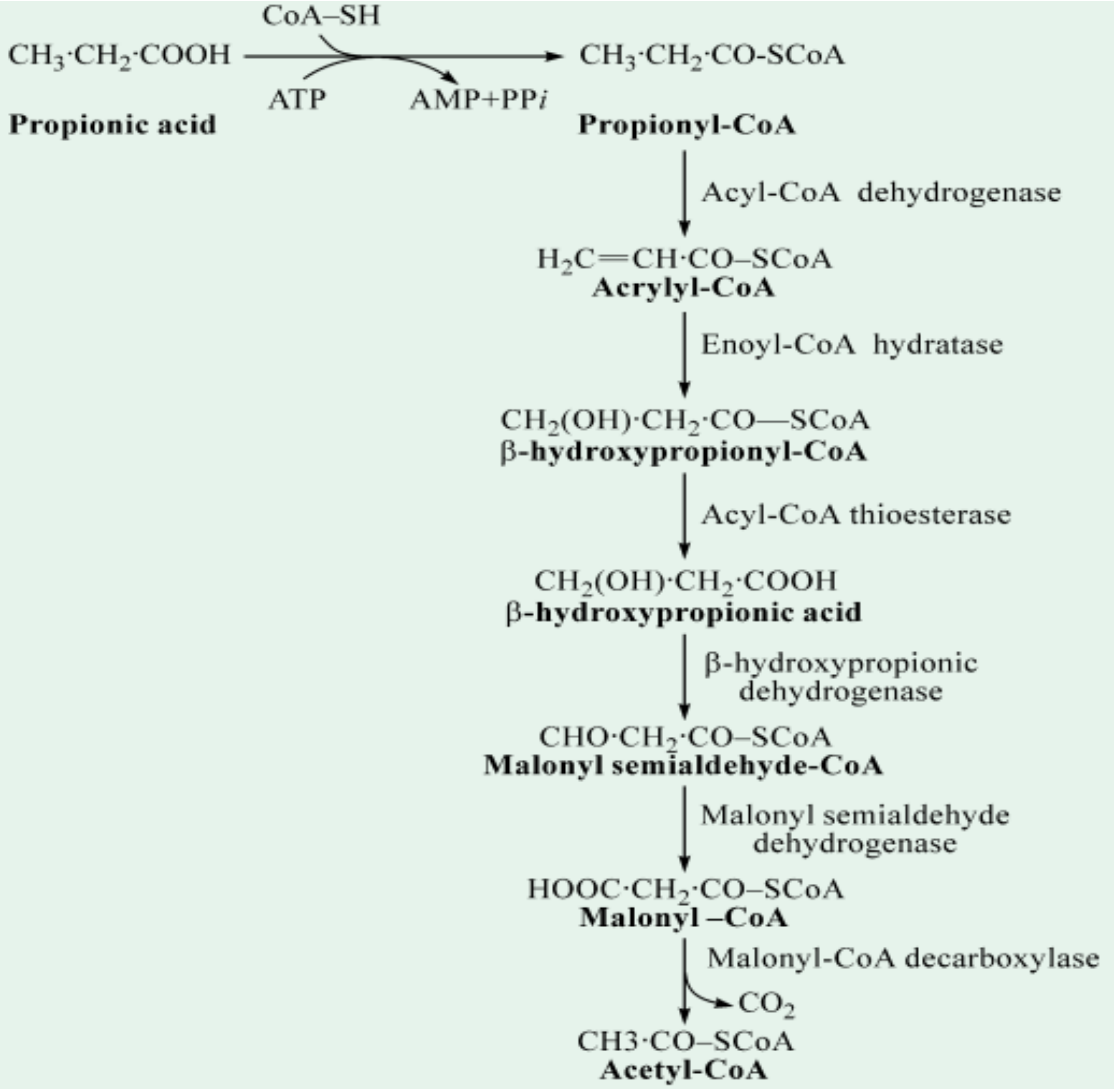
At equilibrium, formation of succinyl-CoA favoured by a ratio of 20: 1 over methylmalonyl-CoA. The succinyl-CoA can then be oxidized via succinate and the citric acid cycle to CO_2 and H_2O. In patients with vitamin B12 deficiency, both propionate and methylmalonate are excreted in the urine in abnormally large amounts. The odd-chain fatty acids are only a small fraction of the total, and only the terminal 3 carbons appear as propionyl-CoA. The metabolism of propionyl-CoA is, therefore, not of quantitative significance in fatty acid oxidation.



(b) β-hydroxypropionate Pathway

This pathway is ubiquitous in plants and is a modified form of β oxidation scheme. It nicely resolves the problem of how plants can cope with propionic acid by a system not involving vitamin B12 as cobamide coenzyme. Since plants have no B12 functional enzymes, the methylmalonate pathway does not operate in them. This pathway, thus, bypasses the B12

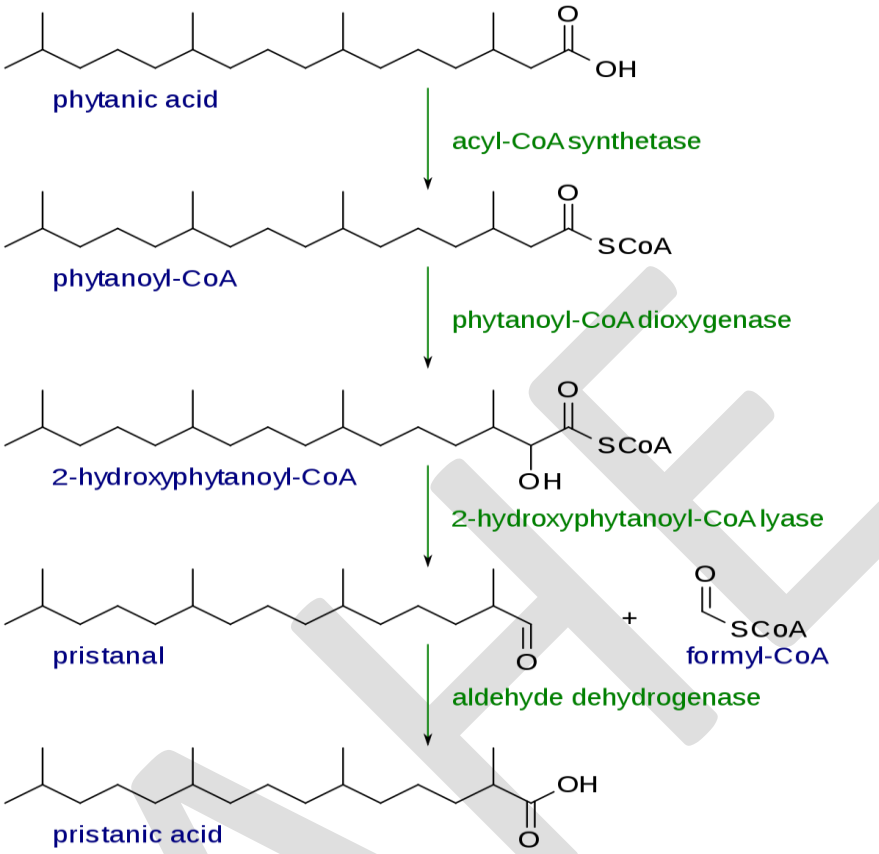
barrier in an effective way.



Oxidation of branched chain fatty acids

Fatty acid degradation in most organisms occurs primarily via the beta-oxidation cycle. In mammals, beta-oxidation occurs in both mitochondria and peroxisomes, whereas plants and most fungi harbor the beta-oxidation cycle only in the peroxisomes. Although the fatty acid oxidation scheme works neatly for even- numbered chain lengths, it can't work completely for fatty acids that contain an odd number of carbons or branched chain fatty acids such as phytanic acid. Beta-oxidation of these compounds leads to propionyl-CoA and acetyl-CoA, rather than to two acetyl-CoA at the final step. The propionyl-CoA is not a substrate for the TCA cycle or other simple

pathways. For example, phytanic acid, found in animal milk, can't be oxidized directly by beta-oxidation because the addition of water is a problem at the branched beta-carbon. The first step in the digestion of this compound is the oxidation of the carbon by molecular oxygen. Then the original carboxyl group is removed as CO2, leaving a shorter chain. This chain can now be accommodated by the beta-oxidation reactions, because the new beta-carbon now lacks a methyl group. In mitochondria, the beta-oxidation pathway includes four reactions that occur in repeating cycles with each fatty acid molecule. In each cycle, a fatty acid is progressively shortened by two carbons as it is oxidized and its energy captured by the reduced energy carriers NADH and FADH2. At the end of each cycle of four reactions, one acetyl-CoA two-carbon unit is released from the end of the fatty acid, which then goes through another round of beta-oxidation, continuing to oxidize and shorten even-chain fatty acids until they are entirely converted to acetyl-CoA. The acetyl-CoA generated in beta-oxidation enters the TCA cycle, where it is further oxidized to CO2, producing more reduced energy carriers, NADH and FADH2. These carriers produced in the TCA cycle, along with those produced directly in beta-oxidation, transfer their energy to the electron transport chain where they drive the creation of the proton gradient that supports mitochondrial ATP production. Another destination of acetyl-CoA is the production of ketone bodies by the liver that are transported to tissues like the heart and brain for energy.



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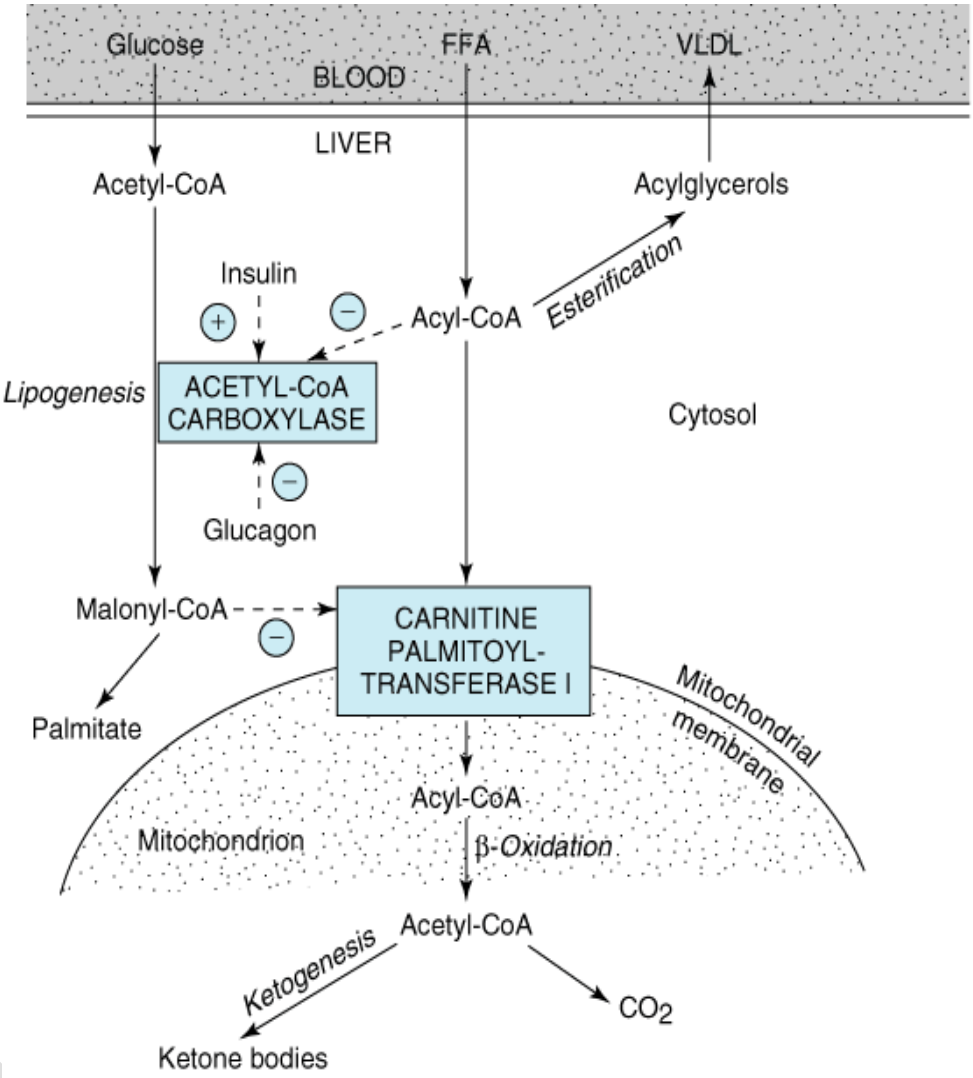
REGULATION OF FATTY ACID OXIDATION

A reciprocal control mechanism for hepatic fatty acid synthesis and oxidation has been discovered. The level of malonyl-CoA determines the rate of fatty acid synthesis, whereas the activity of carnitine acyltransferase determines the rate of fatty acid oxidation. Since malonyl CoA inhibits carnitine acyltransferase, a high rate of fatty acid synthesis results in a low rate of fatty acid oxidation, and vice versa.


Glucose
FFA
VLDL
BLOOD
LIVER
Acetyl-CoA
Acylglycerols
Insulin
Acyl-CoA
Esterification
Lipogenesis
ACETYL-CoA CARBOXYLASE
Cytosol
Glucagon
Malonyl-CoA
CARNITINE PALMITOYL-TRANSFERASE I
Palmitate
Mitochondrial membrane
Acyl-CoA
Mitochondrion
β-Oxidation
Acetyl-CoA
Ketogenesis
CO_2
Ketone bodies

- There is regulation at the level of entry of fatty acids into the oxidative pathway by **carnitine palmitoyl transferase-I** (CPT-I), CPT-I activity is low in the fed state, leading to depression of fatty acid oxidation, and high in starvation, allowing fatty acid oxidation to increase.
- Malonyl-CoA, the initial intermediate in fatty acid biosynthesis (Figure-4), formed by acetyl-CoA carboxylase in the fed state, is a potent inhibitor of CPT-I. Under these conditions, free fatty acids enter the liver cell in low concentrations and are nearly all esterified to acylglycerols and transported out of the liver in very low density lipoproteins (VLDL).
- However, as the concentration of free fatty acids increases with the onset of starvation, acetyl-CoA carboxylase is inhibited directly by acyl-CoA, and [malonyl-CoA] decreases, releasing the inhibition of CPT-I and allowing more acyl-CoA to be -oxidized.
- These events are reinforced in starvation by decrease in the **[insulin]/[glucagon] ratio.**
- Thus, β -oxidation from free fatty acids is controlled by the CPT-I gateway into the mitochondria, and the balance of the free fatty acid uptake not oxidized is esterified.

PEROXISOMAL OXIDATION

Fatty acid oxidation also occurs in peroxisomes when the fatty acid chains are too long to be handled by the mitochondria. The same enzymes are used in peroxisomes as in the mitochondrial matrix, and acetyl-CoA is generated. It is believed that very long chain (greater than C-22) fatty acids, branched fatty acids, some prostaglandins and leukotrienes undergo initial oxidation in peroxisomes until octanoyl-CoA is formed, at which point it undergoes mitochondrial oxidation.

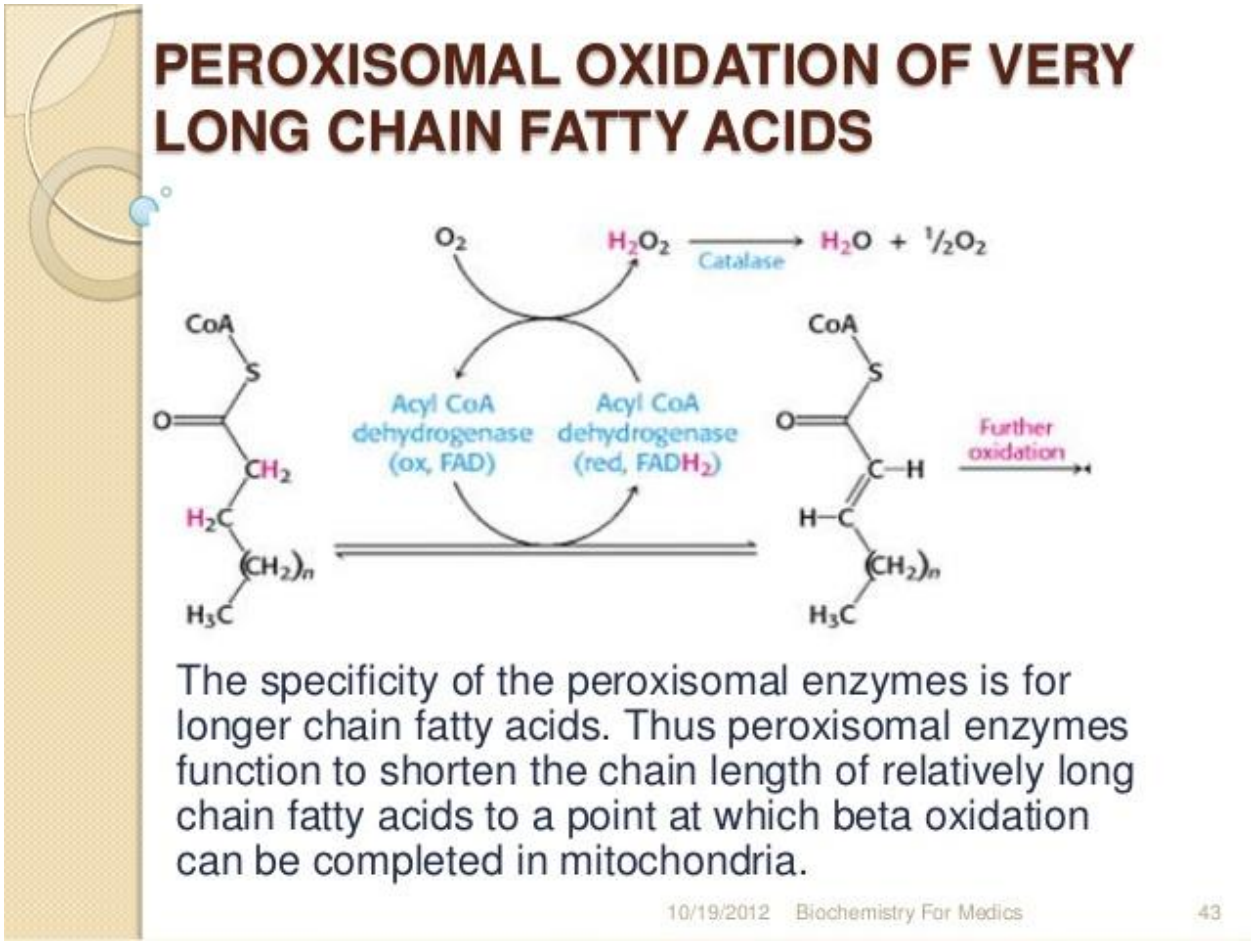
One significant difference is that oxidation in peroxisomes is not coupled to ATP synthesis. Instead, the high-potential electrons are transferred to O_2, which yields H_2O_2. It does generate heat however. The enzyme catalase, found exclusively in peroxisomes, converts the hydrogen peroxide into water and oxygen.

Peroxisomal β-oxidation also requires enzymes specific to the peroxisome and to very long fatty acids. There are three key differences between the enzymes used for mitochondrial and peroxisomal β-oxidation:

1. The NADH formed in the third oxidative step cannot be reoxidized in the peroxisome, so reducing equivalents are exported to the cytosol.

2. β-oxidation in the peroxisome requires the use of a peroxisomal carnitine acyltransferase (instead of carnitine acyltransferase I and II used by the mitochondria) for transport of the activated acyl group into the mitochondria for further breakdown.
3. The first oxidation step in the peroxisome is catalyzed by the enzyme acyl-CoA oxidase.
4. The β-ketothiolase used in peroxisomal β-oxidation has an altered substrate specificity, different from the mitochondrial β-ketothiolase.

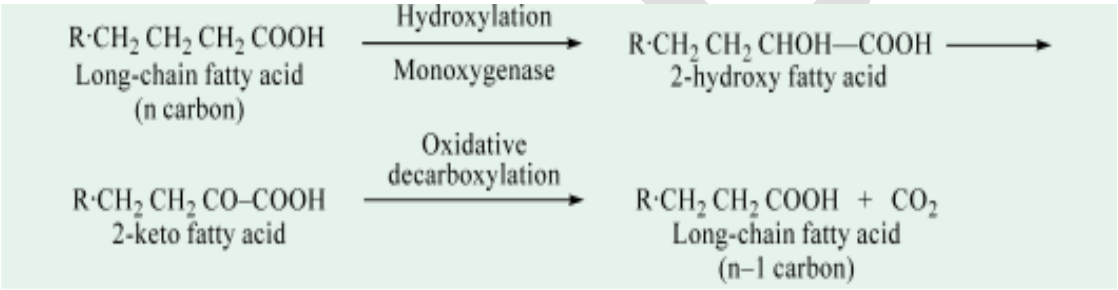
Peroxisomal oxidation is induced by a high-fat diet and administration of hypolipidemic drugs like clofibrate.



α-Oxidation of fatty acids

Although β oxidation is major pathway for the oxidation of fatty acids, two other types of oxidation also occur, α and ω oxidation. α oxidation is the removal of one carbon atom (i.e., α

carbon) at a time from the carboxyl end of the molecule. α oxidation was first observed in seeds and leaf tissues of plants. α oxidation of long-chain fatty acids to 2-hydroxy acids and then to fatty acids with one carbon atom less than the original substrate have been demonstrated in the microsomes of brain and other tissues also. Long-chain α hydroxy fatty acids are constituents of brain lipids, e.g., the C_{24} cerebronic acid (= 2 hydroxylignoceric acid), $CH_3 (CH_2)_{21}$. CH(OH). COOH. These hydroxy fatty acids can be converted to the 2-keto acids, followed by oxidative decarboxylation, resulting in the formation of long-chain fatty acids with an odd number of carbon atoms:



The initial hydroxylation reaction is catalyzed by a mitochondrial enzyme, monoxygenase that requires O_2, Mg^{2+}, NADPH and a heat-stable cofactor. Conversion of the α hydroxy fatty acid to CO_2 and the next lower unsubstituted acid appears to occur in the endoplasmic reticulum and to require O_2, Fe^{2+} and ascorbate.

The salient features of α oxidation are as follows:

1. Only free long-chain fatty acids serve as substrates.
2. Molecular oxygen is indirectly involved.
3. It does not require CoA intermediates.
4. It does not lead to generation of high-energy phosphates.

This mechanism explains the occurrence of α hydroxy fatty acids and of odd-numbered fatty acids in the biomolecules. The latter may, in nature, also be synthesized de novo from propionate. The α oxidation system plays a key role in the capacity of mammalian tissues to oxidize phytanic acid (= 3,7,11,15-tetramethylhexadecanate). Phytanic acid is an oxidation product of phytol and is present in animal fat, cow's milk and foods derived from milk. The phytol presumably originates from plant sources, as it is a substituent of chlorophyll and the side

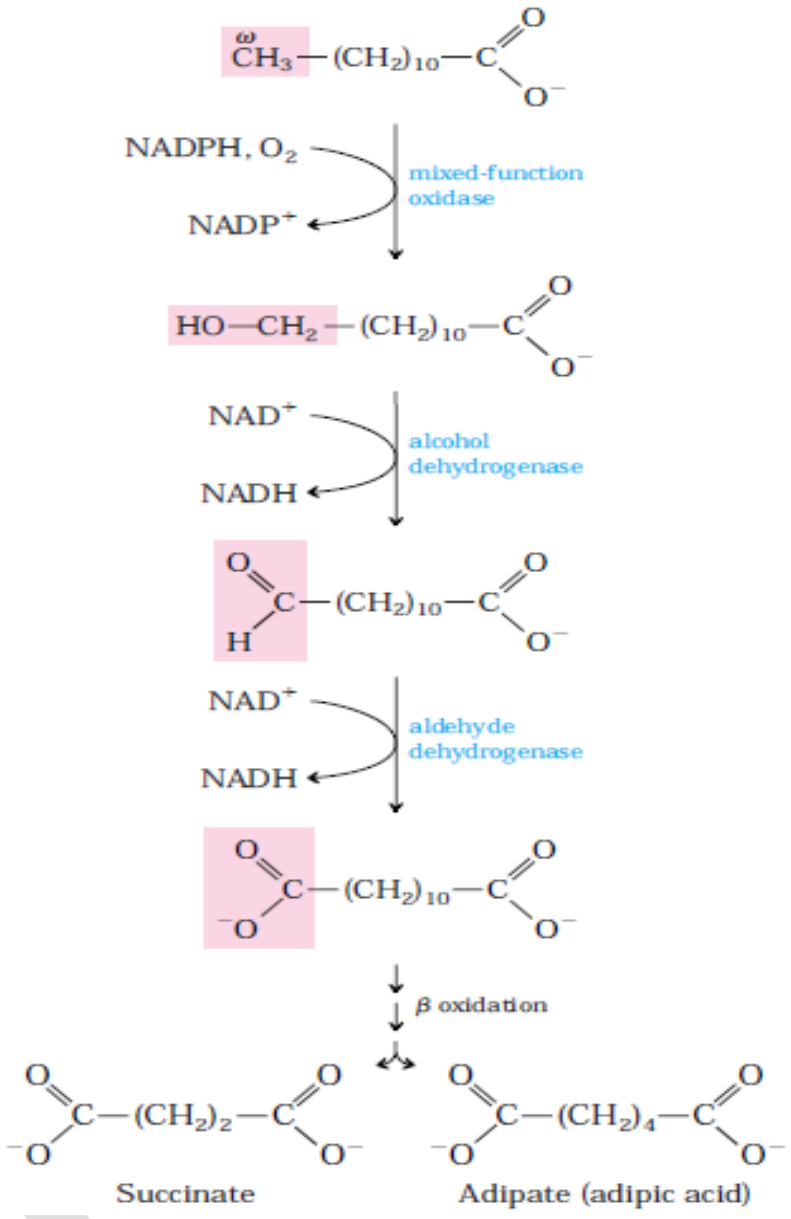
chain of vitamin K_2. Normally, phytanic acid is rarely found in serum lipids because of the ability of normal tissue to degrade (or oxidize) the acid very rapidly. But large amounts of phytanic acid accumulate (as much as 20% of the serum fatty acids and 50% of the hepatic fatty acids) in the tissues and serum of individuals with Refsum's disease, a rare inheritable autosomal recessive disorder affecting the nervous system because of an inability to oxidize this acid. Diets low in animal fat and milk products appear to relieve some of the symptoms of Refsum's disease. The presence of 3-methyl group in phytanic acid blocks β oxidation. In the mitochondria of normal individuals, α hydroxylation of phytanic acid by phytanate α hydroxylase is followed by oxidation by phytanate α oxidase to yield CO_2 and pristanic acid (= 2,6,10, 14-tetramethylpentadecanoic acid), which readily undergoes β oxidation after conversion to its CoA derivative. In Refsum's disease, there is a lack of the enzyme, phytanate α hydroxylase.

ω- Oxidation of fatty acids

Although mitochondrial β oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of the ω (omega) carbon the carbon most distant from the carboxyl group. The enzymes unique to ω oxidation are located (in vertebrates) in the endoplasmic reticulum of liver and kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms. In mammals ω oxidation is normally a minor pathway for fatty acid degradation, but when β oxidation is defective (because of mutation or a carnitine deficiency, for example) it becomes more important.

The first step introduces a hydroxyl group onto the ω carbon. The oxygen for this group comes from molecular oxygen (O_2) in a complex reaction that involves cytochrome P450 and the electron donor NADPH. Reactions of this type are catalyzed by mixedfunction oxidases, described in Box 21–1. Two more enzymes now act on the ω carbon: alcohol dehydrogenase oxidizes the hydroxyl group to an aldehyde, and aldehyde dehydrogenase oxidizes the aldehyde group to a carboxylic acid, producing a fatty acid with a carboxyl group at each end. At this point, either end can be attached to coenzyme A, and the molecule can en ter the mitochondrion and undergo β oxidation by the normal route. In each pass through the β -oxidation pathway, the "double-ended" fatty acid yields dicarboxylic acids such as succinic acid, which can enter the

citric acid cycle, and adipic acid



KETONE BODIES METABOLISM

Ketone body metabolism includes ketone body synthesis (ketogenesis) and breakdown (ketolysis). When the body goes from the fed to the fasted state the liver switches from an organ of carbohydrate utilization and fatty acid synthesis to one of fatty acid oxidationand

ketone body production. This metabolic switch is amplified in uncontrolled diabetes. In these states the fat-derived energy (ketone bodies) generated in the liver enter the blood stream and are used by other organs, such as the brain, heart, kidney cortex and skeletal muscle. Ketone bodies are particularly important for the brain which has no other substantial non-glucose-derived energy source. The two main ketone bodies are acetoacetate (AcAc) and 3-hydroxybutyrate (3HB) also referred to as β-hydroxybutyrate, with acetone the third, and least abundant. Ketone bodies are always present in the blood and their levels increase during fasting and prolonged exercise. After an over-night fast, ketone bodies supply 2–6% of the body's energy requirements, while they supply 30–40% of the energy needs after a 3-day fast. When they build up in the blood they spill over into the urine. The presence of elevated ketone bodies in the blood is termed ketosis and the presence of ketone bodies in the urine is called ketonuria. The body can also rid itself of acetone through the lungs which gives the breath a fruity odour. Diabetes is the most common pathological cause of elevated blood ketones. In diabetic ketoacidosis, high levels of ketone bodies are produced in response to low insulin levels and high levels of counter-regulatory hormones.

KETONE BODIES

The term ketone bodies refers to three molecules, acetoacetate (AcAc), 3-hydroxybutyrate (3HB) and acetone (Figure). 3HB is formed from the reduction of AcAc in the mitochondria. These two predominant ketone bodies are energy-rich compounds that transport energy from the liver to other body tissues. Acetone is a minor product, generated by spontaneous decarboxylation of AcAc and is responsible for the sweet odor on the breath of individuals with ketoacidosis.

Ketone bodies are present in small amounts in the blood of healthy individuals during fasting or prolonged exercise and play a key role in sparing glucose utilization and reducing proteolysis. Unlike most other tissues, the brain cannot utilize fatty acids for energy when blood glucose levels become compromised. In this case, ketone bodies provide the brain with an alternative source of energy, amounting to nearly 2/3 of the brain's energy needs during periods of prolonged fasting and starvation.

Abnormally large quantities of ketone bodies are found in the blood of individuals who are experiencing diabetic ketoacidosis, alcoholic ketoacidosis, salicylate poisoning, and other rare conditions. Ketone bodies stimulate insulin release *in vitro*, generate oxygen radicals and cause lipid peroxidation. Lipid peroxidation and the generation of oxygen radicals may play a role in vascular disease in diabetes[.

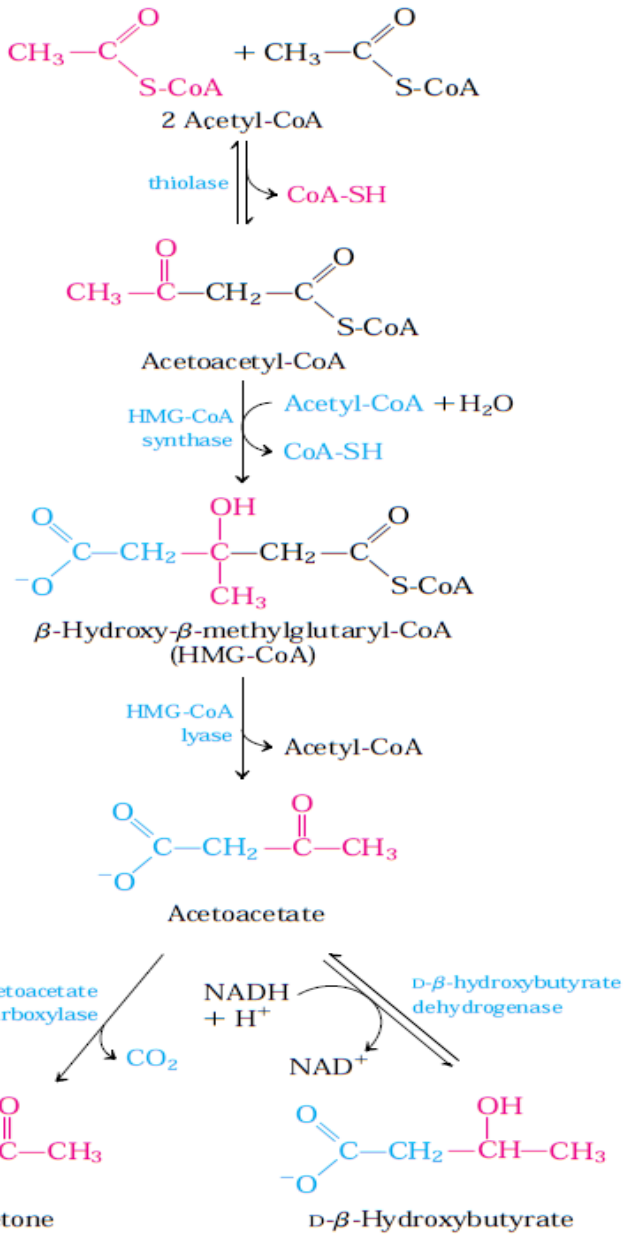
Ketogenesis

Ketogenesis is the process by which fatty acids are transformed into acetoacetate (AcAc) and 3-hydroxybutyrate (3HB) This process takes place in the liver in specialized organelles called mitochondria

The first step in the formation of acetoacetate, occurring in the liver, is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase; this is simply the reversal of the last step of β-oxidation. The acetoacetyl-CoA then condenses with acetyl-CoA to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA), which is cleaved to free acetoacetate and acetyl-CoA. The acetoacetate is reversibly reduced by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-β-hydroxybutyrate. This enzyme is specific for the D stereoisomer; it does not act on L-β-hydroxyacyl-CoAs and is not to be confused with L-β-hydroxyacyl-CoA

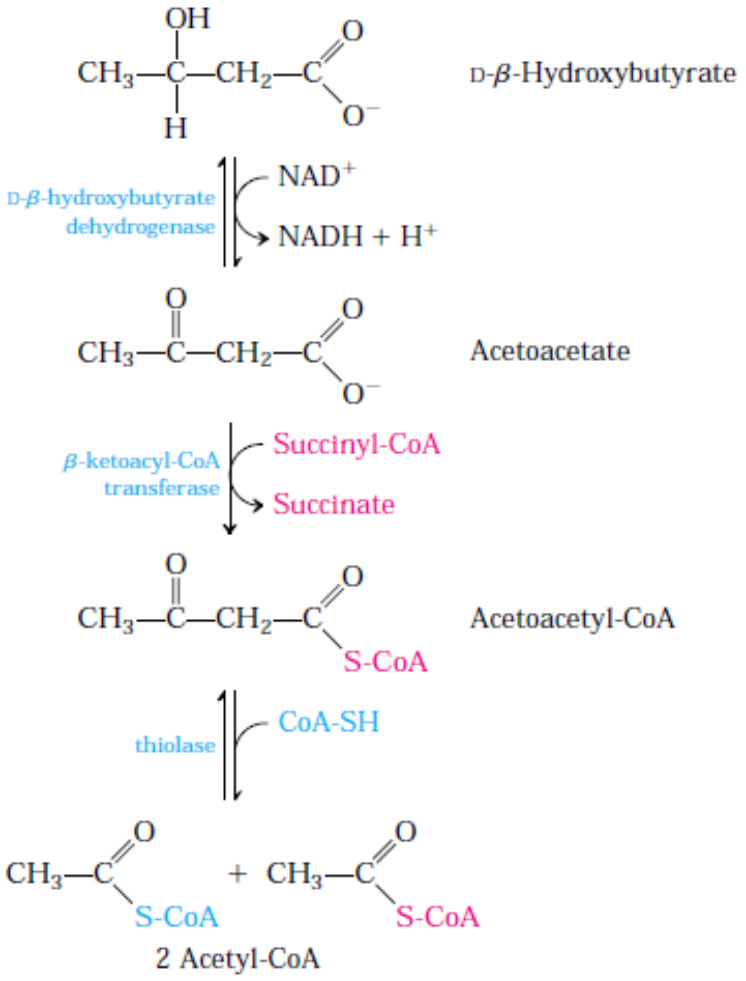
dehydrogenase of the β-oxidation pathway.

In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of acetoacetate decarboxylase. Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic. Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.



In extrahepatic tissues, D-β-hydroxybutyrate is oxidized to acetoacetate by D-β-hydroxybutyrate dehydrogenase. The acetoacetate is activated to its coenzyme A ester by transfer of CoA from succinyl- CoA, an intermediate of the citric acid cycle, in a reaction catalyzed by β-ketoacyl-CoA transferase. The acetoacetyl-CoA is then cleaved by thiolase to yield two acetyl-CoAs, which enter the citric acid cycle. Thus the ketone bodies are used as

fuels. The production and export of ketone bodies by the liver allow continued oxidation of fatty acids with only minimal oxidation of acetyl-CoA. When intermediates of the citric acid cycle are being siphoned off for glucose synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slows and so does acetyl-CoA oxidation. Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl- CoA, β-oxidation slows for want of the free coenzyme. The production and export of ketone bodies free coenzyme A, allowing continued fatty acid oxidation.

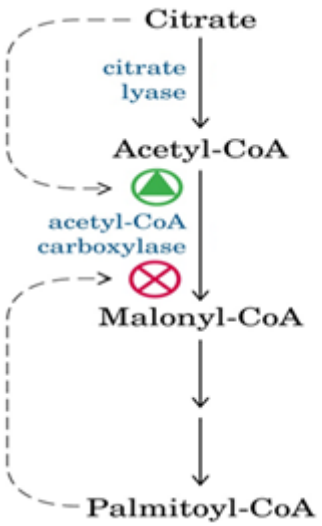


Ketone Bodies Are Overproduced in Diabetes and during Starvation

Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid

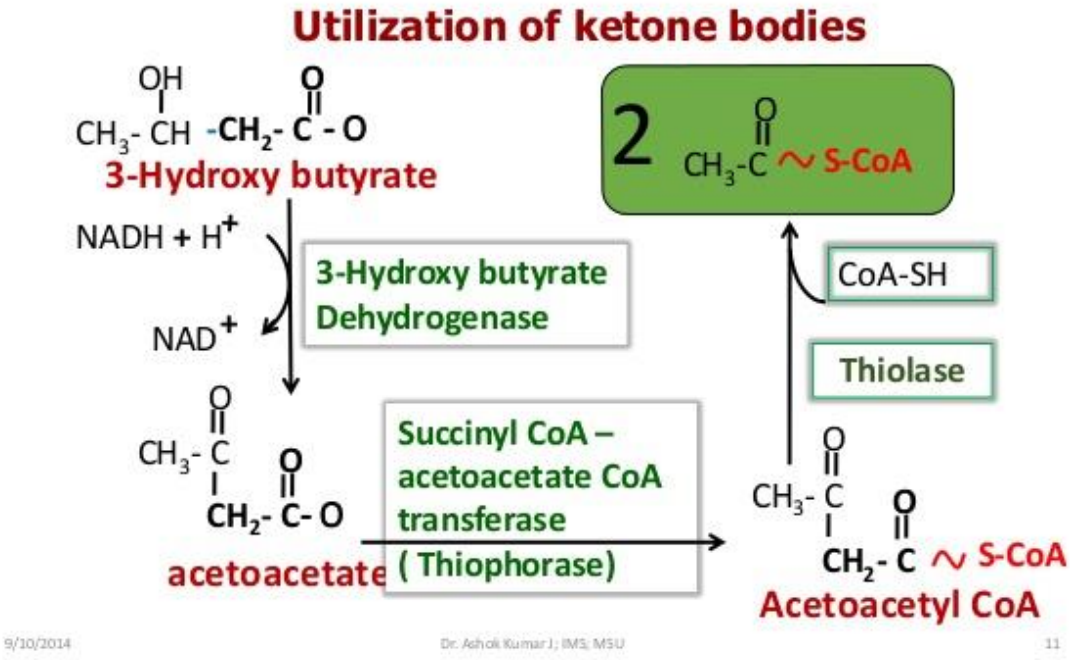
cycle intermediates, diverting acetyl-CoA to ketone body production. In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat. Under these conditions, levels of malonyl- CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to acetyl- CoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis. The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them. The increased blood levels of acetoacetate and D-β-hydroxybutyrate lower the blood pH, causing the condition known as acidosis. Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of untreated diabetics can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of < 3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of ≤ 125 mg/ 24 hr). This condition is called ketosis. Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis).

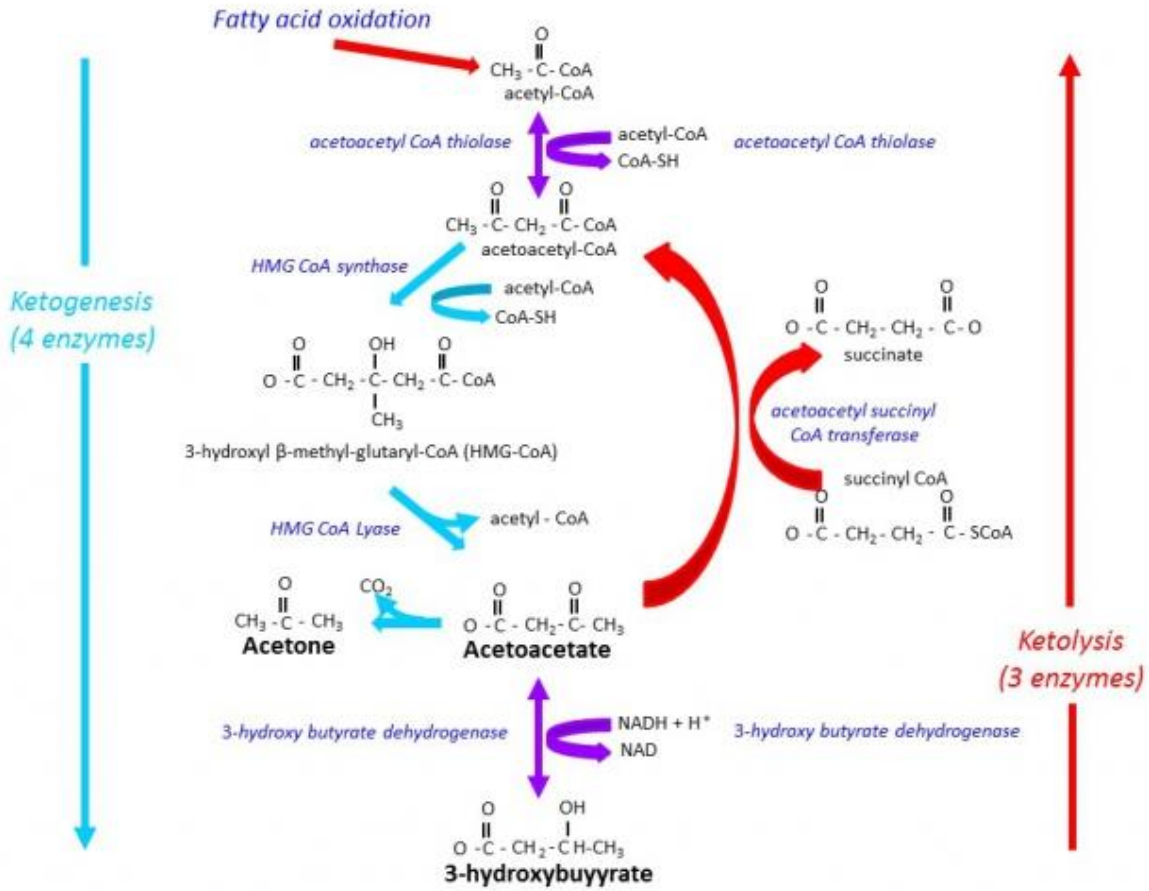
Fatty acid synthesis regulation



Ketolysis

Ketolysis is the process by which ketone bodies produced in the **liver** are converted (in **non-liver tissues**), into acetyl CoA which, on complete oxidation via the tricarboxylic acid cycle and oxidative phosphorylation, provides energy for various intracellular metabolic activities (Figure 3). Ketolysis occurs in the mitochondria of many extrahepatic organs. The central nervous system is particularly dependent on the delivery of ketone bodies produced in the liver for the process of ketolysis, since ketogenesis occurs very slowly if at all in the central nervous system[





KETOACIDOSIS

Ketoacidosis is a metabolic state associated with high concentrations of ketone bodies, formed by the breakdown of fatty acids and the deamination of amino acids. The two common ketones produced in humans are acetoacetic acid and β-hydroxybutyrate.

Ketoacidosis is a pathological metabolic state marked by extreme and uncontrolled ketosis. In ketoacidosis, the body fails to adequately regulate ketone production causing such a severe accumulation of keto acids that the pH of the blood is substantially decreased. In extreme cases ketoacidosis can be fatal.

Ketoacidosis is most common in untreated type 1 diabetes mellitus, when the liver breaks down fat and proteins in response to a perceived need for respiratory substrate. Prolonged alcoholism may lead to alcoholic ketoacidosis.

Ketoacidosis can be smelled on a person's breath. This is due to acetone, a direct by-product of the spontaneous decomposition of acetoacetic acid. It is often described as smelling like fruit or nail polish remover. Ketosis may also give off an odor, but the odor is usually more subtle due to lower concentrations of acetone.

Treatment consists most simply of correcting blood sugar and insulin levels, which will halt ketone production. If the severity of the case warrants more aggressive measures, intravenous sodium bicarbonate infusion can be given to raise blood pH back to an acceptable range. However, serious caution must be exercised with IV sodium bicarbonate to avoid the risk of equally life-threatening hypernatremia

Three common causes of ketoacidosis are alcohol, starvation, and diabetes, resulting in alcoholic ketoacidosis, starvation ketoacidosis, and diabetic ketoacidosis respectively

In diabetic ketoacidosis, a high concentration of ketone bodies is usually accompanied by insulin deficiency, hyperglycemia, and dehydration. Particularly in type 1 diabetics the lack of insulin in the bloodstream prevents glucose absorption, thereby inhibiting the production of oxaloacetate (a crucial molecule for processing Acetyl-CoA, the product of beta-oxidation of fatty acids, in the Krebs cycle) through reduced levels of pyruvate (a byproduct of glycolysis), and can cause unchecked ketone body production (through fatty acid metabolism) potentially leading to dangerous glucose and ketone levels in the blood. Hyperglycemia results in glucose overloading the kidneys and spilling into the urine (transport maximum for glucose is exceeded). Dehydration results following the osmotic movement of water into urine (Osmotic diuresis), exacerbating the acidosis.

BIOSYNTHESIS OF SATURATED FATTY ACIDS

- Glucose entering the TCA cycle is used for the biosynthesis of saturated fatty acids by converting TCA Cycle citrate to acetyl coenzyme~A (acetyl~CoA), and then malonyl~CoA, which is used to produce palmitate.
- The glycerol backbone of TGs comes from glycolytic glycerol-3-phosphate.
- Triglycerides are the primary lipid synthesized, and serve as a starting point for other lipids such as steroids and phospholipids.
- *Biosynthesis of Saturated Fatty acids* primarily occurs in hepatocyte cytoplasm.
- Acetyl CoA and NADPH are both necessary for Biosynthesis of Saturated Fatty acids.

Steps involved in Biosynthesis of Saturated Fatty acids

Acetyl Coenzyme A:

- Acetyl CoA is produced in the matrix of the mitochondria, but fatty acid biosynthesis occurs in the cytosol.
- Citrate synthase frees CoA from acetyl CoA and condenses acetate and oxaloacetate to citrate.
- Matrix membrane transporters for citrate move citrate to the cytosol, where it is acted upon by citrate lyase in the presence of CoA to re-form acetyl CoA and oxaloacetate.
- The oxaloacetate produced is converted to malate, and then to pyruvate, which is transported back to the mitochondrial matrix.
- The conversion of malate to pyruvate releases NADPH into the cytosol, which is necessary for fatty acid biosynthesis. (The hexose monophosphate shunt, pentose phosphate pathway, is the other major source for cytosolic NADPH.)

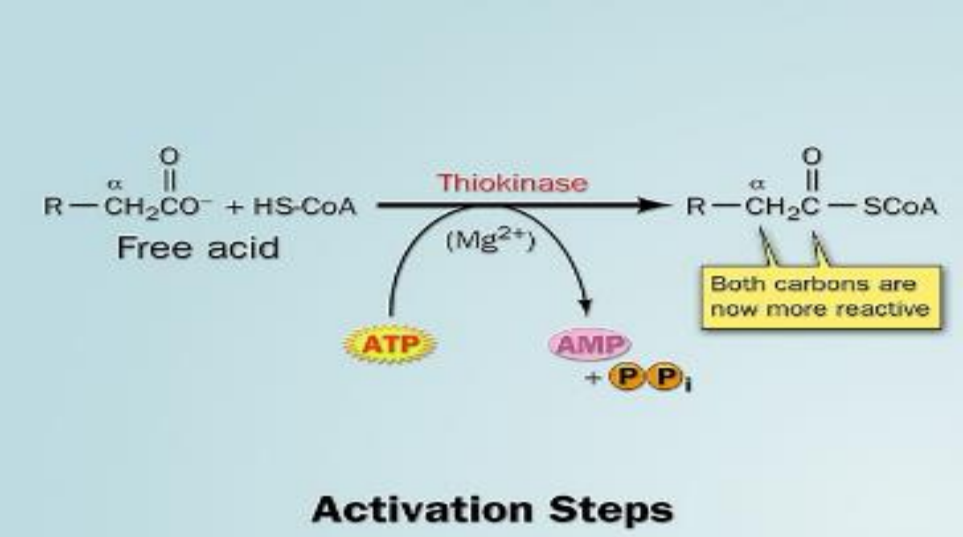
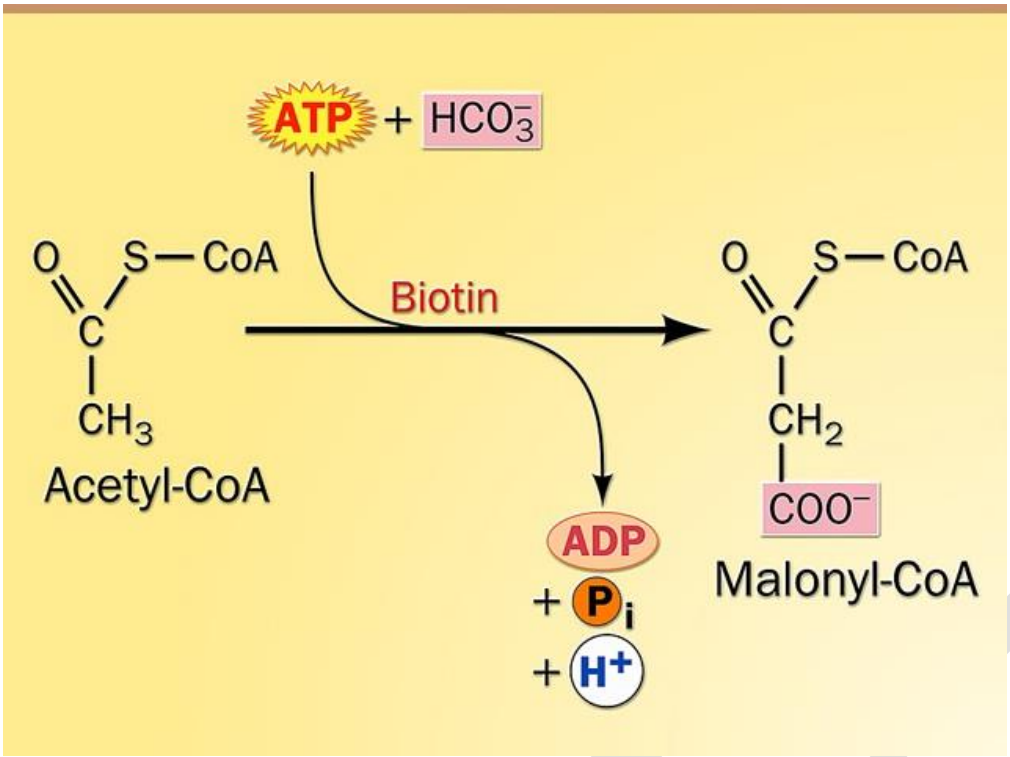


Fig: Synthesis of Malonyl Coenzyme A:

- Acetyl CoA, with the addition of CO_2, and with the hydrolysis of an ATP, is converted to malonyl CoA by acetyl CoA carboxylase (a biotin-dependent enzyme like all carboxylases).
- Acetyl CoA carboxylase (ACC) is, being the first enzyme in the fatty acid biosynthetic pathway, is a regulated enzyme.
- In the short term, allosteric activation by citrate, and allosteric inactivation by malonyl and palmitoyl CoAs, and covalent modification (phosphorylation and dephosphorylation) are the principal regulatory mechanisms.
- ACC is normally present as a tetrameric protomer (inactive form). The active form is the large polymer, which is favored by citrate binding and inhibited by malonyl and palmitoyl CoAs (products of the FA biosynthetic pathway).
- Phosphorylation is regulated by another mechanism, with glucagon and epinephrine activating PKA to phosphorylate (inactivate) ACC, and insulin activating phosphatase to re-activate the enzyme.
- The burden of long-term regulation is carried almost exclusively by up regulating the transcription of the enzyme itself.



Acetyl coA to Malonyl coA

Fatty Acid Synthase Complex (FASC) Dimer:

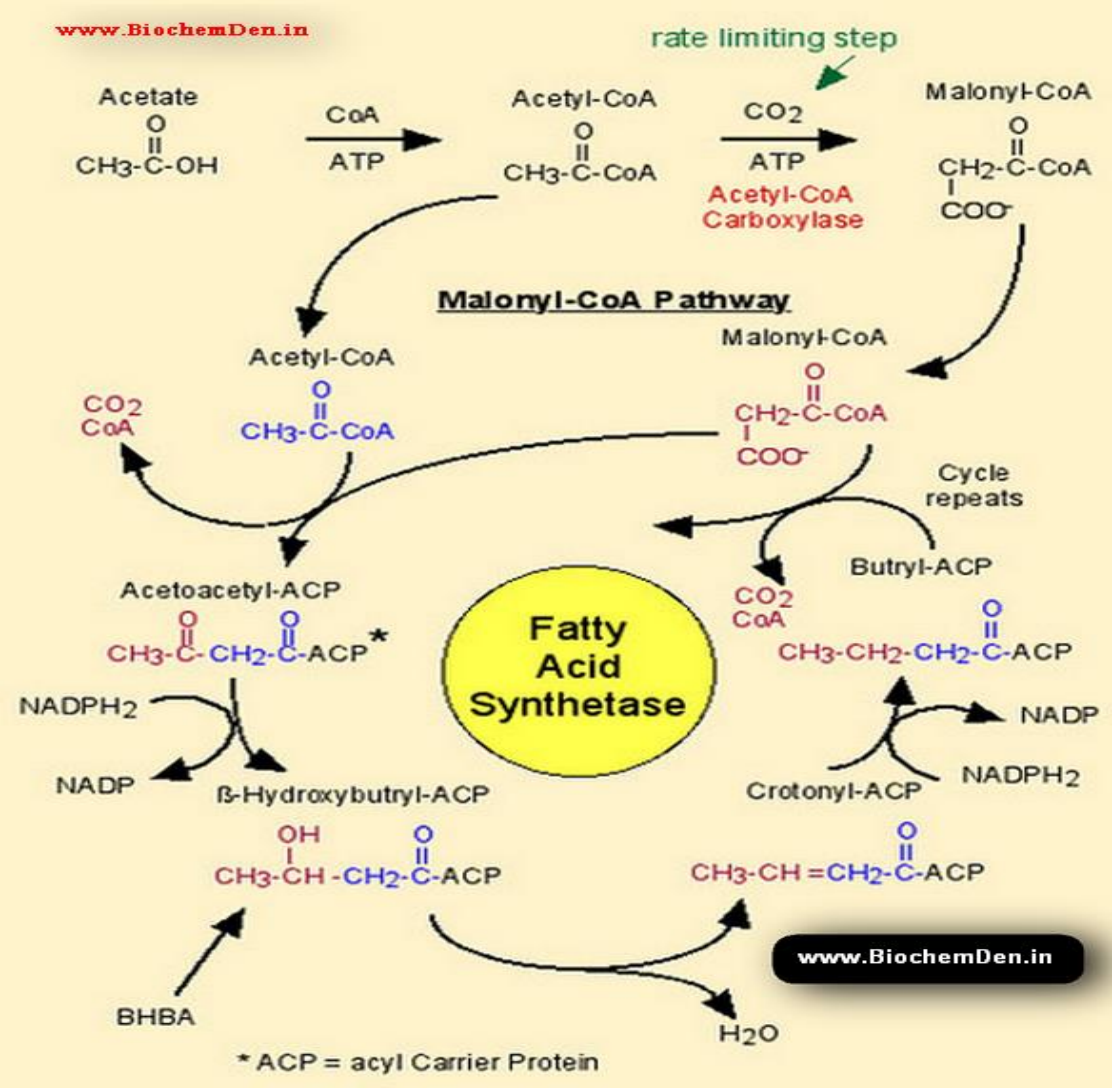
- Seven enzymes and a "carrier" protein: acetyl CoA-ACP transacylase, malonyl CoA-ACP transacylase, β-ketoacyl-ACP synthase (condensing enzyme), β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydratase, enoyl-ACP reductase, palmitoyl thioesterase, and acyl carrier protein (ACP) (containsephosphopentetheine)
- The sulfhydryl group of one ACP unit associates with the enoyl-ACP reductase (ER) subunit of another FASC complex, allowing dimerization of the protein.
- ACP assists in reactions by binding to substrate molecules, such as acetate (from acetyl CoA) and malonate (from malonyl CoA).
- Any time a fatty acid is used in a *biosynthetic reaction in the cell*, it must be in the form of a fatty acyl CoA.

Steps in Biosynthesis of Saturated Fatty acids:

1. Condensation:
    - Acetate (2C) and malonate (3C), as acetyl-ACP and malonyl-ACP

- Releases the non-ACP-bound carboxyl group of malonate as CO_2
- Produces β-acetoacetyl-ACP (4C)

2. Reduction:
   - Produces β-hydroxybutyryl-ACP (4C), with the oxidation of $NADPH_2$ to $NADP^+$
3. Dehydration:
   - Produces crotonyl-ACP (4C) with the release of water
4. Reduction:
   - Produces Butyryl-ACP with the oxidation of $NADPH_2$ to $NADP^+$
5. Repeat:
   - Butyryl-ACP then enters into reaction 1 in the place of malonyl-ACP, undergoing the addition of another two carbons from acetate.
   - The overall reaction uses 8 acetyl CoA, 14 NADPH, 14 H^+ and 1 malonyl CoA to produce a 16-carbon palmitic acid.



Fatty Acid Elongation:

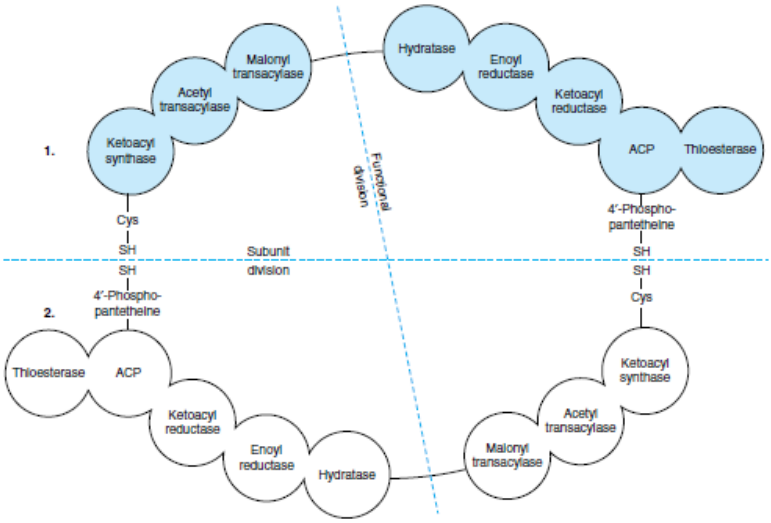
- FASC produces palmitic acid (16C).
- In the endoplasmic reticulum, two-carbon units can be added to palmitate from malonyl CoA.
- In the mitochondria, two-carbon units can be added to 8C fatty acids from acetyl CoA, but only to the extent of 14C fatty acids.

The Fatty Acid Synthase Complex Is a Polypeptide Containing Seven Enzyme Activities

In bacteria and plants, the individual enzymes of the fatty acid synthase system are separate, and the acyl radicals are found in combination with a protein called the acyl carrier

protein (ACP). However, in yeast, mammals, and birds, the synthase system is a multienzyme polypeptide complex that incorporates ACP, which takes over the role of CoA. It contains the vitamin pantothenic acid in the form of 4′-phosphopantetheine. The use of one multienzyme functional unit has the advantages of achieving the effect of compartmentalization of the process within the cell without the erection of permeability barriers, and synthesis of all enzymes in the complex is coordinated since it is encoded by a single gene.

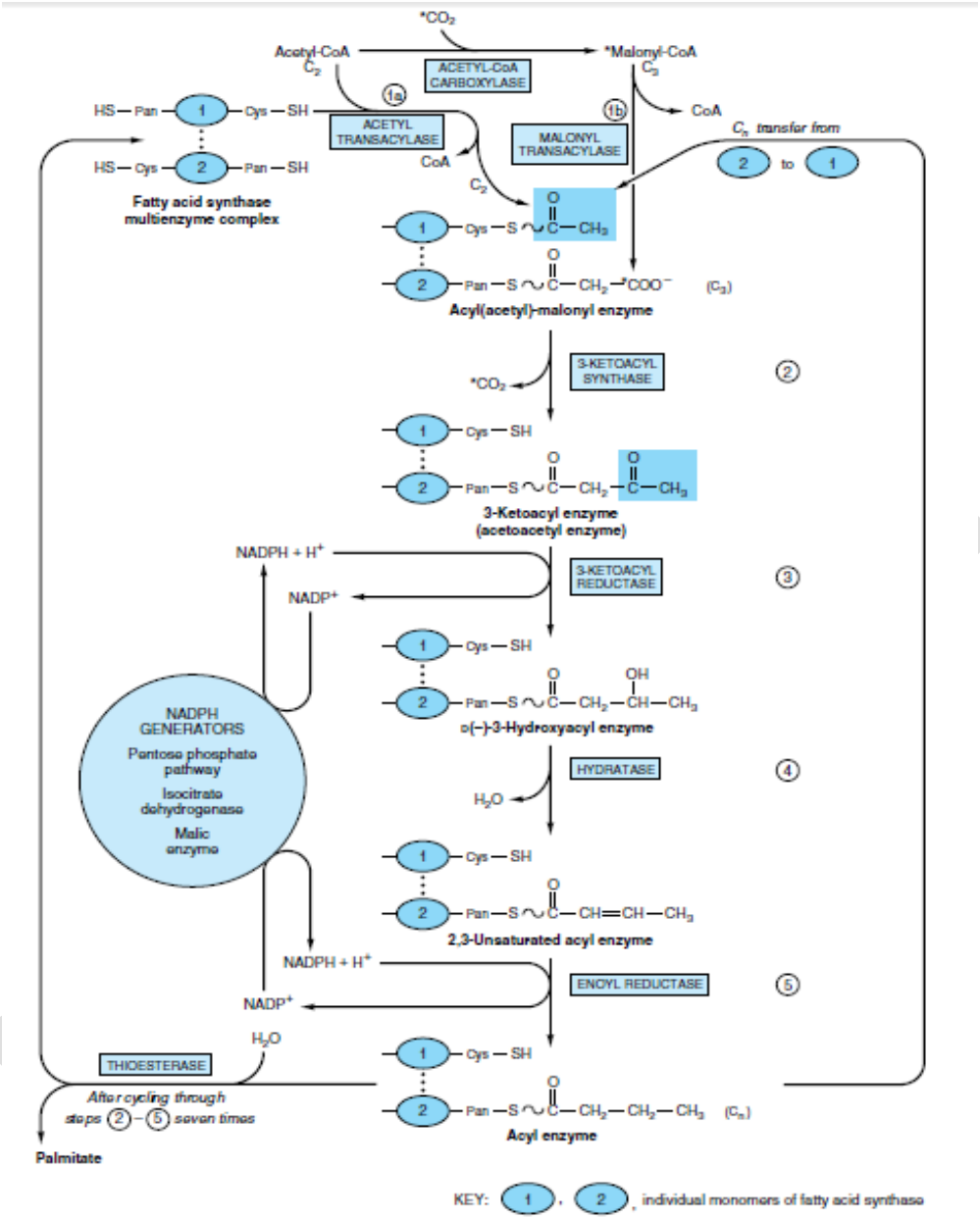
In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain.



Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers, 1 and 2, each consisting of seven enzyme activities and the acyl carrier protein (ACP). (Cys-SH, cysteine thiol.) The -SH of the 4′-phosphopantetheine of one monomer is in close proximity to the -SH of the cysteine residue of the ketoacyl synthase of the other monomer, suggesting a "head-to-tail" arrangement of the two monomers. Though each monomer contains all the partial activities of the reaction sequence, the actual functional unit consists of one-half of one monomer interacting with the complementary half of the other. Thus, two acyl chains are produced simultaneously. The sequence of the enzymes in each monomer is based on Wakil.

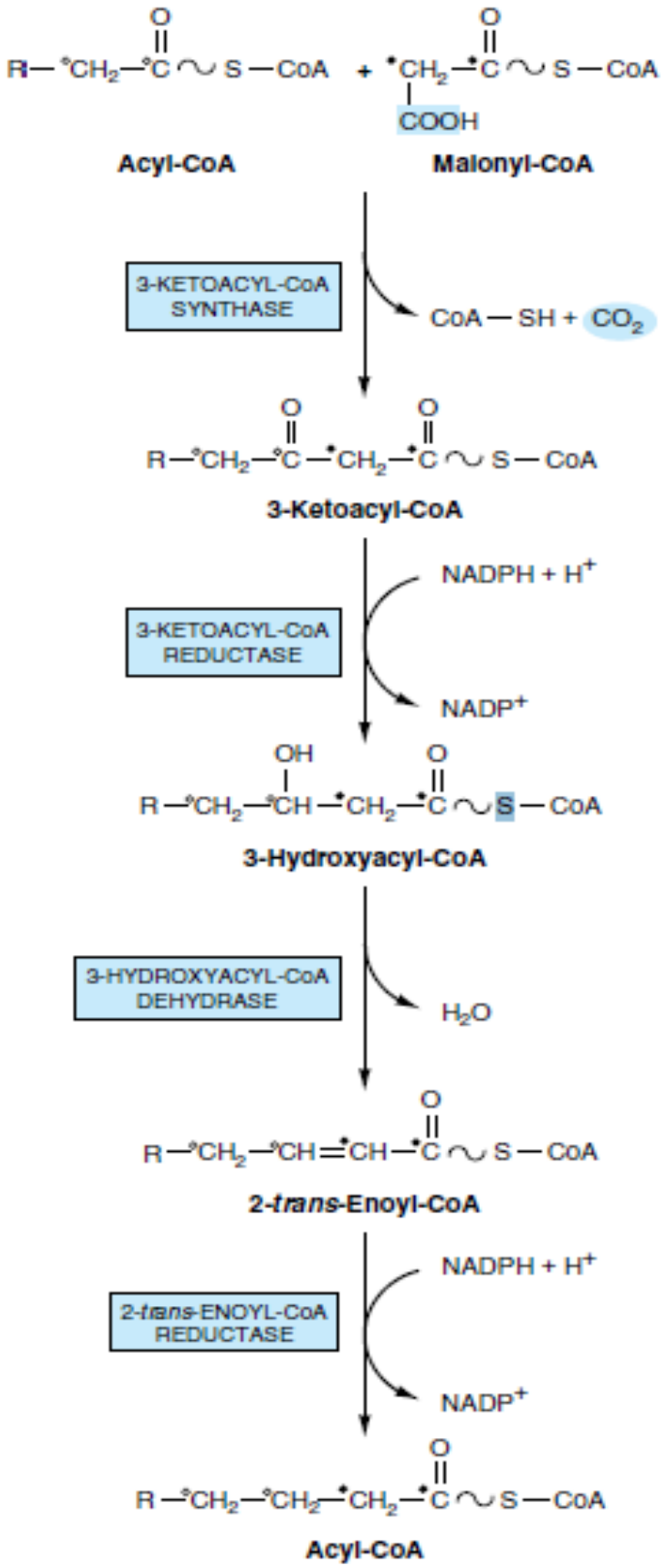
Initially, a priming molecule of acetyl-CoA combines with a cysteine-SH group catalyzed

by acetyl transacylase Malonyl-CoA combines with the adjacent -SH on the 4′-phosphopantetheine of ACP of the other monomer, catalyzed by malonyl transacylase, to form acetyl (acyl)-malonyl enzyme. The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and liberates CO2, forming 3-ketoacyl enzyme (acetoacetyl enzyme), freeing the cysteine -SH group. Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again to form the corresponding saturated acyl-Senzyme. A new malonyl-CoA molecule combines with the -SH of 4′-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine -SH group. The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex, thioesterase (deacylase). The free palmitate must be activated to acyl-CoA before it can proceed via any other acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.

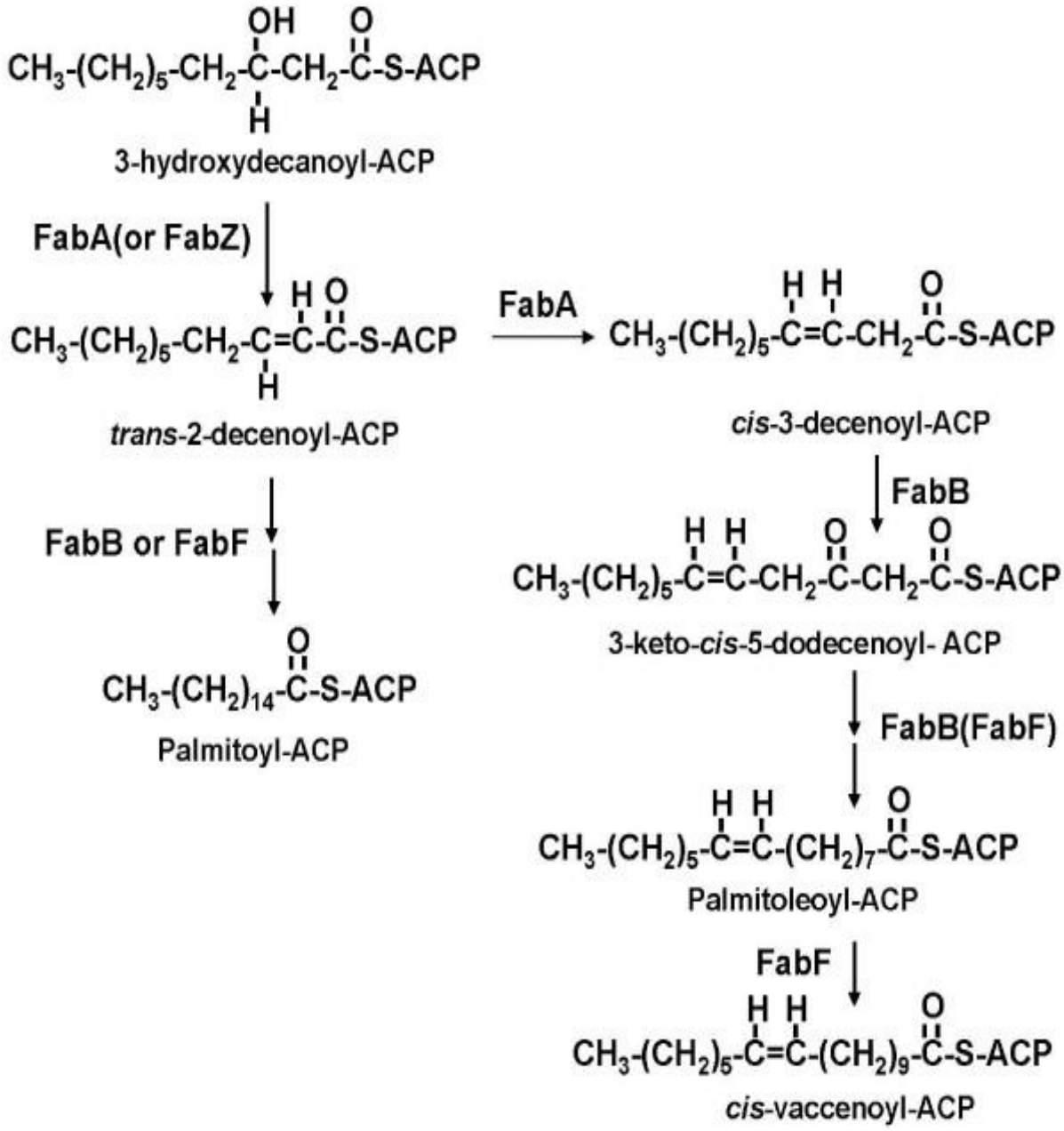


ELONGATION OF FATTY ACID CHAINS occurs in the endoplasmic reticulum

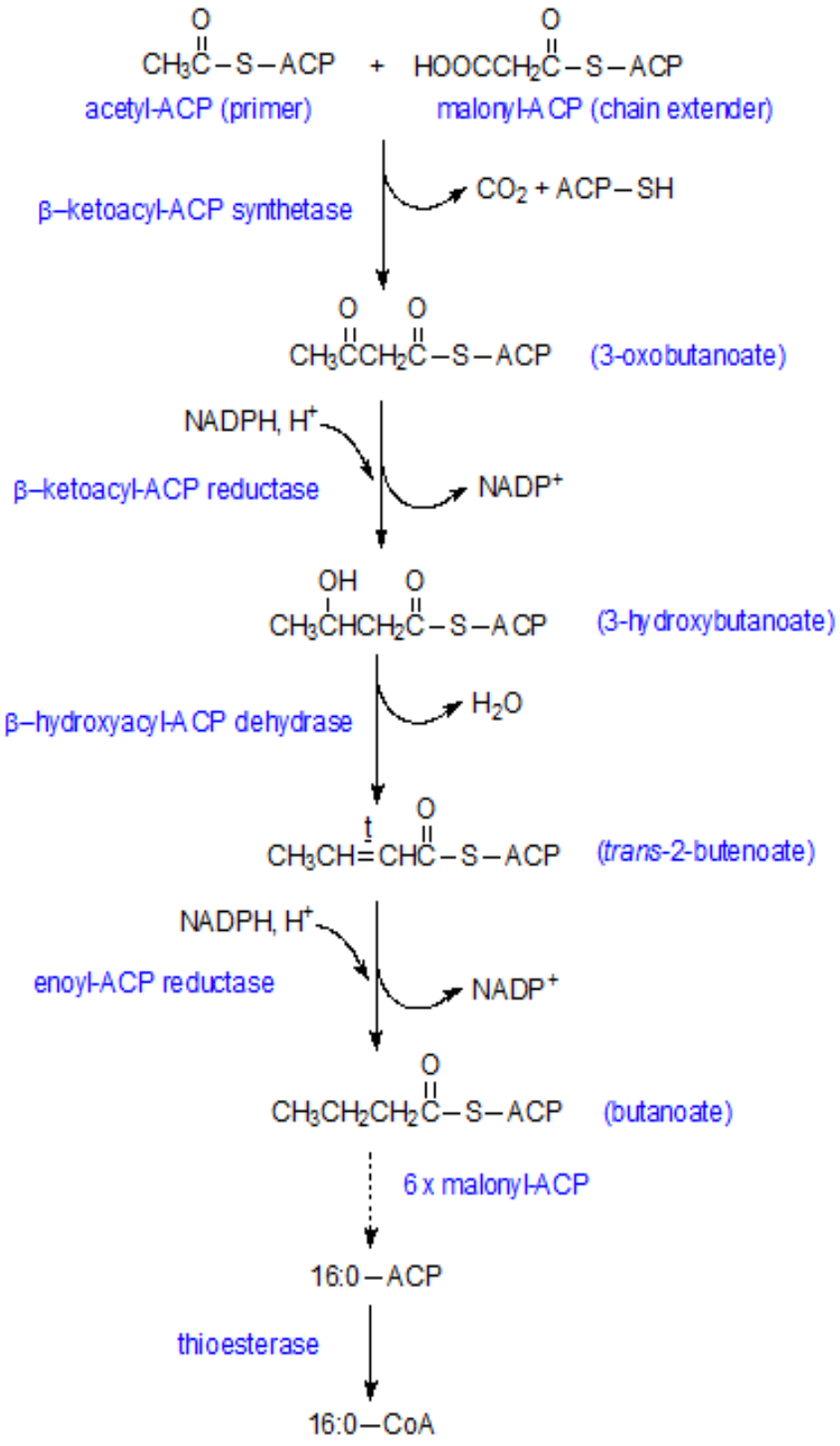
This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as acetyl donor and NADPH as reductant, and is catalyzed by the microsomal fatty acid elongase system of enzymes (Figure). Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids for sphingolipids.


Acyl-CoA
Malonyl-CoA
3-KETOACYL-CoA SYNTHASE
CoA — SH + CO2
3-Ketoacyl-CoA
NADPH + H+
3-KETOACYL-CoA REDUCTASE
NADP+
3-Hydroxyacyl-CoA
3-HYDROXYACYL-CoA DEHYDRASE
H2O
2-trans-Enoyl-CoA
NADPH + H+
2-trans-ENOYL-CoA REDUCTASE
NADP+
Acyl-CoA

Biosynthesis of unsaturated fatty acid



Biosynthesis of odd chain fatty acid


CH3C(=O)–S–ACP + HOOCCH2C(=O)–S–ACP
acetyl-ACP (primer)
malonyl-ACP (chain extender)
β–ketoacyl-ACP synthetase
CO2 + ACP–SH
CH3C(=O)CH2C(=O)–S–ACP (3-oxobutanoate)
NADPH, H+
β–ketoacyl-ACP reductase
NADP+
CH3CH(OH)CH2C(=O)–S–ACP (3-hydroxybutanoate)
β–hydroxyacyl-ACP dehydrase
H2O
CH3CH=CHC(=O)–S–ACP (trans-2-butenoate)
NADPH, H+
enoyl-ACP reductase
NADP+
CH3CH2CH2C(=O)–S–ACP (butanoate)
6 x malonyl-ACP
16:0–ACP
thioesterase
16:0–CoA

THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc, and to provide energy for use between meals in animals, including humans, that take their food at spaced intervals. Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake, on a fat diet, or when there is a deficiency of insulin, as in diabetes mellitus. These latter conditions are associated with increased concentrations of plasma free fatty acids, and an inverse relationship has been demonstrated between hepatic lipogenesis and the concentration of serum-free fatty acids. Lipogenesis is increased when sucrose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway.

Short- & Long-Term Mechanisms Regulate Lipogenesis

Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression governing rates of synthesis of enzymes.

POSSIBLE QUESTIONS
UNIT IV

PART A (1 Mark)
Question number 1 – 20 (Online examination)

PART B (2 Marks)

1. How is cholesterol transported?
2. Write a note on β-oxidation of saturated fatty acids
3. Give a note on fatty acid synthase complex
4. Give a note on riacyl glycerol
5. Write a note on triacyl glycerol
6. Add note about ω oxidation
7. Give note on peroxisomal oxidation

8. Give short note on carnitine transporter
9. List the ketone bodies and its importance
10. Add note on respiratory distress syndrome

PART C (6 Marks)

11. Write notes on fatty acid transport to mitochondria
12. Give a detail about fatty acid transport to mitochondria
13. Explain in detail about β oxidation of saturated fatty acids
14. Write notes on synthesis of odd and even chain fatty acids and regulation.
15. Write notes on:
    (i) Synthesis of saturated fatty acids
    (ii) Synthesis of unsaturated fatty acids
16. Explain phospholipid biosynthesis
17. Discuss on synthesis of fatty acids by fatty acid synthase complex.
18. Explain the metabolism of ketone bodies

Karpagam Academy of Higher Education
Department of Biochemistry
Metabolism of Carbohydrates and Lipids (17BCU301) MCQ
UNIT IV

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1							
2		What is the most common electron carrier in biological systems?	FAD	Coenzyme A	NAD	NADP	NAD
3							
4						Coenzyme Q	
5		What is not a compound in the electron transport system?	NADH dehydrogenase	Flavoproteins	NADPH dehydrogenase		NADPH dehydrogenase
6		The coupling of ATP synthesis to electron transport is known as	Oxidative phosphorylation	Chemiosmosis	ATP synthesis	Proton motive force	Oxidative phosphorylation
7		Which of the following drives the synthesis of ATP by ATP synthase in oxidative phosphorylation?	Distribution of electric potential across a membrane	Distribution of Cytochrome oxidase	Distribution of NADH	Distribution of FADH	Distribution of electric potential across a membrane
8		Which is not a metabolic intermediate used in amphibolic pathways?			Acetyl CoA		
9				Fructose-1,6-bisphosphate			Fructose-1,6-bisphosphate
10			Glyceraldehyde-3-phosphate			Oxaloacetic acid	
11		The compound having the lowest redox potential amongst the following is	Hydrogen	NAD	Cytochrome b	Cytochrome a	Hydrogen
12		The compound having the highest redox potential amongst the following is	Coenzyme Q	NAD	Cytochrome c	Cytochrome b	Cytochrome c
13		Superoxide radicals can be detoxified by	Cytochrome c	Cytochrome b	Cytochrome a	Coenzyme Q	Cytochrome c
14							
15			FAD				FAD
16		Which of the following is a coenzyme?		Ca^{2+}	Mg^{2+}	CO_2	
17		An exergonic reaction is one in which	Electrons are added to a molecule	Electrons are removed from a molecule	The products have more free energy than the reactants	The reactants have more free energy than the products	The reactants have more free energy than the products
18		The main endergonic reaction that is driven by most of the body's exergonic reactions is the	Oxidation of $FADH_2$	Synthesis of ATP	Reduction of NAD	Hydrolysis of ATP	Synthesis of ATP
19		The "universal energy carrier" is	FAD	$FADH_2$	Glucose	Adenosine triphosphate	Adenosine triphosphate
20		If molecule A accepts electrons from molecule B, molecule A is	Reduced agent	A reducing agent	An oxidizing agent	An exergonic agent	An oxidizing agent
21		Any oxidation reaction must be coupled to	The synthesis of ATP	The availability of oxygen	An exergonic reaction	A reduction reaction	A reduction reaction
22		If a molecule accepts a hydrogen atom, it becomes	Hydrolyzed	Dehydrated	Oxidized	Reduced	Reduced
23		Nicotinamide adenine dinucleotide (NAD) is	A vitamin	An oxidizing agent	A reducing agent	A coenzyme	A coenzyme
24							
25		In the electron transport chain, the hydrogen ions enter the inner compartment of	ATP synthase				
26		mitochondria through special channels formed by		Coenzyme A	Acetyl CoA	Oxygen	ATP synthase
27							
28		Which process produces both NADH and $FADH_2$?	The citric acid cycle	Glycolysis	The electron transport system	Fermentation	The citric acid cycle
29		Which process involves chemiosmotic phosphorylation?	The citric acid cycle	The electron transport system	Glycolysis	Fermentation	The electron transport system
30		Which of these pairs of processes are anaerobic?	Fermentation and glycolysis	Fermentation and the citric acid cycle	Glycolysis and the citric acid cycle	The citric acid cycle and the electron transport system	Fermentation and glycolysis
		ETC is located in the	Outer mitochondrial membrane	Inner mitochondrial membrane	Mitochondrial matrix	Nucleus	Inner mitochondrial membrane
		Coenzyme Q catalyzes electron transport between	FADH and cytochrome b	It is the last member in the ETC	NADH and ubiquinone	Cytochrome Q and cytochrome c	FADH and cytochrome b
		The enzymes of ETC belong to the following classes except	Oxidases	Dehydrogenases	Peroxidases	Reductases	Peroxidases
		Which of the electron carriers is soluble and mobile	CoQ	Cytochrome c	Cytochrome a	Cytochrome b	CoQ
		During chemiosmosis in aerobic respiration, protons are pumped	Out of the cell	Out of the mitochondria into the cell cytoplasm	Out of the mitochondrial matrix into the outer compartment of the mitochondria	Out of the cell cytoplasm into the matrix of the mitochondria	Out of the mitochondrial matrix into the outer compartment of the mitochondria
		The final electron acceptor in lactic acid fermentation is:	NAD^+	Pyruvate	O_2	Lactic acid	Pyruvate
		β-oxidation of long chain fatty acids occours primarily in _____	Cytosol	Peroxisomes	Mitochondria	Golgi apparatus	Mitochondria
		β-oxidation of palmitic acid produces a net synthesis of how many ATP molecules?	109	129	24	38	129
		ω-Oxidation of fatty acids occurs mainly in _____	Liver	Brain	Muscles	Adipose tissue	Brain
		The enzyme involved ω-oxidation are located in	golgi complex	Cytoplasm	Endoplasmic recticulum	Mitochondria	Endoplasmic recticulum
		The rate of fatty acid oxidation is increased by _____	Phospholipids	Glycolipids	Amino lipids	Spingolipids	Phospholipids
		Enzymes catalyzing electron transport are present mainly in the	Ribosomes	Endoplasmic reticulum	Lysosomes	Inner mitochondrial membrane	Inner mitochondrial membrane
		The power house of the cell is	Nucleus	Cell membrane	Mitochondria	Lysosomes	Mitochondria
		Starch is a	Polysaccharide	Monosaccharide	Disaccharide	Trisaccharide	Polysaccharide
		Oxidation is a	Gain of the electron	Removal of an electron	Gain of an oxygen	Removal of an oxygen	Removal of an electron
		Reduction is a	Removal of an oxygen	Gain of an oxygen	Gain of the electron	Removal of an electron	Gain of the electron
		Electron transport and phosphorylation can be uncoupled by compounds that increase the permeability of the inner mitochondrial membrane to	Electrons	Protons	Uncouplers	Couplers	Protons
		The function of an electron in the electron transport chain is	To transfer energy from complex II to complex I	To pump hydrogen ions using complex II	To use its free energy to pump protons against their concentration gradient	To combine with phosphate when ATP is synthesized	To use its free energy to pump protons against their concentration gradient
		In aerobic cellular respiration, which generates more ATP?	Substrate-level phosphorylation	Chemiosmosis	Both generate the same amount of ATP	Neither generates any ATP	Chemiosmosis
		Coenzyme Q is involved in electron transport as	Directly to O_2	A water-soluble electron donor	Covalently attached cytochrome cofactor	A lipid-soluble electron carrier	A lipid-soluble electron carrier
		Which of the following is a coenzyme associated with cellular respiration?	NAD^+	O_2	FAD	NAD and FAD	NAD and FAD
		The _____ connects glycolysis to the Citric Acid cycle.	Electron transport system	Transition reaction	Cristae	Mitochondrial intermembrane	Transition reaction
		When electrons are removed from pyruvate in the transition reaction, they are accepted by _____	acetyl Co-A	FAD^+	NAD^+	ATP	NAD^+
		The electron transport system is located in the _____	Stroma	Matrix	Cytosol	Cristae	Cristae
		When O_2 is reduced during the electron transport system, _____ is produced	H_2O	CO_2	ADP	Glucose	H_2O
		The final electron acceptor in the electron transport system is _____	$FADH_2$	O_2	Coenzyme Q	Cytochrome b	O_2
		Oxidative phosphorylation occurs in	Mitochondria	Nucleus	Cell Membrane	Ribosomes	Mitochondria
		Electrons in electron transport chains reduces oxygen in to	Carbondioxide	Water	Carbonmonoxide	Ozone	Water
		Process of flow of electrons generated by oxidation of NADH and $FADH_2$ is called	Phosphorylation	Electron transport chain	Dehydrogenation	Oxidative phosphorylation	Electron transport chain
		Electron transport chain consists of how many complexes	7	5	3	4	4
		Major components of electron transport chain are arranged in order of redox potential which is	Decreasing	Increasing	Variable	Alternatively increasing and decreasing	Increasing
		Cytochromes are enzymes which function as electron transfer agent in	Hydrolysis	Conjugation reaction	Transamination	Oxidation and reduction	Oxidation and reduction
		Oxidative phosphorylation is a process for	Phosphorylation of glucose	Generating creatine phosphate	Generating ATP	Utilizing ATP	Generating ATP

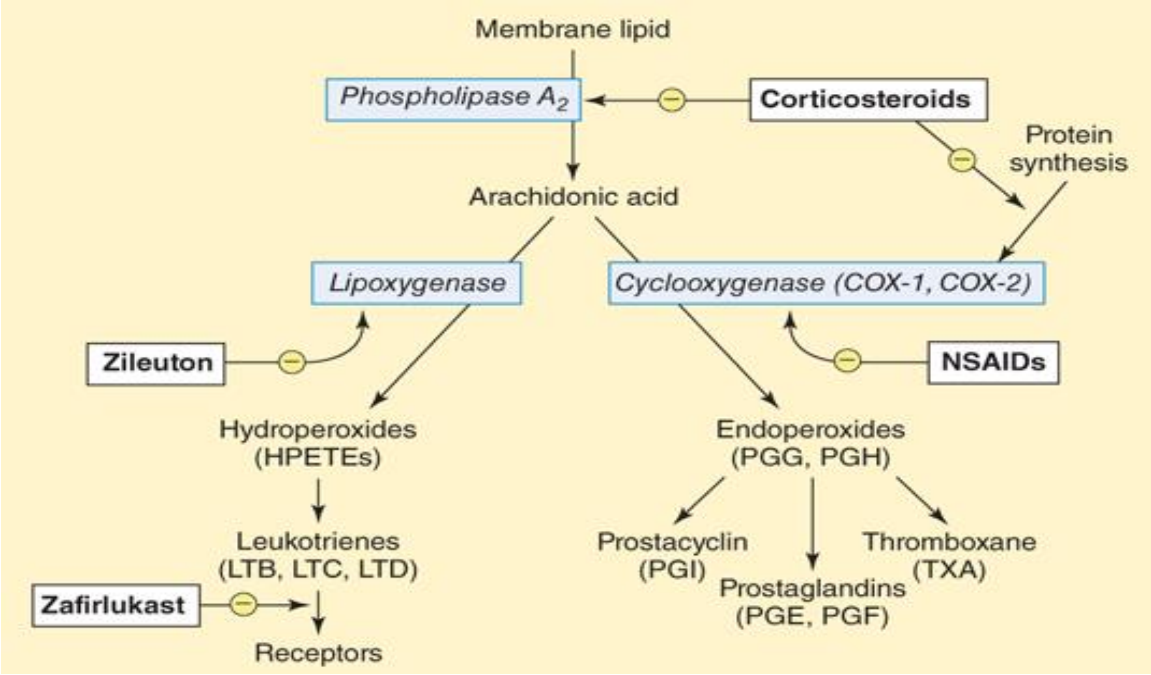
UNIT-V
SYLLABUS

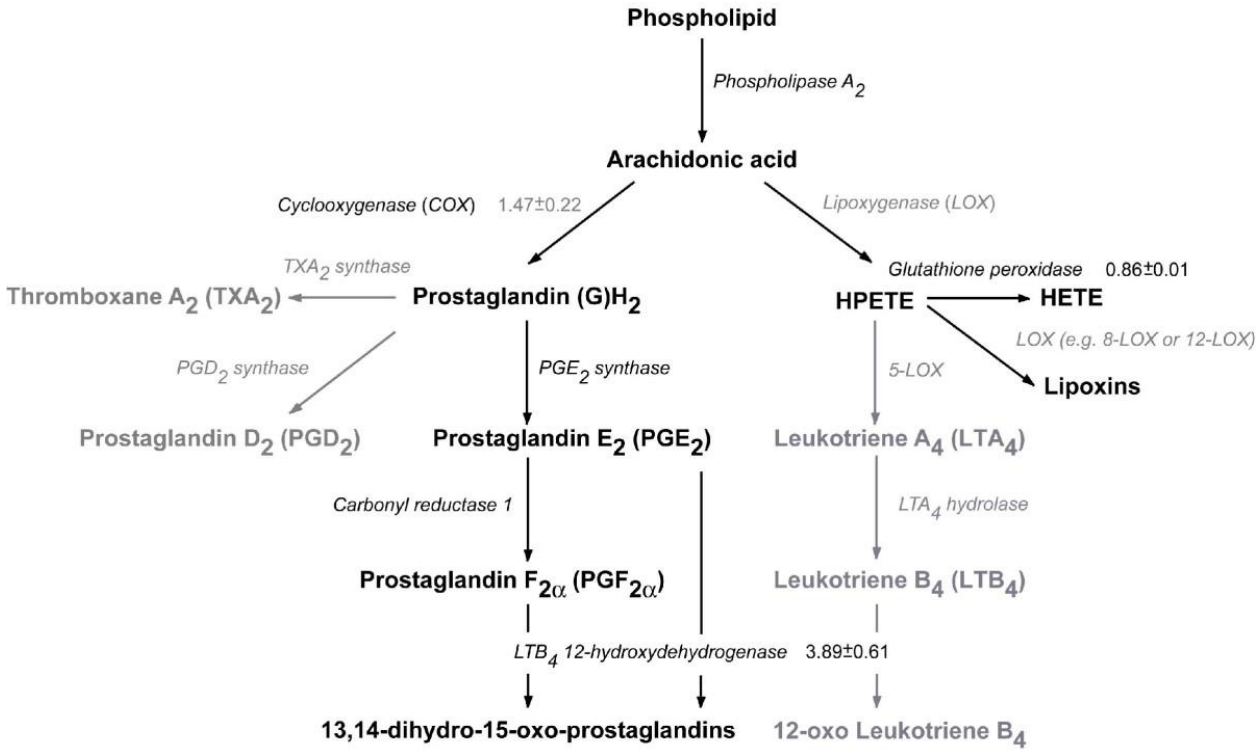
Biosynthesis of eicosanoids, cholesterol, steroids and isoprenoids : Synthesis of prostagladins, leukotrienes and thromboxanes. Synthesis of cholesterol, regulation of cholesterol synthesis. Synthesis of steroids and isoprenoids.

Biosynthesis of membrane lipids : Synthesis of membrane phospholipids in prokaryotes and eukaryotes, respiratory distress syndrome, biosynthesis of triacylglycerol, biosynthesis of plasmalogens, sphingolipids and glycolipids, lipid storage diseases.

Starve-feed cycle: Well-fed state, early fasting state, fasting state, early re-fed state, energy requirements, reserves and caloric homeostasis, five phases of glucose homeostasis.

SYNTHESIS OF PROSTAGLANDINS,LEUCOTRIENES AND THROMBOXANES





METABOLISM OF CHOLESTEROL

Cholesterol is an essential molecule in many animals, including humans, but is not required in the mammalian diet- all cells can synthesize it from simple precursors. It is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain.

The isoprene units that are the essential intermediates in the pathway from acetate to cholesterol are also precursors to many other natural lipids and the mechanisms by which isoprene units are polymerized are similar in all these pathways.

Cholesterol Is Made from Acetyl-CoA in Four Stages

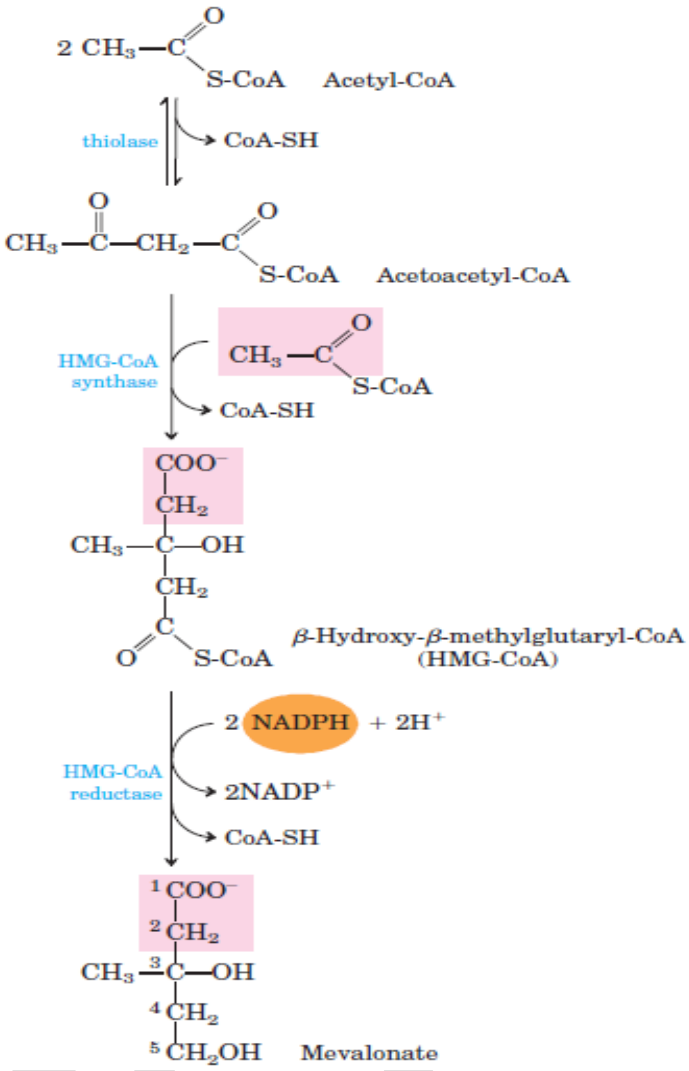
Cholesterol, like long-chain fatty acids, is made from acetyl-CoA, but the assembly plan is quite different. In early experiments, animals were fed acetate labelled with 14C in either the

methyl carbon or the carboxyl carbon. Synthesis takes place in four stages, 1 condensation of three acetate units to form a six-carbon intermediate, mevalonate; 2 conversion of mevalonate to activated isoprene units; 3 polymerization of six 5-carbon isoprene units to form the 30-carbon linear squalene; and 4 cyclization of squalene to form the four rings of the steroid nucleus, with a further series of changes (oxidations, removal or migration of methyl groups) to produce cholesterol. The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by NADPH while ATP provides energy. For the production of one mole of cholesterol, 18 moles of acetyl-CoA, 36 moles of ATP and 16 moles of NADPH are required.

Stage 1 Synthesis of Mevalonate from Acetate

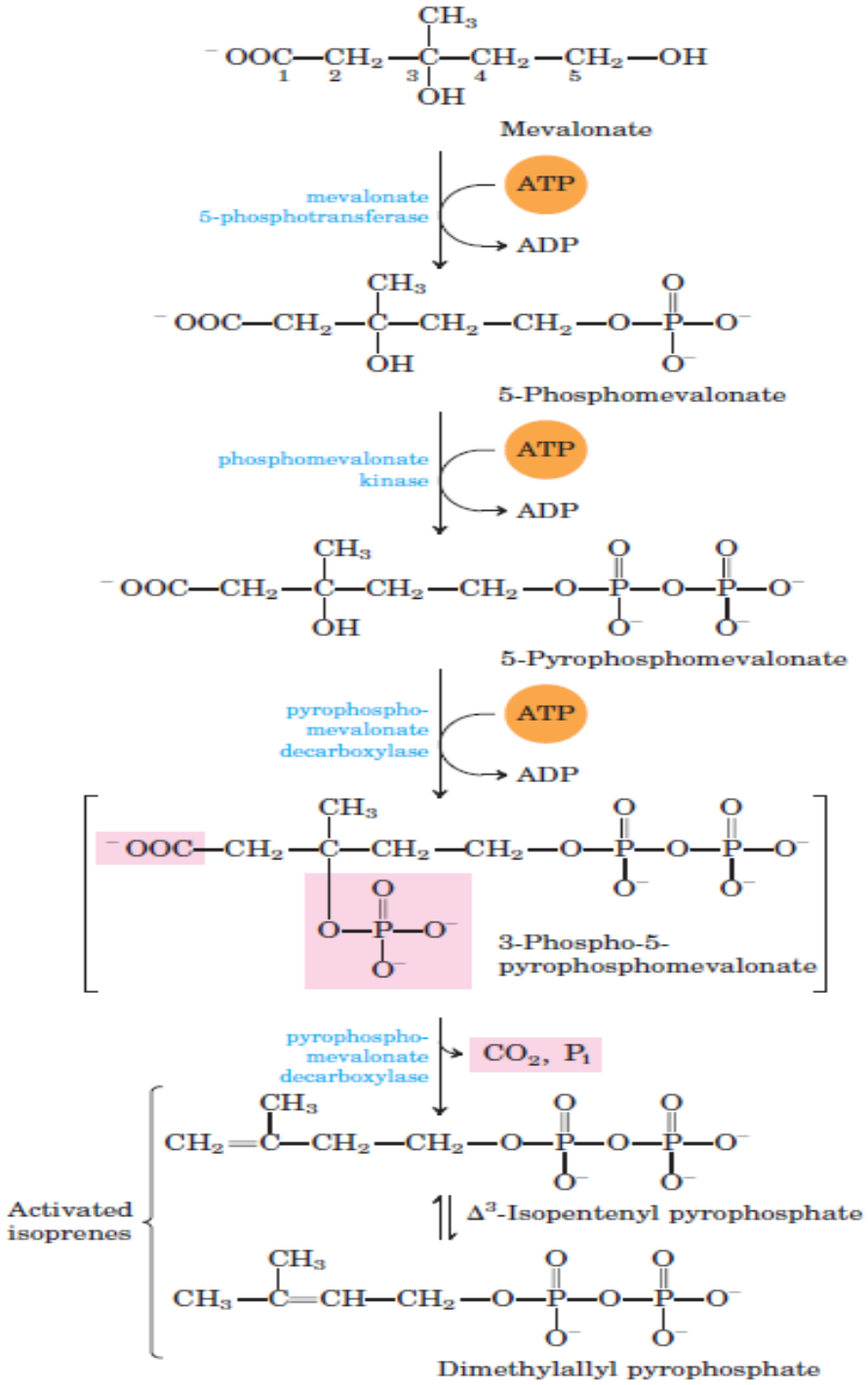
The first stage in cholesterol biosynthesis leads to the intermediate mevalonate. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which condenses with a third molecule of acetyl-CoA to yield the six-carbon compound β-hydroxy- β -methylglutaryl-CoA (HMG-CoA). These first two reactions are catalyzed by thiolase and HMG-CoA synthase, respectively. The cytosolic HMG-CoA synthase in this pathway is distinct from the mitochondrial isozyme that catalyzes HMG-CoA synthesis in ketone body formation. The third reaction is the committed and rate-limiting step: reduction of HMG-CoA to mevalonate, for which each of two molecules of NADPH donates two electrons. HMG-CoA reductase, an integral membrane protein of the smooth ER, is the major point of regulation on the pathway to cholesterol.

Formation of mevalonate from acetyl-CoA: The origin of C-1 and C-2 of mevalonate from acetyl-CoA is shown in pink.



Stage 2 Conversion of Mevalonate to Two Activated Isoprenes

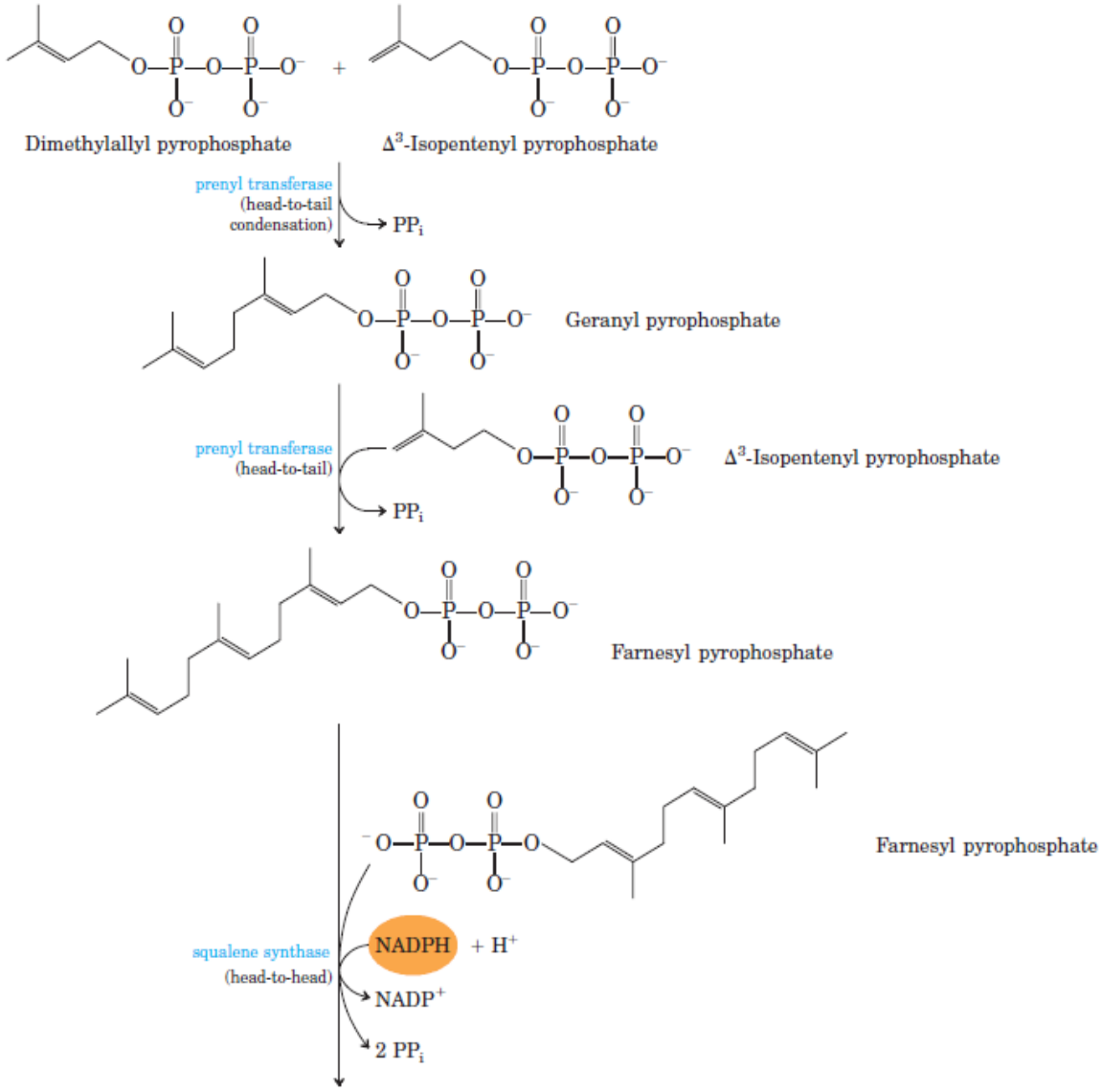
In the next stage of cholesterol synthesis, three phosphate groups are transferred from three ATP molecules to mevalonate. The phosphate attached to the C-3 hydroxyl group of mevalonate in the intermediate 3-phospho-5-pyrophosphomevalonate is a good leaving group; in the next step, both this phosphate and the nearby carboxyl group leave, producing a double bond in the five-carbon product, Δ^3-isopentenyl pyrophosphate. This is the first of the two activated isoprenes central to cholesterol formation. Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, dimethylallyl pyrophosphate.

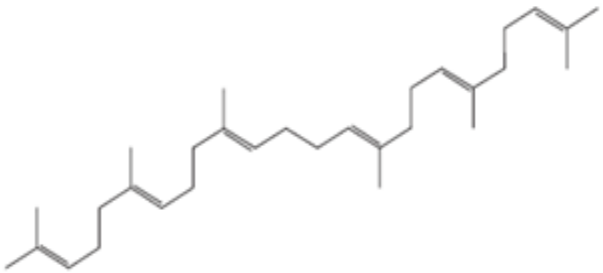


Stage 3 Condensations of Six Activated Isoprene Units to Form Squalene

Isopentenyl pyrophosphate and dimethylallyl pyrophosphate now undergo a head-to-tail

condensation, in which one pyrophosphate group is displaced and a 10-carbon chain, geranyl pyrophosphate, is formed. Geranyl pyrophosphate undergoes another head-to-tail condensation with isopentenyl pyro-phosphate, yielding the 15-carbon intermediate farnesyl pyrophosphate. Finally, two molecules of farnesyl pyrophosphate join head to head, with the elimination of both pyrophosphate groups, to form squalene.

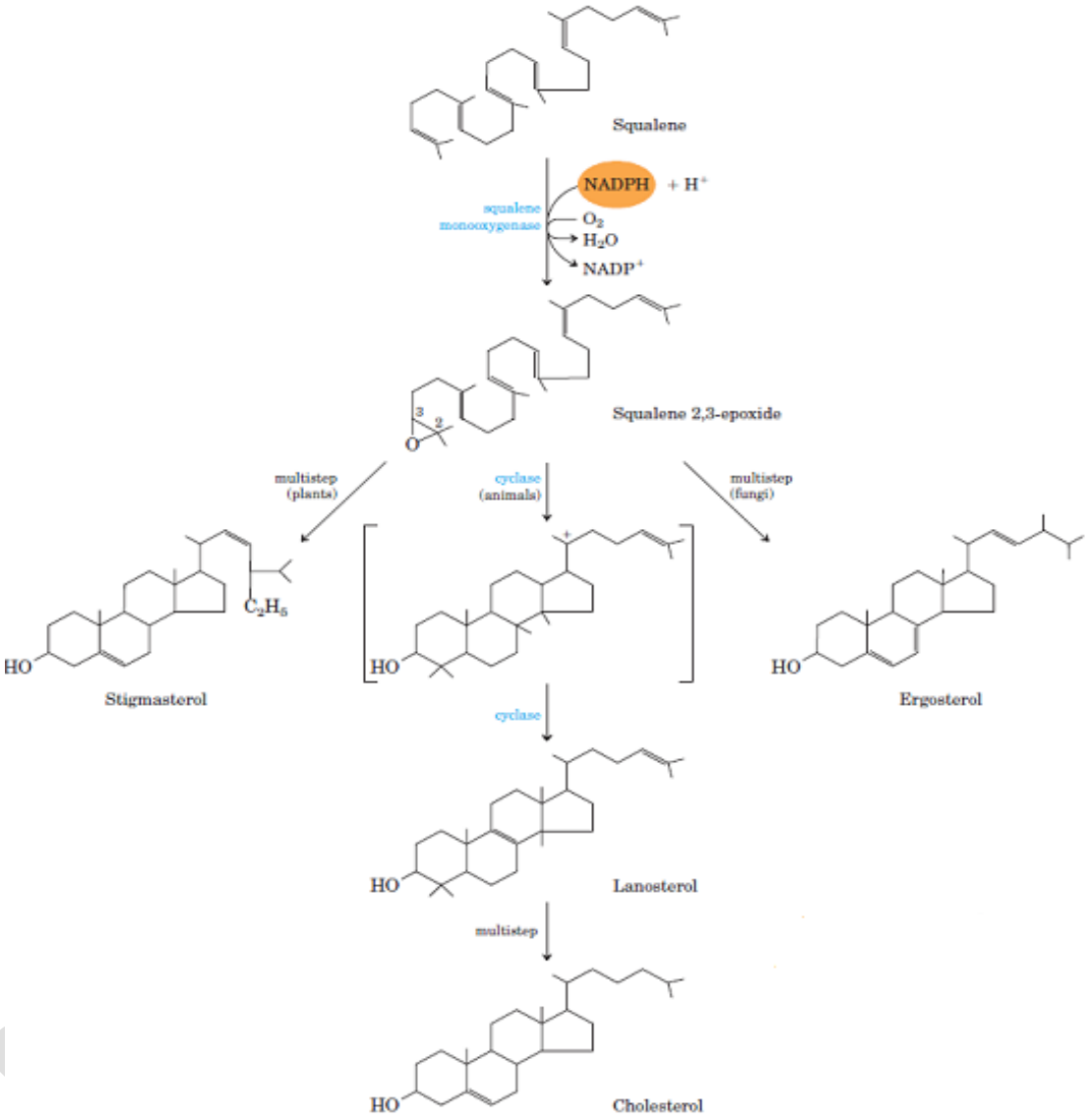




Squalene

Stage 4 Conversion of Squalene to the Four-Ring Steroid Nucleus

When the squalene molecule is represented the relationship of its linear structure to the cyclic structure of the sterols becomes apparent.



Ring closure converts linear squalene to the condensed steroid Nucleus: The first step in this sequence is catalyzed by a mixed-function oxidase (a monooxygenase), for which the cosubstrate is NADPH. The product is an epoxide, which in the next step is cyclized to the steroid nucleus. The final product of these reactions in animal cells is cholesterol; in other organisms, slightly different sterols are produced.

The action of squalene monooxygenase adds one oxygen atom from O_2 to the end of the squalene chain, forming an epoxide. This enzyme is another mixed-function oxidase NADPH reduces the other oxygen atom of O_2 to H_2O. The double bonds of the product, squalene 2,3-

epoxide, are positioned so that a remarkable concerted reaction can convert the linear squalene epoxide to a cyclic structure. In animal cells, this cyclization results in the formation of lanosterol, which contains the four rings characteristic of the steroid nucleus. Lanosterol is finally converted to cholesterol in a series of about 20 reactions that include the migration of some methyl groups and the removal of others. Cholesterol is the sterol characteristic of animal cells; plants, fungi and protists make other, closely related sterols instead. They use the same synthetic pathway as far as squalene 2, 3-epoxide, at which point the pathways diverge slightly, yielding other sterols, such as stigmasterol in many plants and ergosterol in fungi. Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase step. The reduced synthesis of cholesterol in starving animals is accompanied by a decrease in the activity of the enzyme. However, it is only hepatic synthesis that is inhibited by dietary cholesterol. HMG-CoA reductase in liver is inhibited by mevalonate, the immediate product of the pathway, and by cholesterol, the main product.

Summary

In the first stage of β -oxidation, four reactions remove each acetyl-CoA unit from the carboxyl end of a saturated fatty acyl–CoA: (1) dehydrogenation of the α and β carbons (C-2 and C-3) by FAD-linked acyl-CoA dehydrogenases, (2) hydration of the resulting *trans*-Δ^2 double bond by enoyl-CoA hydratase, (3) dehydrogenation of the resulting L- β hydroxyacyl-CoA by NAD-linked β -hydroxyacyl-CoA dehydrogenase, and (4) CoA-requiring cleavage of the resulting β -ketoacyl-CoA by thiolase, to form acetyl-CoA and a fatty acyl–CoA shortened by two carbons. The shortened fatty acyl–CoA then re enters the sequence.

In the second stage of fatty acid oxidation, the acetyl-CoA is oxidized to CO_2 in the citric acid cycle. A large fraction of the theoretical yield of free energy from fatty acid oxidation is recovered as ATP by oxidative phosphorylation, the final stage of the oxidative pathway. Malonyl-CoA, an early intermediate of fatty acid synthesis, inhibits carnitine acyltransferase I, preventing fatty acid entry into mitochondria. This blocks fatty acid breakdown while synthesis is occurring.

Cholesterol is formed from acetyl-CoA in a complex series of reactions, through the intermediates β -hydroxy- β -methylglutaryl-CoA, mevalonate and two activated isoprenes, dimethylallyl pyrophosphate and isopentenyl pyrophosphate. Condensation of isoprene units

produces the noncyclic squalene, which is cyclized to yield the steroid ring system and side chain. Cholesterol synthesis is under hormonal control and is also inhibited by elevated concentrations of intracellular cholesterol, which acts through covalent modification and transcriptional regulation mechanisms.

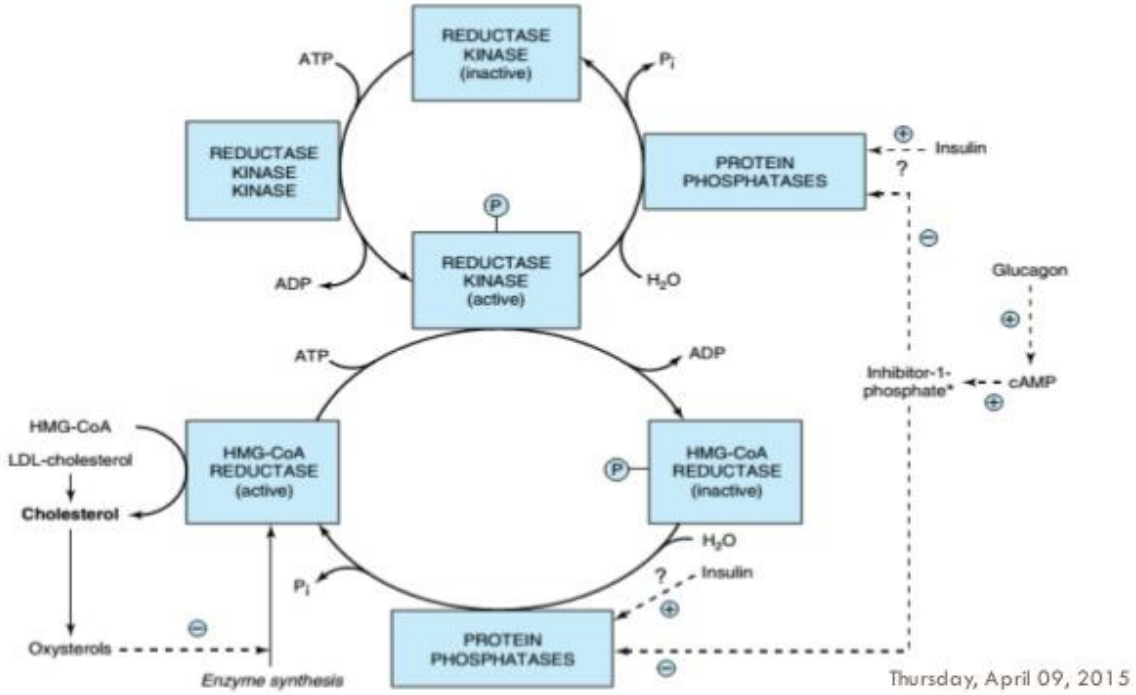
REGULATION OF CHOLESTEROL SYNTHESIS

Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase step.

Following mechanisms are involved at the regulatory step-

- Competitive inhibition
- Feed back inhibition
- Covalent modification(Role of hormones)
- Sterol mediated regulation of transcription

Regulation of Cholesterol synthesis is controlled by HMG-CoA reductase



Thursday, April 09, 2015

Regulation of cholesterol synthesis

1- Feed back inhibition.

- Cholesterol is a feed back inhibitor of HMG-CoA reductase → decrease cholesterol synthesis.
- HMG-CoA reductase activity is the rate limiting step in the biosynthesis of cholesterol and subjected to different controls.

2- Hormonal regulation of cholesterol biosynthesis.

- HMG- reductase is controlled by Glucagon and Insulin.
- Glucagon → increase formation of inactive (phosphorylated) form of the enzyme → decrease cholesterol biosynthesis.
- Insulin → increase formation of active (unphosphorylated) form of the enzyme → increase cholesterol biosynthesis.

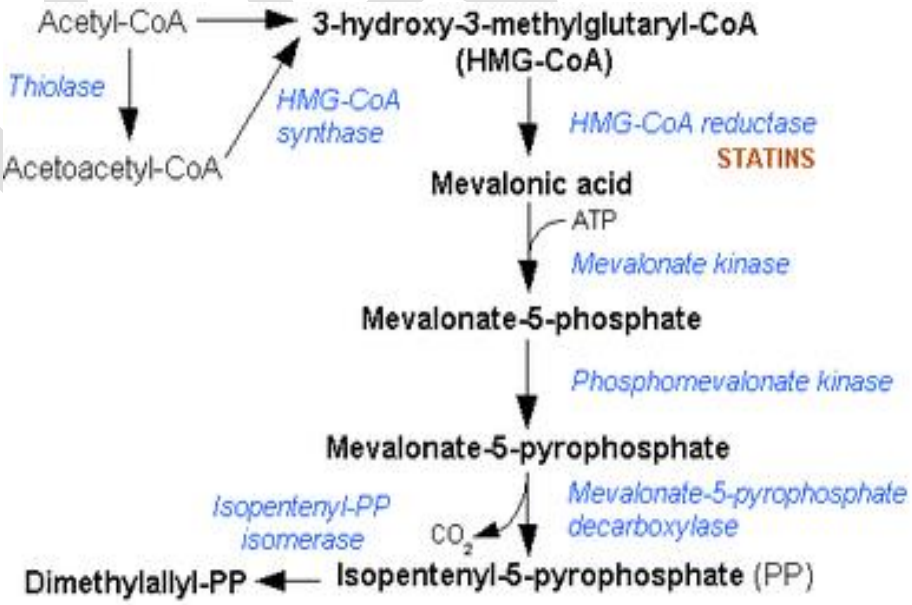
3- Sterol-mediated regulation of transcription.

- cholesterol taken by cells from lipoproteins → decrease biosynthesis of cholesterol by decrease of transcription of DNA of the HMG-CoA reductase enzyme.

4- Inhibition of drugs.

Lovastatin and Mevastatin are competitive inhibitors of HMG-CoA reductase.

SYNTHESIS OF STEROIDS AND ISOPRENOIDS



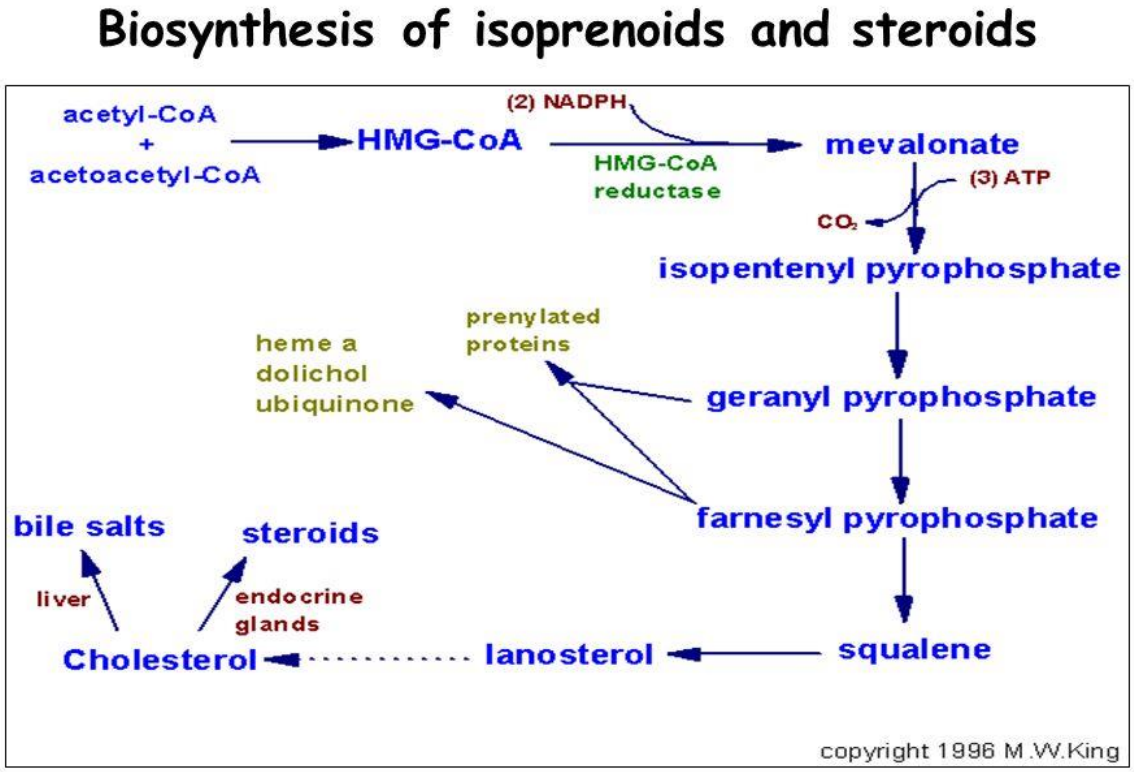


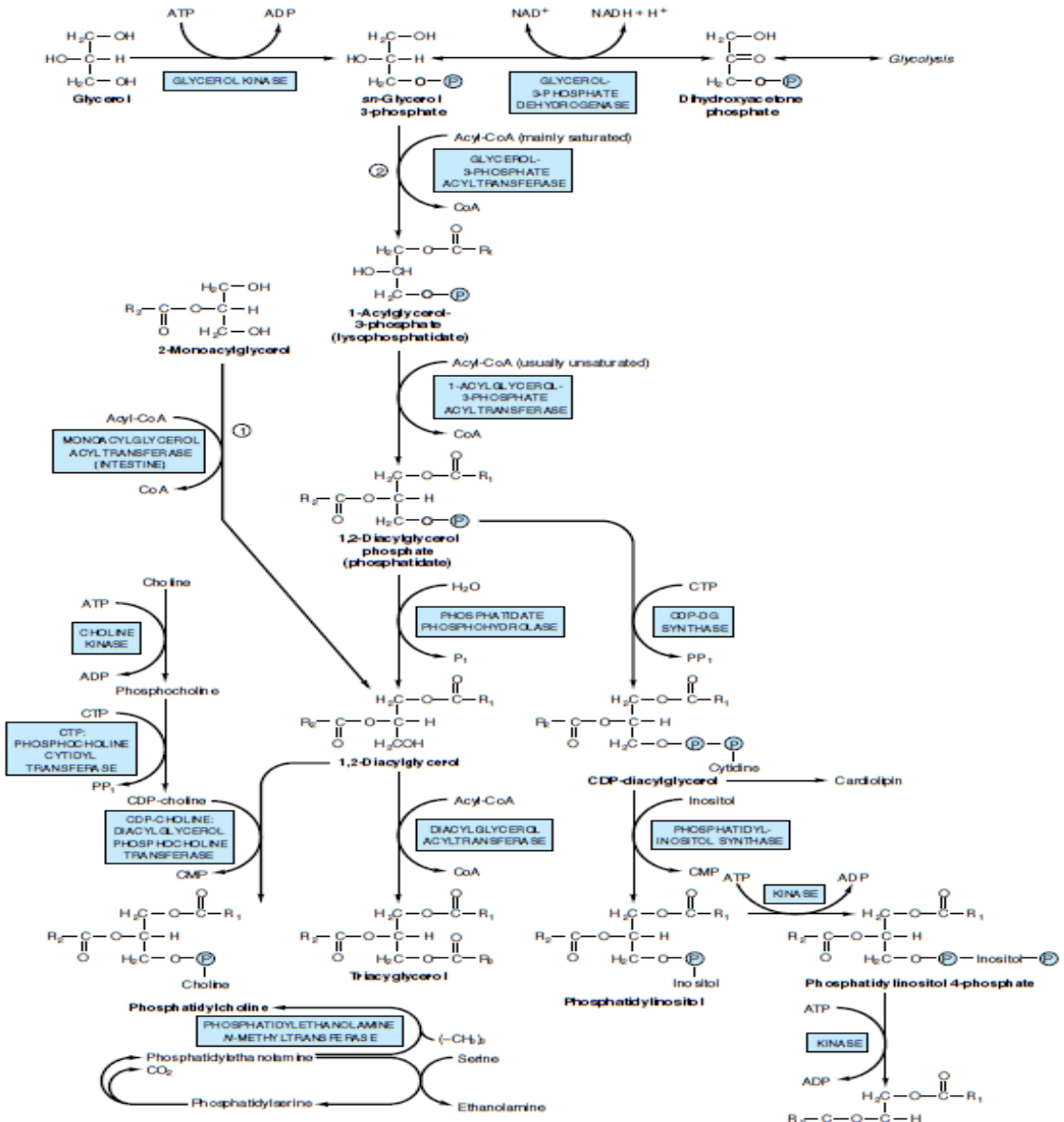
Figure is found on http://web.indstate.edu/thcme/mwking/cholesterol.html

BIOSYNTHESIS OF MEMBRANE PHOSPHOLIPIDS

The diacylglycerol is activated by condensation of phosphatidic acid with cytidine triphosphate (CTP) to form CDP-diacylglycerol, with the elimination of pyrophosphate. Displacement of CMP through nucleophilic attack by the hydroxyl group of serine or by the C-1 hydroxyl of glycerol 3-phosphate yields phosphatidylserine or phosphatidylglycerol 3-phosphate, respectively. The latter is processed further by cleavage of the phosphate monoester (with release of P_i) to yield phosphatidylglycerol. Phosphatidylserine and phosphatidylglycerol can serve as precursors of other membrane lipids in bacteria. Decarboxylation of the serine moiety in phosphatidylserine, catalyzed by phosphatidylserine decarboxylase, yields phosphatidylethanolamine. In *E. coli,* condensation of two molecules of phosphatidylglycerol, with elimination of one glycerol, yields cardiolipin, in which two diacylglycerols are joined through a common head group.

Eukaryotes Synthesize Anionic Phospholipids from CDP-Diacylglycerol

In eukaryotes, phosphatidylglycerol, cardiolipin, and the phosphatidylinositols are synthesized by the same strategy used for phospholipid synthesis in bacteria. Phosphatidylglycerol is made exactly as in bacteria. Cardiolipin synthesis in eukaryotes differs slightly: phosphatidylglycerol condenses with CDP-diacylglycerol not another molecule of phosphatidylglycerol as in *E. coli*. Phosphatidylinositol is synthesized by condensation of CDP-diacylglycerol with inositol.

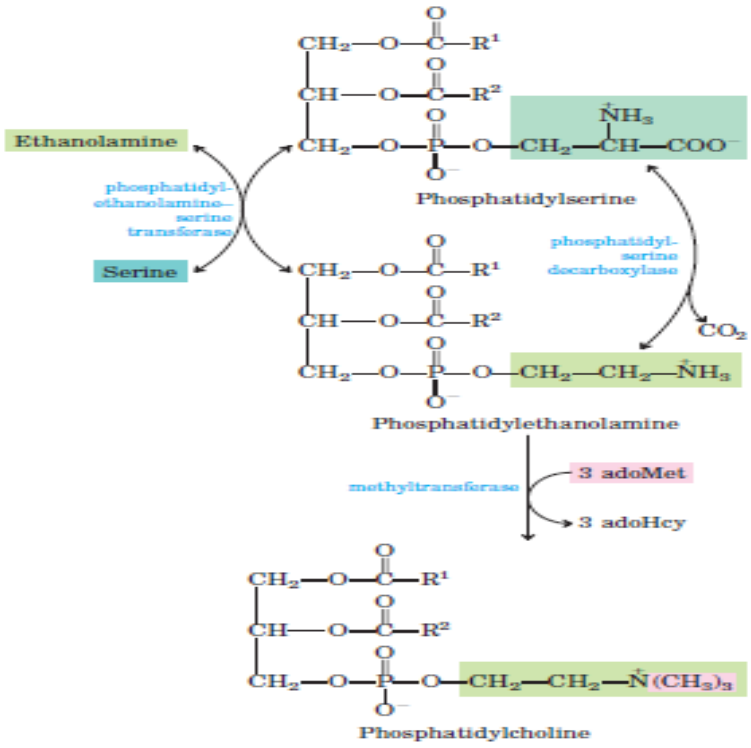


Biosynthesis of triacylglycerol and phospholipids. (①, Monoacylglycerol pathway; ②, glycerol phosphate pathway.) Phosphatidylethanolamine may be formed from ethanolamine by a pathway similar to that shown for the formation of phosphatidylcholine from choline.

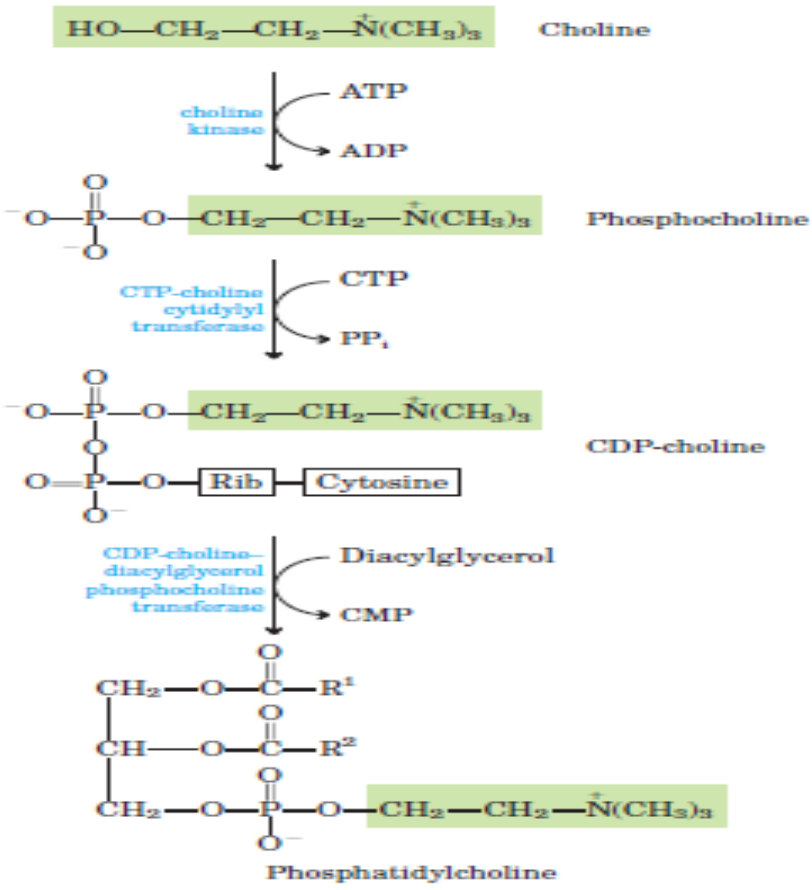
Specific phosphatidylinositol kinases then convert phosphatidylinositol to its phosphorylated derivatives. Phosphatidylinositol and its phosphorylated products in the plasma membrane play a central role in signal transduction in eukaryotes.

Eukaryotic Pathways to Phosphatidylserine, Phosphatidylethanolamine, and Phosphatidylcholine Are Interrelated

Yeast, like bacteria, can produce phosphatidylserine by condensation of CDP-diacylglycerol and serine, and can synthesize phosphatidylethanolamine from phosphatidylserine in the reaction catalyzed by phosphatidylserine decarboxylase. In mammalian cells, an alternative route to phosphatidylserine is a head-group exchange reaction, in which free serine displaces ethanolamine. Phosphatidylethanolamine may also be converted to phosphatidylcholine (lecithin) by the addition of three methyl groups to its amino group; *S*adenosylmethionine is the methyl group donor for all three methylation reactions. In mammals, phosphatidylserine is not synthesized from CDP-diacylglycerol; instead, it is derived from phosphatidylethanolamine via the head-group exchange reaction.



Choline is reused ("salvaged") by being phosphorylated then converted to CDP-choline by condensation with CTP. A diacylglycerol displaces CMP from CDP-choline, producing phosphatidylcholine.



An analogous salvage pathway converts ethanolamine obtained in the diet to phosphatidylethanolamine. In the liver, phosphatidylcholine is also produced by methylation of phosphatidylethanolamine (with *S*-adenosylmethionine, as described above), but in all other tissues phosphatidylcholine is produced only by condensation of diacylglycerol and CDP-choline. Although the role of lipid composition in membrane function is not entirely understood, changes in composition can produce dramatic effects. Researchers have isolated fruit flies with mutations in the gene that encodes ethanolamine kinase. Lack of this enzyme eliminates one pathway for phosphatidylethanolamine synthesis, thereby reducing the amount of this lipid in cellular membranes. Flies with this mutation—those with the genotype *easily shocked*—exhibit transient paralysis following electrical stimulation or mechanical shock that would not affect wild-type flies.

DEGRADATION OF PHOSPHOLIPIDS

Phospholipids are degraded by phospholipases which cleave the phosphodiester bonds.

These enzymes are found in mammalian tissues, pancreatic juice, snake venom and in some toxins. Certain pathogenic bacteria produce phospholipases which help in the spread of infection by dissolving cell membranes.

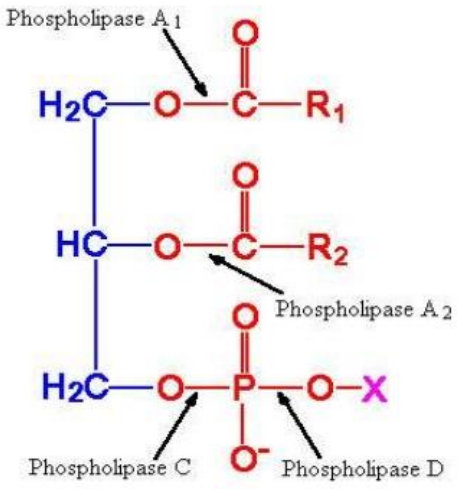
Phospholipase A_1 specifically cleaves the fatty acid at C_1 position of phospholipids resulting in lysophospholipid. The latter can be further acted by lysophospholipase, phospholipase B to remove the second acyl group at C_2 position.

Phospholipase A2 hydrolyses the fatty acid at C_1 position of phospholipids. Snake venom and bee venom are rich sources of phospholipase A2. This enzyme is found in many tissues and pancreatic juice. Phospholipase A2 acts on phosphatidyl inositol to liberate arachidonic acid, the substrate for the synthesis of prostaglandins.

Phospholipase C specifically cleaves the bond between phosphate and glycerol of phospholipids. This enzyme is present in lysosomes of hepatocytes. The toxins isolated from clostridia and other bacilli contain phospholipase C.

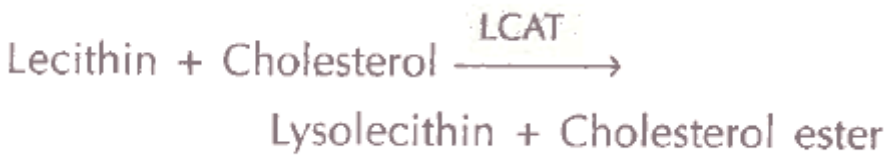
Phospholipase D hydrolyses and removes the nitrogenous base from phospholipids. This enzyme is mostly found in plant sources (cabbage, cotton, seed etc.). The degraded products of phospholipids enter the metabolic pool and are utilized for various purposes.

Degradation of phospholipids



Role of LCAT in lecithin metabolism

Lecithin-cholesterol acyl transferase (LCAT) is a plasma enzyme, synthesized in the liver. LCAT activity is associated with apo A1 of HDL. This enzyme esterifies cholesterol by transferring acyl group from the second position of lecithin



The above reaction is responsible for the reverse cholesterol transport mediated by HDL

RESPIRATORY DISTRESS SYNDROME

Infant respiratory distress syndrome (IRDS), also called neonatal respiratory distress syndrome,respiratory distress syndrome of newborn, or increasingly surfactant deficiency disorder (SDD), and previously called hyaline membrane disease (HMD), is a syndrome in premature infants caused by developmental insufficiency of pulmonary surfactant production and structural immaturity in the lungs. It can also be a consequence of neonatal infection. It can also result from a genetic problem with the production of surfactant associated proteins. IRDS affects about 1% of newborn infants and is the leading cause of death in preterm infants.[The incidence decreases with advancing gestational age, from about 50% in babies born at 26–28 weeks, to about 25% at 30–31 weeks. The syndrome is more frequent in infants of diabetic mothers and in the second born of premature twins.

IRDS is distinct from pulmonary hypoplasia, another leading cause of neonatal death that involves respiratory distress.

Signs and symptoms

RDS begins shortly after birth and is manifest by fast breathing, a fast heart rate, chest wall retractions (recession), expiratory grunting, nasal flaring and blue discoloration of the skin during breathing efforts.

As the disease progresses, the baby may develop ventilatory failure (rising carbon dioxide concentrations in the blood), and prolonged cessations of breathing ("apnea"). Whether treated or not, the clinical course for the acute disease lasts about 2 to 3 days. During the first day the

patient worsens and requires more support. During the second day the baby may be remarkably stable on adequate support and resolution is noted during the third day, heralded by a prompt diuresis. Despite huge advances in care, IRDS remains the most common single cause of death in the first month of life in the developed world. Complications include metabolic disorders (acidosis, low blood sugar), patent ductus arteriosus, low blood pressure, chronic lung changes, and bleeding in the brain. The disease is frequently complicated by prematurity and its additional defects in other organ function.

Pathophysiology

The lungs of infants with respiratory distress syndrome are developmentally deficient in a material called surfactant, which helps prevent collapse of the terminal air-spaces (the future site of alveolar development) throughout the normal cycle of inhalation and exhalation. This deficiency of surfactant is related to an inhibition from the insulin that is produced in the newborn especially in diabetic mothers.

Pulmonary surfactant is a complex system of lipids, proteins and glycoproteins that are produced in specialized lung cells called Type II cells or Type II pneumocytes. The surfactant is packaged by the cell in structures called lamellar bodies, and extruded into the air-spaces. The lamellar bodies then unfold into a complex lining of the air-space. This layer reduces the surface tension of the fluid that lines the alveolar air-space. Surface tension is responsible for approximately 2/3 of the inward elastic recoil forces. In the same way that a bubble will contract to give the smallest surface area for a given volume, so the air/water interface means that the liquid surface will tend toward being as small as possible, thereby causing the air-space to contract. By reducing surface tension, surfactant prevents the air-spaces from completely collapsing on exhalation. In addition, the decreased surface tension allows re-opening of the air-space with a lower amount of force. Therefore, without adequate amounts of surfactant, the air-spaces collapse and are very difficult to expand.

Microscopically, a pulmonary surfactant deficient lung is characterized by collapsed air-spaces alternating with hyper-expanded areas, vascular congestion and, in time, hyaline membranes. Hyaline membranes are composed of fibrin, cellular debris, red blood cells,

rare neutrophils and macrophages. They appear as an eosinophilic, amorphous material, lining or filling the air spaces and blocking gas exchange. As a result, blood passing through the lungs is unable to pick up oxygen and unload carbon dioxide. Blood oxygen levels fall and carbon dioxide rises, resulting in rising blood acid levels and hypoxia. Structural immaturity, as manifest by decreased number of gas-exchange units and thicker walls, also contributes to the disease process. Therapeutic oxygen and positive-pressure ventilation, while potentially life-saving, can damage the lung.

Dignosis

The diagnosis is made by the clinical picture and the chest xray, which demonstrates decreased lung volumes (bell-shaped chest), absence of the thymus (after about 6 hours), a small (0.5–1 mm), discrete, uniform infiltrate

Prevention

Giving the mother glucocorticoids speeds the production of surfactant. For very premature deliveries, a glucocorticoid is given without testing the fetal lung maturity.

Treatment

Oxygen is given with a small amount of continuous positive airway pressure ("CPAP"), and intravenous fluids are administered to stabilize the blood sugar, blood salts, and blood pressure. If the baby's condition worsens, an endotracheal tube (breathing tube) is inserted into the trachea and intermittent breaths are given by a mechanical device. An exogenous preparation of surfactant, either synthetic or extracted from animal lungs, is given through the breathing tube into the lungs.

BIOSYNTHEIS OF TRIACYL GLYCEROL

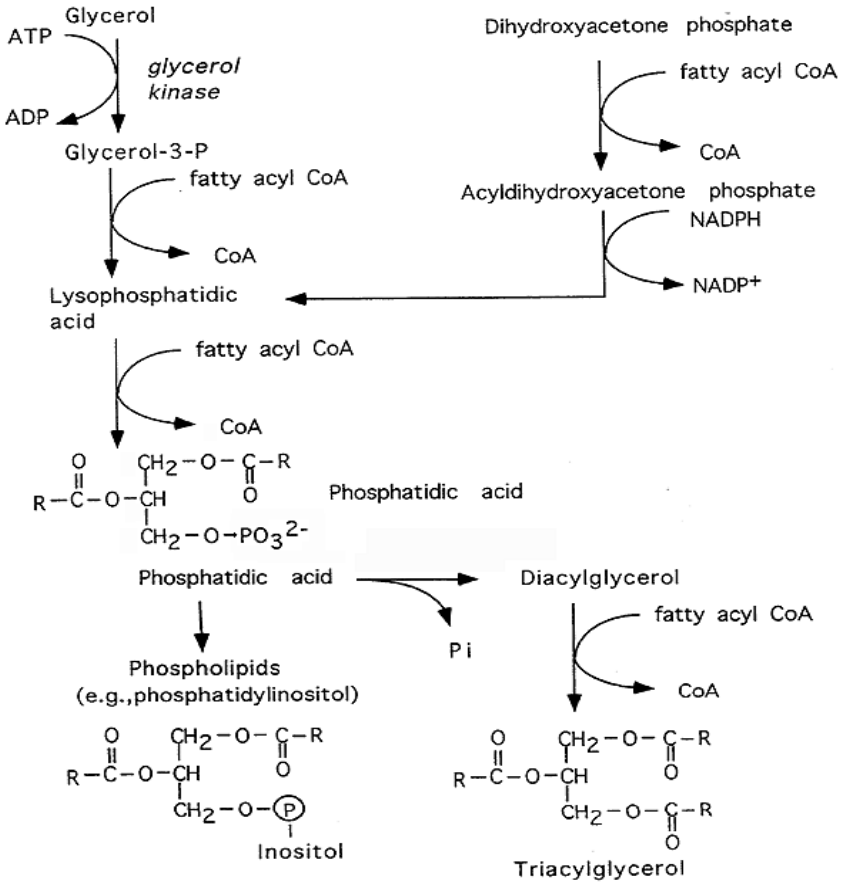
Triacylglycerols the body fuel reserve

Lipids constitute about "15-20% of the body weight in humans. Blood lipids (or blood fats) are lipids in the blood, either free or bound to other molecules. Blood lipids are mainly fatty acids and cholestero Triacylglycerols (formerly triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerols (TC; also called neutral fat or depot fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in

contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals Triacylglycerols are the most predominant storage form of energy. There are two main reasons for fat being the fuel reserve of the body

1. Triacylglycerols (TC) are highly concentrated form of energy, yielding 9 Cal/g, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form.
2. The triacylglycerols are non-polar and hydrophobic in nature, hence stored in pure form without any association with water (anhydrous form). On the other hand, glycogen and proteins are polar. One gram of glycogen combines with 2 g of water for storage.

For the two reasons stated above, one gram of fat stored in the body yields nearly six times as much energy as one gram of (hydrated) glycogen. Fats can support the body's energy needs for long periods of food deprivation. In extreme cases, humans can fast and survive for 60-90 days, and the obese persons can survive even longer (6 months to one year) without food.



Other important body lipids

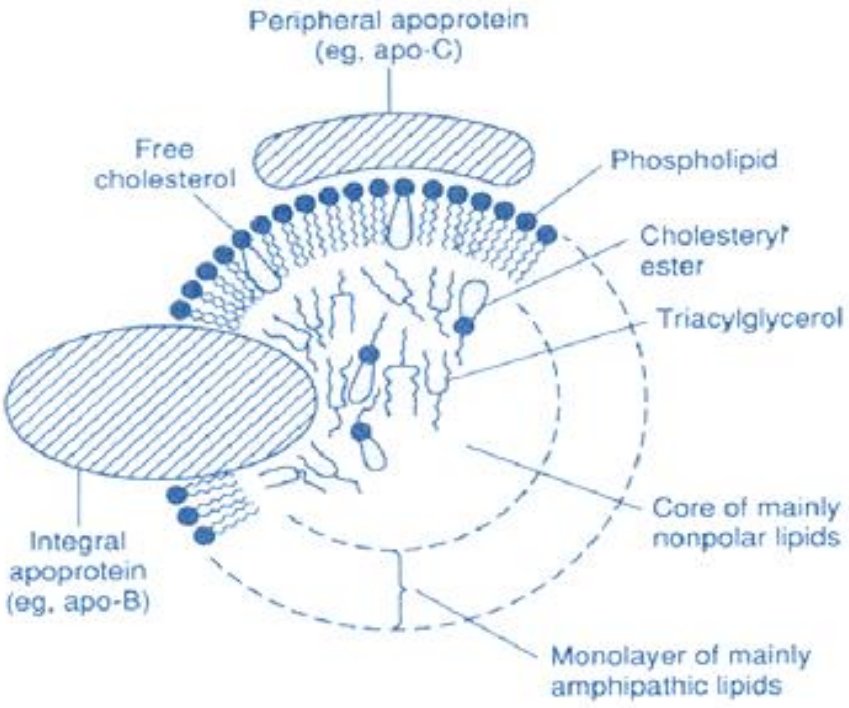
Phospholipids, glycolipids and cholesterol are major components of cell membranes. Cholesterol is also a precursor for bile acids and steroid hormones. Arachidonic acid an unsaturated fatty acid is the substrate for the synthesis of certain intercellular regulators prostagalndins, thromboxanes, prostacycilns etc.

Transport of Lipids

The insoluble lipids are solubilized in association with proteins to form lipoproteins in which form lipids are transported in the blood stream. Free lipids are undetectable in blood. Chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and albumin-free fatty acids are the different lipoprotein complexes that transport lipids in the blood stream.

Lipoproteins

Lipoproteins are molecular complexes that consist of lipids and proteins (conjugated proteins). They function as transport vehicles for lipids in blood plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization.



Five major classes of lipoproteins are identified in human plasma, based on their separation by electrophoresis.

1. Chylomicrons: They are synthesized in the intestine and transport exogenous (dietary) triacylglycerol to various tissues. They consist of highest (99%) quantity of lipid and lowest (1%) concentration of protein. The chylomicrons are the least in density and the largest in size, among the lipoproteins.
2. Very low density lipoproteins (VLDL): They are produced in liver and intestine and are responsible for the transport of endogenously synthesized triacylglycerols.
3. Low density lipoproteins (LDL): They are formed from VLDL in the blood circulation. They transport cholesterol from liver to other tissues.

4. High density lipoproteins (HDL): They are mostly synthesized in liver. Three different fractions of HDL (1, 2 and 3) can be identified by ultracentrifugation HDL particles transport cholesterol from peripheral tissues to liver (reverse cholesterol transport).
5. Free fatty acids-albumin: Free fatty acids in the circulation are in a bound form to albumin. Each molecule of albumin can hold about 20-30 molecules of free fatty acids. This lipoprotein cannot be separated by electrophoresis.

Apolipoproteins (apoproteins)

The protein components of lipoproteins are known as apolipoproteins or, simply, apoproteins. They perform the following functions

1. Act ass tructuracl omponentso f lipoproteins.
2. Recognize the cell membrane surface receptors.
3. Activate enzymes involved in lipoprotein metabolism.

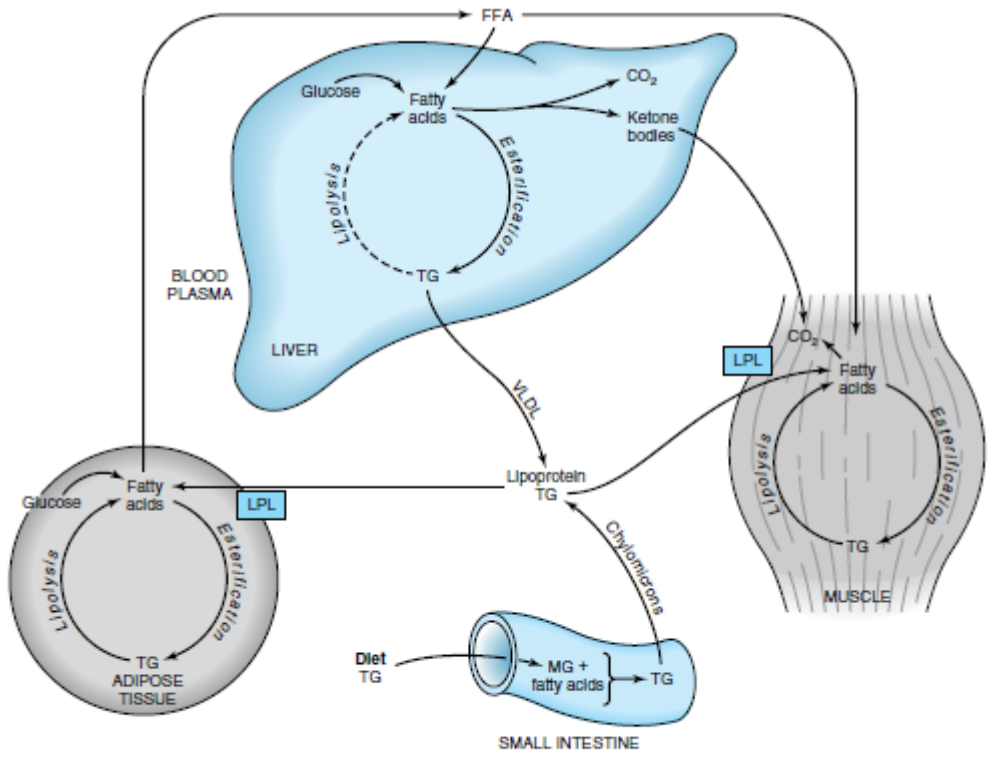
Biomedical Importance

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. They have the common property of being (1) relatively insoluble in water and (2) soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves. Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria, and serving also as the means of transporting lipids in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, eg, obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

Fate of dietary lipids

Lipids in the diet are mainly triacylglycerol and are hydrolyzed to monoacylglycerols and fatty acids in the gut, then reesterified in the intestinal mucosa. Here they are packaged with protein and secreted into the lymphatic system and thence into the blood stream as chylomicrons,

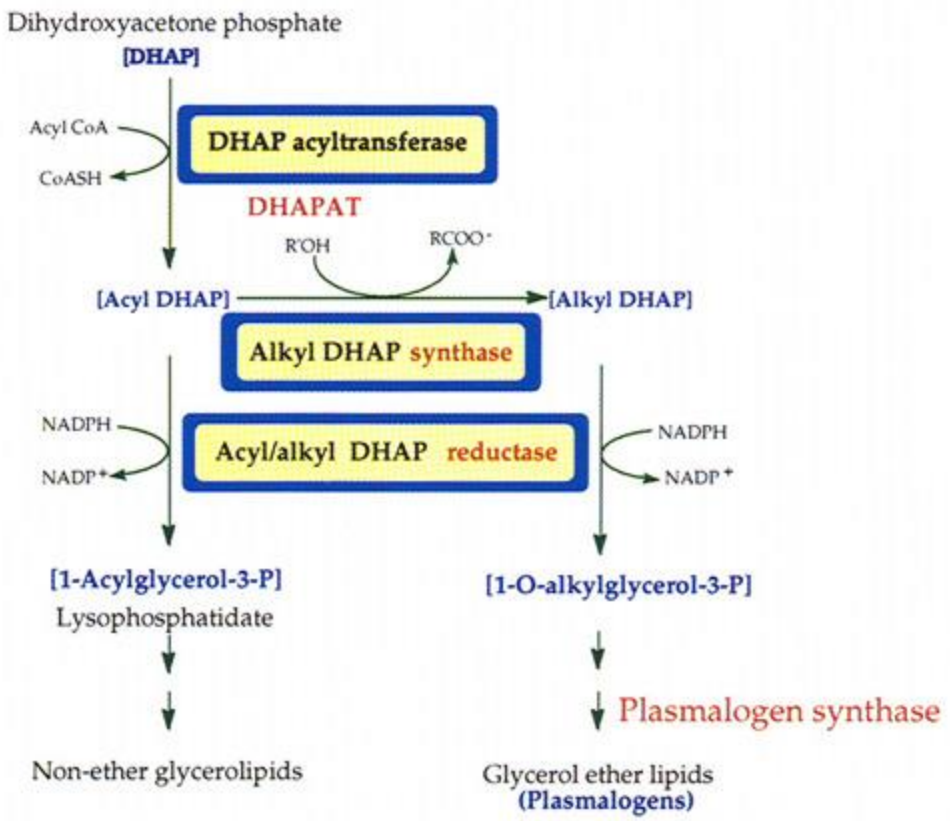
the largest of the plasma lipoproteins. Chylomicrons also contain other lipidsoluble nutrients, eg, vitamins. Unlike glucose and amino acids, chylomicron triacylglycerol is not taken up directly by the liver. It is first metabolized by tissues that have lipoprotein lipase, which hydrolyzes the triacylglycerol, releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel. The other major source of long-chain fatty acid is synthesis (lipogenesis) from carbohydrate, mainly in adipose tissue and the liver.



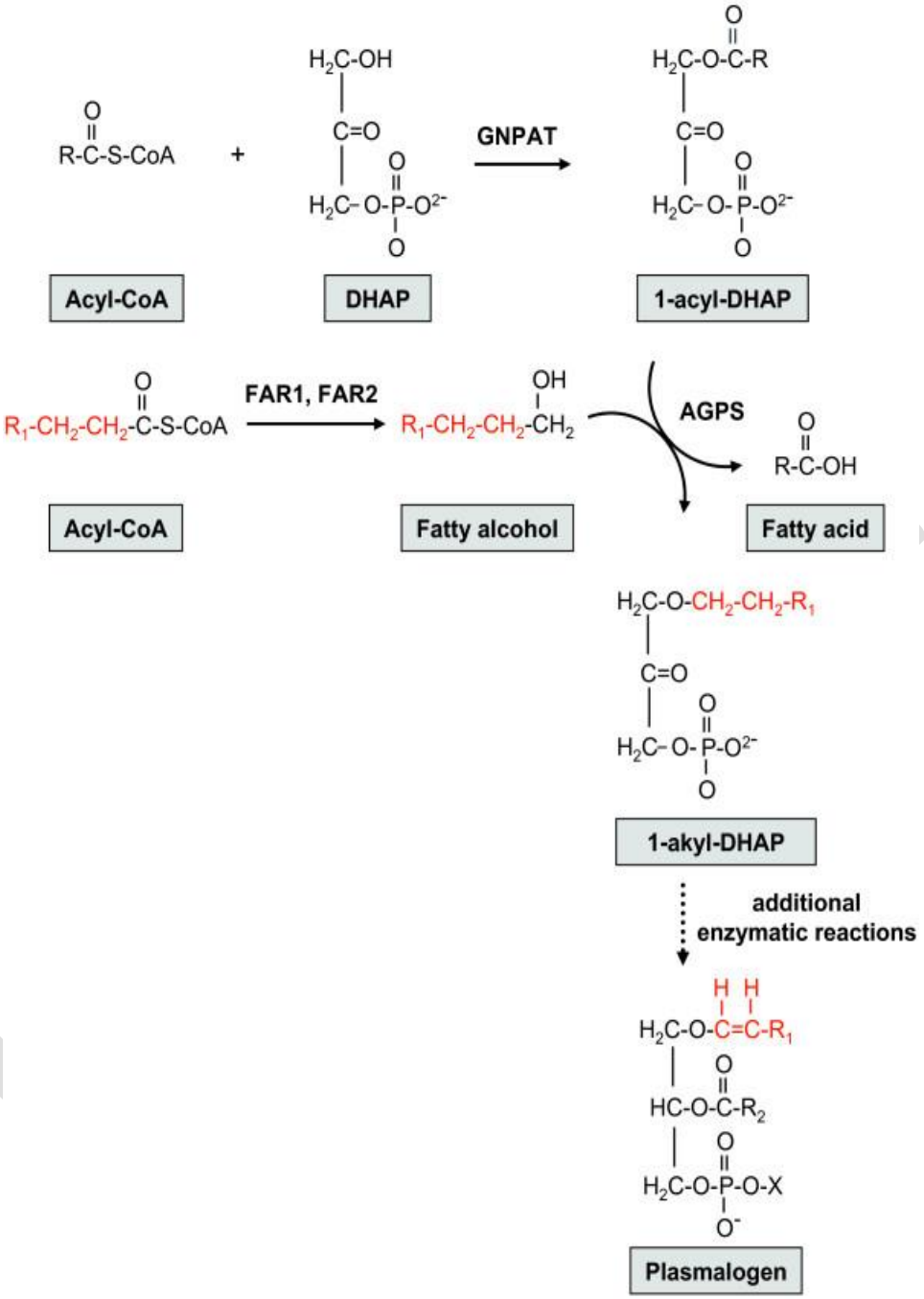
Transport and fate of major lipid substrates and metabolites. (FFA, free fatty acids; LPL, lipoprotein lipase; MG, monoacylglycerol; TG, triacylglycerol; VLDL, very low density lipoprotein.)

Adipose tissue triacylglycerol is the main fuel reserve of the body. On hydrolysis (lipolysis) free fatty acids are released into the circulation. These are taken up by most tissues (but not brain or erythrocytes) and esterified to acylglycerols or oxidized as a fuel. In the liver, triacylglycerol arising from lipogenesis, free fatty acids, and chylomicron remnants is secreted into the circulation as very low density lipoprotein (VLDL). This triacylglycerol undergoes a fate similar to that of chylomicrons. Partial oxidation of fatty acids in the liver leads to ketone body production (keto- genesis). Ketone bodies are transported to extrahepatic tissues, where they act as a fuel source in starvation.

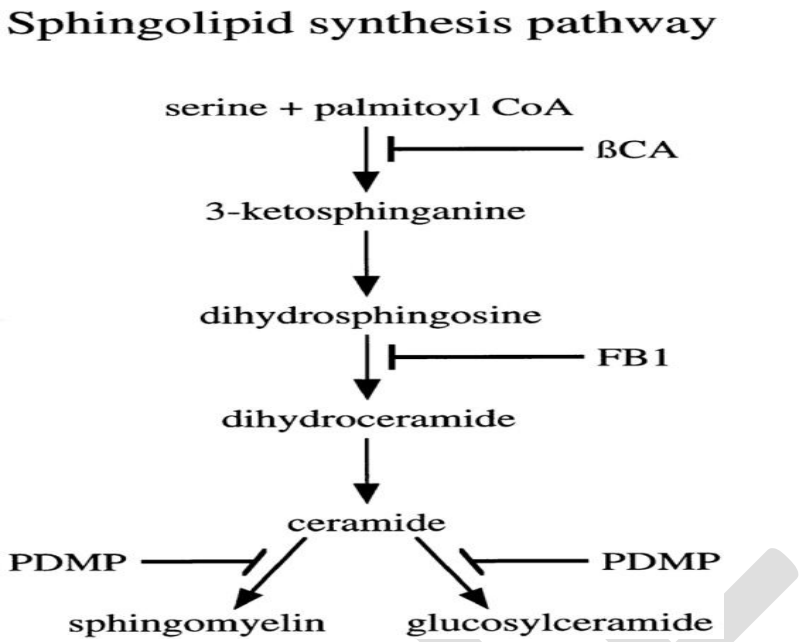
BIOSYNTHESIS OF PLASMALOGENS



Acyl dihydroxyacetone phosphate pathway.



SPHINGOLIPIDS



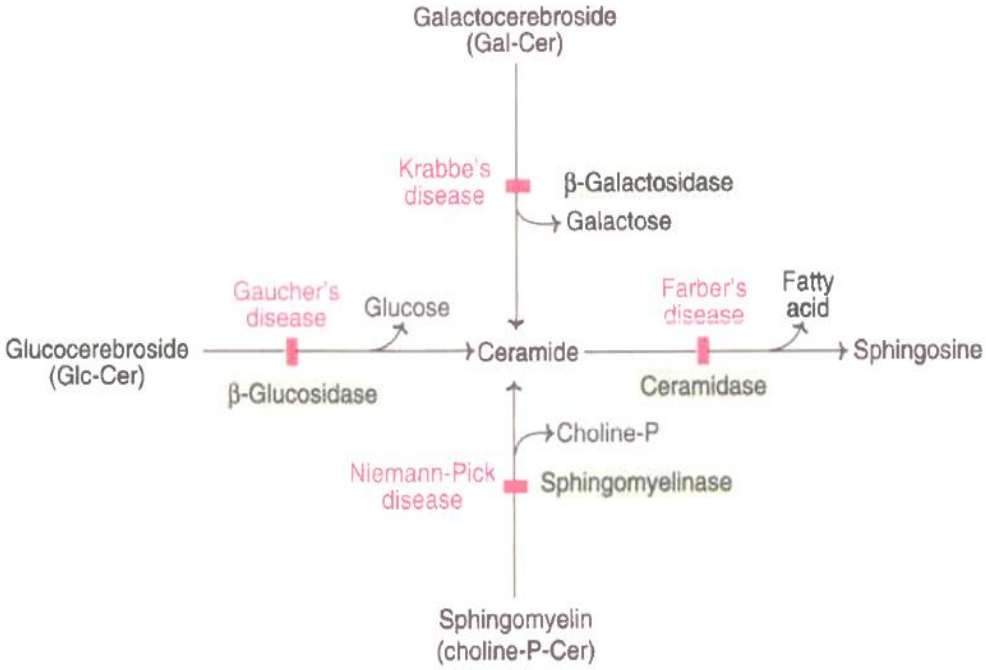
Degradation of sphingomyelins

The enzyme sphingomyelinase of lysosomes hydrolyses sphingomyelins to ceramide and phosphorylcholine. Ceramide formed can be further degraded to sphingosine and free fatty acid. Niemann-Pick disease: It is an inherited disorder due to a defect in the enzyme sphingomyelinase. This causes accumulation of sphingomyelins in liver and spleen, resulting in the enlargement of these organs. Victims of Niemann-Pick disease suffer from severe mental retardation, and death may occur in early childhood.

Farber's disease: A defect in the enzyme ceramidase results in Farber's disease. This disorder is characterized by skeletal deformation, subcutaneous nodules, dermatitis and mental retardation. It is fatal in early life"

BIOSYNTHESIS OF GLYCOLIPIDS

Glycolipids are derivatives of ceramide (sphingosine bound to fatty acid), hence they are more appropriately known as glycosphingolipids. The simplest form of glycosphingo lipids are cerebrosides containing ceramide bound to monosaccharides. Galactocerebroside (Gal-Cer) and glucocerebrosid (Glu-Cer) are the common glycosphingolipids Galactocerebrosidies a major component of membrane lipids in the nervous tissue (high in myelin sheath). Glucocerebroside is an intermediate in the synthesis and degradation of complex glycosphingo lipids.



LIPID STORAGE DISEASES

These are a group of inherited diseases that are often manifested in childhood due to deficiency of sphingolipid degrading enzymes (in lysosomes) .

all are autosomal recessive disorders except Fabry disease (X-linked)

Common features:

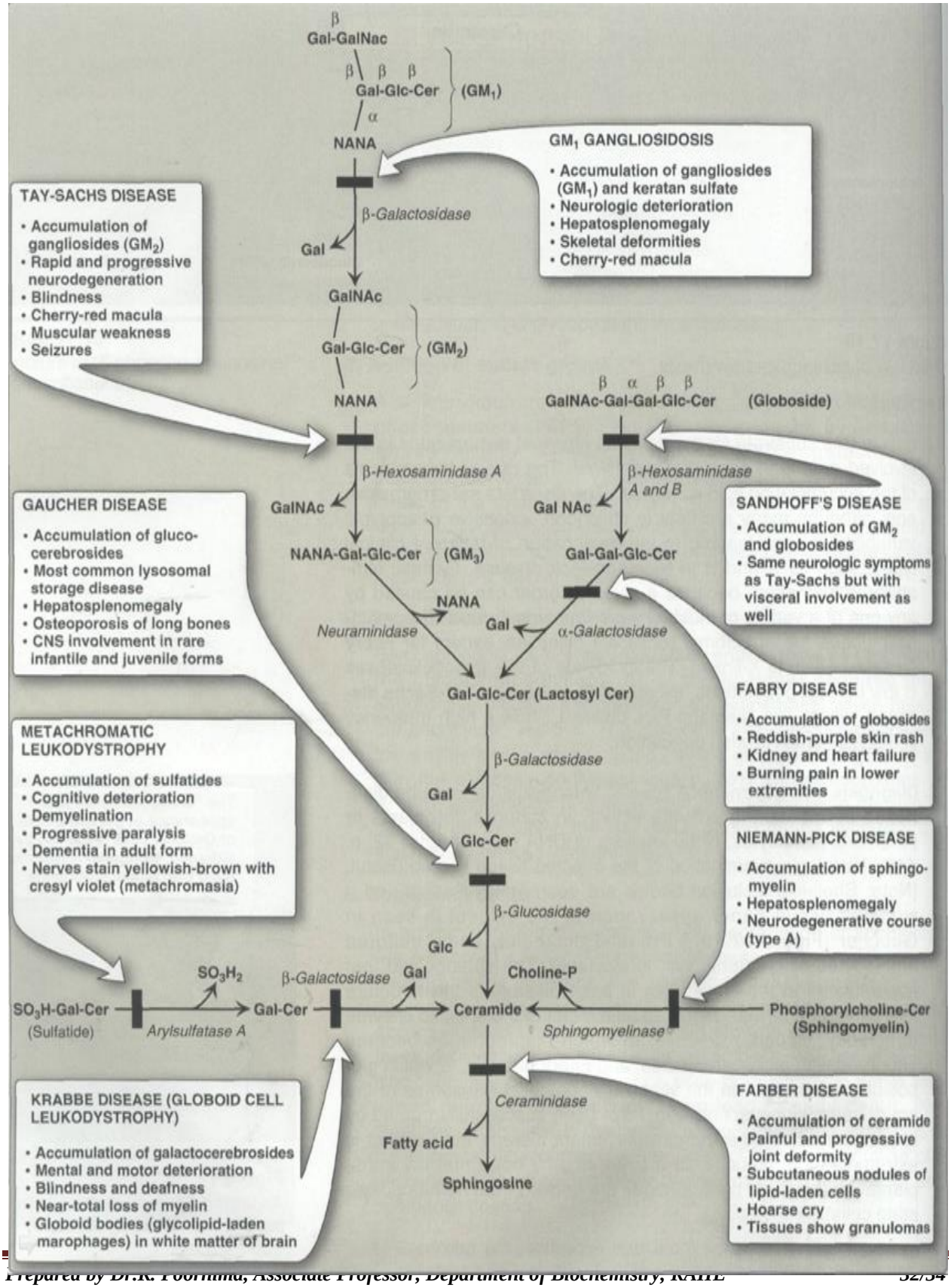
(1) Complex lipids containing ceramide accumulate in cells, particularly neurons, causing neurodegeneration and shortening the life span.

(2) The rate of synthesis of the stored lipid is normal.

(3) The enzymatic defect is in the lysosomal degradation pathway of sphingolipids.

Disease	Enzyme Deficiency	Lipid Accumulating[1]	Clinical Symptoms
Tay-Sachs disease	Hexosaminidase A	Cer—Glc—Gal(NeuAc)—GalNAc G_{M2} Ganglioside	Mental retardation, blindness, muscular weakness.
Fabry's disease	α-Galactosidase	Cer—Glc—Gal—Gal Globotriaosylceramide	Skin rash, kidney failure (full symptoms only in males; X-linked recessive).
Metachromatic leukodystrophy	Arylsulfatase A	Cer—Gal—OSO_3 3-Sulfogalactosylceramide	Mental retardation and psychologic disturbances in adults; demyelination.
Krabbe's disease	β-Galactosidase	Cer—Gal Galactosylceramide	Mental retardation; myelin almost absent.
Gaucher's disease	β-Glucosidase	Cer—Glc Glucosylceramide	Enlarged liver and spleen, erosion of long bones, mental retardation in infants.
Niemann-Pick disease	Sphingomyelinase	Cer—P—choline Sphingomyelin	Enlarged liver and spleen, mental retardation; fatal in early life.
Farber's disease	Ceramidase	Acyl—Sphingosine Ceramide	Hoarseness, dermatitis, skeletal deformation, mental retardation; fatal in early life.

[1]NeuAc, *N*-acetylneuraminic acid; Cer, ceramide; Glc, glucose; Gal, galactose. —, site of deficient enzyme reaction.


β
Gal-GalNac
β β β
Gal-Glc-Cer (GM1)
α
NANA
GM1 GANGLIOSIDOSIS
• Accumulation of gangliosides (GM1) and keratan sulfate
• Neurologic deterioration
• Hepatosplenomegaly
• Skeletal deformities
• Cherry-red macula
TAY-SACHS DISEASE
• Accumulation of gangliosides (GM2)
• Rapid and progressive neurodegeneration
• Blindness
• Cherry-red macula
• Muscular weakness
• Seizures
β-Galactosidase
Gal
GalNAc
Gal-Glc-Cer (GM2)
NANA
β α β β
GalNAc-Gal-Gal-Glc-Cer (Globoside)
β-Hexosaminidase A
β-Hexosaminidase A and B
GalNAc
Gal NAc
GAUCHER DISEASE
• Accumulation of gluco-cerebrosides
• Most common lysosomal storage disease
• Hepatosplenomegaly
• Osteoporosis of long bones
• CNS involvement in rare infantile and juvenile forms
NANA-Gal-Glc-Cer (GM3)
Gal-Gal-Glc-Cer
SANDHOFF'S DISEASE
• Accumulation of GM2 and globosides
• Same neurologic symptoms as Tay-Sachs but with visceral involvement as well
NANA
Neuraminidase
Gal
α-Galactosidase
Gal-Glc-Cer (Lactosyl Cer)
FABRY DISEASE
• Accumulation of globosides
• Reddish-purple skin rash
• Kidney and heart failure
• Burning pain in lower extremities
METACHROMATIC LEUKODYSTROPHY
• Accumulation of sulfatides
• Cognitive deterioration
• Demyelination
• Progressive paralysis
• Dementia in adult form
• Nerves stain yellowish-brown with cresyl violet (metachromasia)
β-Galactosidase
Gal
Glc-Cer
β-Glucosidase
Glc
NIEMANN-PICK DISEASE
• Accumulation of sphingo-myelin
• Hepatosplenomegaly
• Neurodegenerative course (type A)
SO3H2
β-Galactosidase
Gal
Choline-P
SO3H-Gal-Cer
(Sulfatide)
Arylsulfatase A
Gal-Cer
Ceramide
Sphingomyelinase
Phosphorylcholine-Cer
(Sphingomyelin)
KRABBE DISEASE (GLOBOID CELL LEUKODYSTROPHY)
• Accumulation of galactocerebrosides
• Mental and motor deterioration
• Blindness and deafness
• Near-total loss of myelin
• Globoid bodies (glycolipid-laden marophages) in white matter of brain
Ceraminidase
FARBER DISEASE
• Accumulation of ceramide
• Painful and progressive joint deformity
• Subcutaneous nodules of lipid-laden cells
• Hoarse cry
• Tissues show granulomas
Fatty acid
Sphingosine

Metabolic disorders of cerebrosides

Gaucher's disease: This is due to a defect in the enzyme β-glucosidase. As a result, tissue glucocerebroside levels increase. This disorder is commonly associated with enlargement of liver and spleen, osteoporosis, pigmentation of skin, anemia, mental retardation etc. Sometimes, Gaucher's disease is fatal.

Krabbe's disease: Defect in the enzyme β-galactosidase results in the accumulation of galactocerebrosides. A total absence of myelin in the nervous tissue is a common feature. Severe mental retardation, convulsions, blindness, deafness etc. are seen. Krabbe's disease is fatal in early life.

Gangliosides are complex glycosphingolipids mostly found in ganglion cells. They contain one or more molecules of N-acetylneuraminic acid (NANA) bound ceramide ligosaccharides Defect in the degradation of gangliosides causes gangliosidosis, Tay-Sach's disease etc.

Sphinognlipidoses: Lipid storage diseases, representing Iysosomal storage defects, are inherited disorders. They are characterized by the accumulation of complex lipids. The term sphingolipidoses is often used to collectively refer to the genetic disorders that lead to the accumulation of any one of the sphingolipids (glycosphingolipids and sphingomyelins).

POSSIBLE QUESTIONS
UNIT V

PART A (1 Mark)
Question number 1 – 20 (Online Examination)

PART B (2 Marks)

1. Write a note on Lecotriens
2. List the importance of prostaglandins
3. Draw the structure of cholesterol
4. List te membrane phospholipids
5. Add note on sphingo lipids
6. Add note glucose homeostatis
7. Give a note on glycolipids
8. List out lipid storage diseases
9. Give a account on synthesis of steroids
10. Regulation of cholesterol synthesis

PART C (6 Marks)

1. Discuss in detail about the synthesis of prostagladins
2. Give a detailed account on synthesis of sphingolipids and glycolipids
3. Write notes on Synthesis of steroids and isoprenoids
4. List out the lipid storage diseases
5. Write notes on leukotrienes and thromboxanes
6. Give a detail account on biosynthesis of triacylglycerol
7. Give a detail account on five phases of glucose homeostasis.
8. Explain the biosynthesis of cholesterol

Karpagam Academy of Higher Education
Department of Biochemistry
Metabolism of Carbohydrates and Lipids (17BCU301) MCQ
UNIT V

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	5	Which enzyme is involved in lipid digesion?	Elastase	lactase	Lipase	Lactate dehydrogenase	Lipase
2	5	Digesion of triglycerides requires _____	Bile salts	Bile pigments	Intrinsic factor	Bile acids	Bile salts
3	5	Absorption of fats occurs mainly in_____	Stomach	Duodenum	Jejunum	Ileum	Jejunum
4	5	Fatty acids are degraded mainly by_____	ω-oxidation	α- oxidation	β-oxidation	HMP shunt	β-oxidation
5	5	Majority of the absorbed fat appears in the form of_____	VLDL	LDL	HDL	Chylomicrones	Chylomicrones
6	5	The end product of fatty acid synthesis in mammals is_____	Arachidonic acid	Linoleic acid	Stearic acid	Palmitic acid	Stearic acid
7	5	The key regulatory enzyme of fatty acid synthesis is _____	Acyl coA synthetase	Acetyl coA carboxylase	Keto acyl synthase	Thioesterase	Acetyl coA carboxylase
8	5	NADPH required for fatty acid synthesis can be generated from _____	HMP shunt	Glycolysis	TCA cycle	Urea cycle	HMP shunt
9	5	Which of the following inhibits the acetylCoA carboxylase a rate limiting enzymes of carbohydrate metabolism?	Citrate	ATP	Malonyl CoA	Acyl CoA	Acyl CoA
10	5	Malonyl-CoA A is a direct inhibitor of which enzyme of fatty acid					
11	5	oxidation_____	Carnitine Acyl Transferase –I	Carnitine Acyl Transferase –II	Thiokinase	Acyl co A synthetase	Carnitine Acyl Transferase –I
12	5	Cholestrol is the precursor of_____	Steroid hormones	Vitamin A	Urea	Folic acid	Steroid hormones
13	5	The committed step in cholesterol biosynthesis	Formation of squaline	Formation of HMG CoA	Formation of mevalonic acid from HMG CoA	Cyclisation of squaline to lanodterol	Formation of mevalonic acid from HMG CoA
14	5	The principle building block of fatty acid is _____	Succynyl CoA	Acetyl CoA	Propionyl CoA	Acetoacetyl CoA	Acetyl CoA
15	5	Biosynthesis of fatty acid requires which vitamin?	Riboflavin	Pyridoxine	Thiamin	Pantothenic acid	Pantothenic acid
16	5	ACP is involved in the synthesis of _____	Phospholipids	Fatty acids	Glycogen	Triglycerides	Fatty acids
17	5	The main catabolic end product of cholesterol is _____	Acetyl CoA	Propionyl CoA	Coprosterol	Bile acids	Bile acids
18	5	The fattyacid synthase complex comprises two monomers, each containing_____	2 enzymes	5 enzymes	7 enzymes	10 enzymes	7 enzymes
19	5						
20	5	Bile acid are derived from_____	Cholesterol	Amino acids	Fatty acids	Bilirubin	Cholesterol
21	5	The major storage form of lipids is_____	Esterified cholesterol	Glycerophospholipids	Triglycerides	Sphingolipids	Triglycerides
22	5	The principal precursors of glycerophospholipids are	Phospholipids	Spingolipids	Diacylglycerols	Spingomyelins	Diacylglycerols
23	5	The important lipid involved in cell adhesion and cell recognition is	Phospholipids	Cholesterol	Glycospingolipids	Ceramide	Glycospingolipids
24	5	Acyl Carrier Protein contains the vitamin_____	Biotin	Lipoic acid	Pantothenic acid	Folic acid	Pantothenic acid
25	5	The starting material for the process of ketogenesis is_____	Acetyl CoA	Oxaloacetate	Pyruvate	Citrate	Acetyl CoA
26	5	Which among the following is the most complex sphingolipid_____	Cerebroside	Gangleoside	Globoside	Ceramide	Gangleoside
27	5	How many double bonds occur in Arachidonic acid_____	1	2	3	4	4
28	5	Which of the following is essential fatty acid_____	Linolenic acid	Arachidonic acid	Oleic acid	Palmitic acid	Linolenic acid
29	5	A genetic disorder caused by the accumulation of sphingomyelin in brain is called_____	Tay-Sach syndrome	Gout	Niemann-Pick Disease	Gauche's disease	Niemann-Pick Disease
30	5	Lipid molecule involved in the bio-signaling pathway that include membrane turnover and exocytosis is _____.	Phosphatidylinositol	Phosphatidyl glycerol	Myoinositol	Phosphatidyl glycerol and Myoinositol	Phosphatidylinositol
31	5	Most abundant membrane lipid in the biosphere is_____	Phospholipid	Galactolipid	Sphingolipid	Ether lipid	Galactolipid
32	5	What is the molecular formula of cholesterol?	$C_{27}H_{45}OH$	$C_{28}H_{47}OH$	$C_{29}H_{47}OH$	$C_{23}H_{41}OH$	$C_{27}H_{45}OH$
33	5	Enzymes for beta oxidation of fatty acids are located in_____	Mitochondria	Mitochondria and cytoplasm	Mitochondria and Golgi	Mitochondria and peroxisome	Mitochondria
34	5	Cerebroside may also classified as____	Phospholipid	Sphingolipid	Aminolipid	Glycolipid	Sphingolipid
35	5	Glyco-sphingolipids are a combination of_____	Glycerol with two galactose residues	Ceramide with one or more sugar residues	Sphingosine with galactose and ceramide	Sphingosine with glucose	Ceramide with one or more sugar residues
36	5	Spingomyelins contain a complex amino alcohol named as_____	Serine	Lysolecithin	Spingosine	Glycol	Spingosine
37	5						
38	5	The key regulatory enzyme of cholesterol synthesis is_____	HMG- Co A synthase	HMG Co A lyase	HMG Co A reductase	Mevalonate kinase	HMG Co A reductase
39	5	The enzyme 'Thiolase' catalyzes the conversion of_____	2 Acetyl co A to Acetoacetyl co A	Acetyl co A to Malonyl co A	Fatty acid to Fatty Acyl co A	Succinyl co A to succinate	2 Acetyl co A to Acetoacetyl co A
40	5	The enzyme involved in mammalian signal transduction is	Phospholipase A	Phospholipase B	Phospholipase C	Phospholipase D	Phospholipase D
41	5	In alpha oxidation which of the following products is released ?	Co A	CO_2	H_2O	Acetyl co A	CO_2
42	5	Which of the following is a break down product of odd chain fatty acids?	Acetyl co A only	Acetyl co A and Butyryl co A	Acetyl co A and Propionyl co A	Malonyl co A	Acetyl co A and Propionyl co A
43	5	All the 27 carbon atoms of cholesterol are derived from_____	Acetyl co A	Acetoacetyl co A	Propionyl co A	Succinyl co A	Acetyl co A
44	5	NADPH is synthesized by the action of which of the following enzymes?	Glucose-6-P dehydrogenase	Pyruvate dehydrogenase	Acetyl co A carboxylase	Lipoprotein lipase	Glucose-6-P dehydrogenase
45	5	How many carbons are removed from fatty acyl co A in one turn of β- oxidation spiral ?	1	2	3	4	2
46	5	What is the role of Thiolase in the β- oxidation of fatty acids?	Cleaves of Co A	Cleaves the bond between α- and β-carbons	Adds H_2O across the double bond	Generates NADH	Cleaves the bond between α- and β-carbons
47	5	The key enzyme for the utilization of ketone bodies is_____	Thiolase	Thiophorase	Thiokinase	Thioesterase	Thiophorase
48	5	High content of triglycerides are seen in_____	LDL	HDL	VLDL	Chylomicrones	Chylomicrones
49	5	Gangliosides are glycolipids occurring in_____	Liver	Brain	Kidney	Muscle	Brain
50	5	The prostaglandins are synthesized from _____	Aracadonic acid	Oleic acid	Linoleic acid	Linolenic acid	Aracadonic acid
51	5	Prostaglandins are liberated in the circulation by the stimulation of _____	Anterior pitutary glands	Posterior pitutary glands	Adrenal gland	Thyroid gland	Adrenal gland
52	5	The synthesis of prostaglandins is inhibited by_____	Aspirin	Arsenite	Fluoride	Cyanide	Aspirin
53	5	HDL is synthesized and secreted from_____	Pancrease	Liver	Kidney	Muscle	Liver
54	5	Fatty liver caused by_____	CH_3Cl	CCL_4	$MgSO_4$	CH_3COOH	CCL_4
55	5	Ketosis generally occurs in_____	Nephritis	Oedema	Infective hepatic disease	Coronary thrombosis	Infective hepatic disease
56	5	Ketone bodies are utilized in _____	Mitochondria	Extrahepatic tissues	Nuclei	Chromosomes	Extrahepatic tissues
57	5	Eicosanoids are formed from _____	Arachidonate	Palmitate	Stearate	Butyrate	Arachidonate
58	5	The excreation of ketone bodies in the urine involves the deficiency of _____	Na^+	Fe^{++}	Ca^{++}	Mg^{++}	Na^+