KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOTECHNOLOGY

I. M.Sc. BIOTECHNOLOGY (SEMESTER – I)

BIOCHEMISTRY AND MICROBIOLOGY (19BTP101)

SYLLABUS

Unit - 1

Introduction: Chemical basis of life; Composition of living matter; Water – properties, pH, ionization and hydrophobicity; Emergent properties of biomolecules in water; Biomolecular hierarchy; Biomolecules - Structure and properties of carbohydrates, amino acids, proteins, fatty acids, nucleosides, nucleotides, polynucleotides, Ribonucleic acids and deoxy ribonucleic acids, nucleoprotein complexes.

Unit - 2

Metabolisms: carbohydrates, lipids (fatty acid oxidation and biosynthesis), amino acids biosynthesis, nucleotides (de novo synthesis and salvage pathways). Disorders of lipid, carbohydrate, nucleic acid, amino acid metabolism. Inborn errors of metabolism. Metabolomics.

Unit - 3

Bioenergetics: TCA Cycle, glycolysis, gluconeogenesis, Pentose phosphate shunt, Embden-Meyerhof pathway, urea cycle, interconnection of pathways, Metabolic regulation, Bioenergetics: Respiratory chain, ATP cycle, energy-rich compounds.

Unit - 4

Microbial Diversity and Techniques: Diversity- Bacteria, fungi, algae - distribution, reproduction, characteristics, nutrition. Techniques - staining, Microscopy - Principle, types, applications. Microbial growth - nutrients, media, isolation, maintenance, preservation, curve, measurements, factors, regulation.

Unit - 5

Applications, Diseases and Control measures: Causative agent, pathology, diagnosis, control and treatment of Bacterial - TB, Cholera and Typhoid. Protozoan – Amoebiasis and Malaria. Viral - AIDS. Control of microorganisms – drugs, chemotheraphy, antimicrobial agents.

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II. M.Sc. BIOTECHNOLOGY (SEMESTER – III)

LECTURE PLAN- BIOCHEMISTRY AND MICROBIOLOGY (19BTP101)

S. No.	Lecture duration (Hr)	Topic to be covered	Support materials/ Page no.
UNIT - I			
1	1	**Introduction:** Chemical basis of life; Composition of living matter	T1: 2-12; T2: 43-70
2	1	Water – properties, pH, ionization and hydrophobicity;	T1: 22-37; T2: 43-70
3	1	Emergent properties of biomolecules in water	
4	1	Structure and properties of carbohydrates,	T3: 64-72
5	1	amino acids	T1: 67-78
6	1	proteins	R1: 1-119
7	1	fatty acids	T1: 386-448
8	1	nucleosides, nucleotides, polynucleotides, Ribonucleic acids and deoxy ribonucleic acids	T2: 271-302
9	1	nucleoprotein complexes	T3: 107-110
UNIT - II			
10	1	**Metabolisms:** carbohydrates	T2: 527-646
11	1	lipids (fatty acid oxidation and biosynthesis)	T1: 940-980; T2: 413-509
12	1	amino acids biosynthesis	T2: 413-509
13	1	nucleotides (de novo synthesis and salvage pathways)	T1: 789-822; T4: 293-302
14	1	Disorders of lipid	T3: 239-245
15	1	carbohydrate	T2: 527-646
16	1	nucleic acid	T3: 239-245
17	1	amino acid metabolism	T2: 413-509

18	1	Inborn errors of metabolism. Metabolomics	T4: 293-302
UNIT – III			
19	1	**Bioenergetics**: TCA Cycle	T2: 527-646; T3: 112-125
20	1	glycolysis, gluconeogenesis	T1: 593-637
21	1	Pentose phosphate shunt	T2: 527-568
22	1	Embden- Meyerhof pathway	T3: 134-140
23	1	urea cycle, interconnection of pathways	T1: 789-822
24	1	Metabolic regulation	T3: 210
25	1	Bioenergetics: Respiratory chain	T4: 80-84
26	1	ATP cycle	T3: 239-245
27	1	Energy-rich compounds	T2: 569-646
UNIT – IV			
28	1	**Microbial Diversity and Techniques**: Diversity, distribution, reproduction, characteristics, nutrition of Bacteria	T5: 3-72
29	1	Fungi	
30	1	Algae	
31	1	Staining techniques	T6: 55-75
32	1	Microscopy - Principle, types, applications	T6: 872-899
33	1	Microbial growth - nutrients, media	T5: 73-133
34	1	Isolation, maintenance, preservation	
35	1	Growth curve measurements	
36	1	Factors and regulation	
37	1	Revision	
UNIT – V			
38	1	**Applications, Diseases and Control measures:** Causative agent, pathology, diagnosis, control and treatment of Bacterial - TB	T5: 788-823; T6: 609-634
39	1	Cholera	T5: 788-823
40	1	Typhoid	

41	1	Protozoan – Amoebiasis	T5: 850-876
42	1	Malaria	
43	1	Viral - AIDS	T5: 824-849
44	1	Control of microorganisms – drugs	T5: 469-542
45	1	chemotherapy	
46	1	antimicrobial agents	
47	1	Revision	
48	1	Test	

Textbooks

T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

T2: Nelson, D.L., & Cox, M.M. (2013). Lehninger: *Principles of Biochemistry* (6th ed.). New York: W.H. Freeman and Company.

T3: Fundamentals of Biochemistry (S. Chand & Co, 2002) – J.L.Jain.

T4: Murray, R.K., Bender, D.A., Botham, K.M.,& Kennelly, P.J., (2012). *Harper's illustrated Biochemistry* (29th ed.). London : McGraw-Hill Medical.

T5: Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993). *Microbiology* (5th ed.). McGraw Hill Book Company.

T6: Dubey, R.C., Maheshwari, D.K.(2006). A textbook of microbiology (1^{st} ed.). S. Chand & Company Ltd. New Delhi.

Reference

R1: Introduction to Protein Structure (Taylor & Francis Group, 1999) – Branden & Tooze

UNIT - I

SYLLABUS

Introduction: Chemical basis of life; Composition of living matter; Water – properties, pH, ionization and hydrophobicity; Emergent properties of biomolecules in water; Biomolecular hierarchy; Biomolecules - Structure and properties of carbohydrates, amino acids, proteins, fatty acids, nucleosides, nucleotides, polynucleotides, Ribonucleic acids and deoxy ribonucleic acids, nucleoprotein complexes.

KAHE

Carbon is Important to Life

In its metabolism of food and respiration, an animal consumes glucose ($C_6H_{12}O_6$), which combines with oxygen (O_2) to produce carbon dioxide (CO_2), water (H_2O), and energy, which is given off as heat. The animal has no need for the carbon dioxide and releases it into the atmosphere. A plant, on the other hand, uses the opposite reaction of an animal through photosynthesis. It intakes carbon dioxide, water, and energy from sunlight to make its own glucose and oxygen gas. The glucose is used for chemical energy, which the plant metabolizes in a similar way to an animal. The plant then emits the remaining oxygen into the environment. Cells are made of many complex molecules called macromolecules, which include proteins, nucleic acids (RNA and DNA), carbohydrates, and lipids. The macromolecules are a subset of organic molecules (any carbon-containing liquid, solid, or gas) that are especially important for life. The fundamental component for all of these macromolecules is carbon. The carbon atom has unique properties that allow it to form covalent bonds to as many as four different atoms, making this versatile element ideal to serve as the basic structural component, or "backbone," of the macromolecules.

Structure of Carbon

Individual carbon atoms have an incomplete outermost electron shell. With an atomic number of 6 (six electrons and six protons), the first two electrons fill the inner shell, leaving four in the second shell. Therefore, carbon atoms can form four covalent bonds with other atoms to satisfy the octet rule. The methane molecule provides an example: it has the chemical formula CH_4. Each of its four hydrogen atoms forms a single covalent bond with the carbon atom by sharing a pair of electrons. This results in a filled outermost shell.

Table 1: The frequency of elements in the earth's crust and in the human body

frequency			
in the earth's crust		in the human body	
element	atomic %	element	atomic %
O	62.5	H	60.3
Si	21.2	O	25.5
Al	6.47	C	10.5
Na	2.64	N	2.42
Ca	1.94	Na	0.73
Fe	1,92	Ca	0.23
Mg	1.84	P	0.13
P	1.42	S	0.13
C	0.08	K	0.04
N	0.0001	Cl	0.03

Among the elements in the earth's crust, oxygen, silicon and metals occur in the largest quantity. Living matter consists of four elements in 99%, these are hydrogen, oxygen, carbon and nitrogen. These four elements are called organogenic elements. Organogenic elements rank among non-metallic elements. There is a wide variety of their molecules formed by covalent bond. The existence of the numerous varieties of their molecules can be explained by the specific properties of carbon atom (it can form single and double bond as well with itself, nitrogen and oxygen) Phosphorus and sulphur are also essential in the construction of the living matters. Phosphorus can be found mostly in ester ulinkage, while sulphur is attached to the carbon atom by covalent bond. Sulphur and phosphorus together with organogenic elements are called biogenic elements To the normal life function of living organisms of other elements are also essential, but these occur in a much smaller quantity in them. Na, K, Ca, Mg belong to macro elements as they are present in plant in quantities more than 0.1% on a dry matter weight basis, and in humans and animals more than 0,005%. Cl, I, Fe, Zn, Mn, Co, Cu, Mo, Se, B are micro elements while their amount in human organisms is smaller than the above-mentioned quantities. Apart from the listed ones living beings contain other elements but these are not essential. Their biological role is not known yet. The composition of molecular constituents that can be found in the living organisms are represented through the example of Escherichia coli bacterium.

The chemical composition of Escherichia coli bacterium

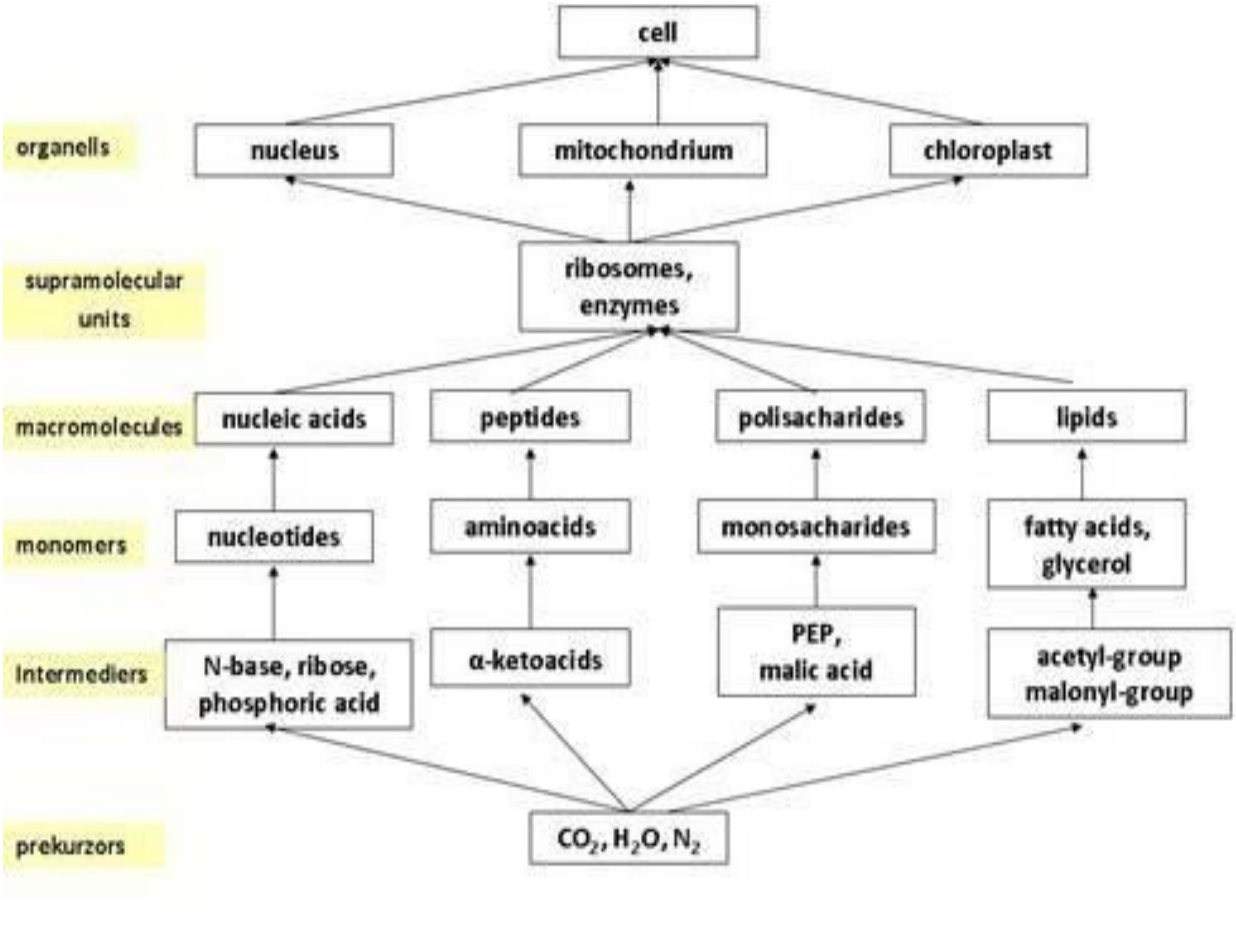
compound	Percentage of total weight of cell %
water	70
proteins	15
nucleic acids	7
carbohydrates	3
lipids	2
other organic matter	2
inorganic ions	1

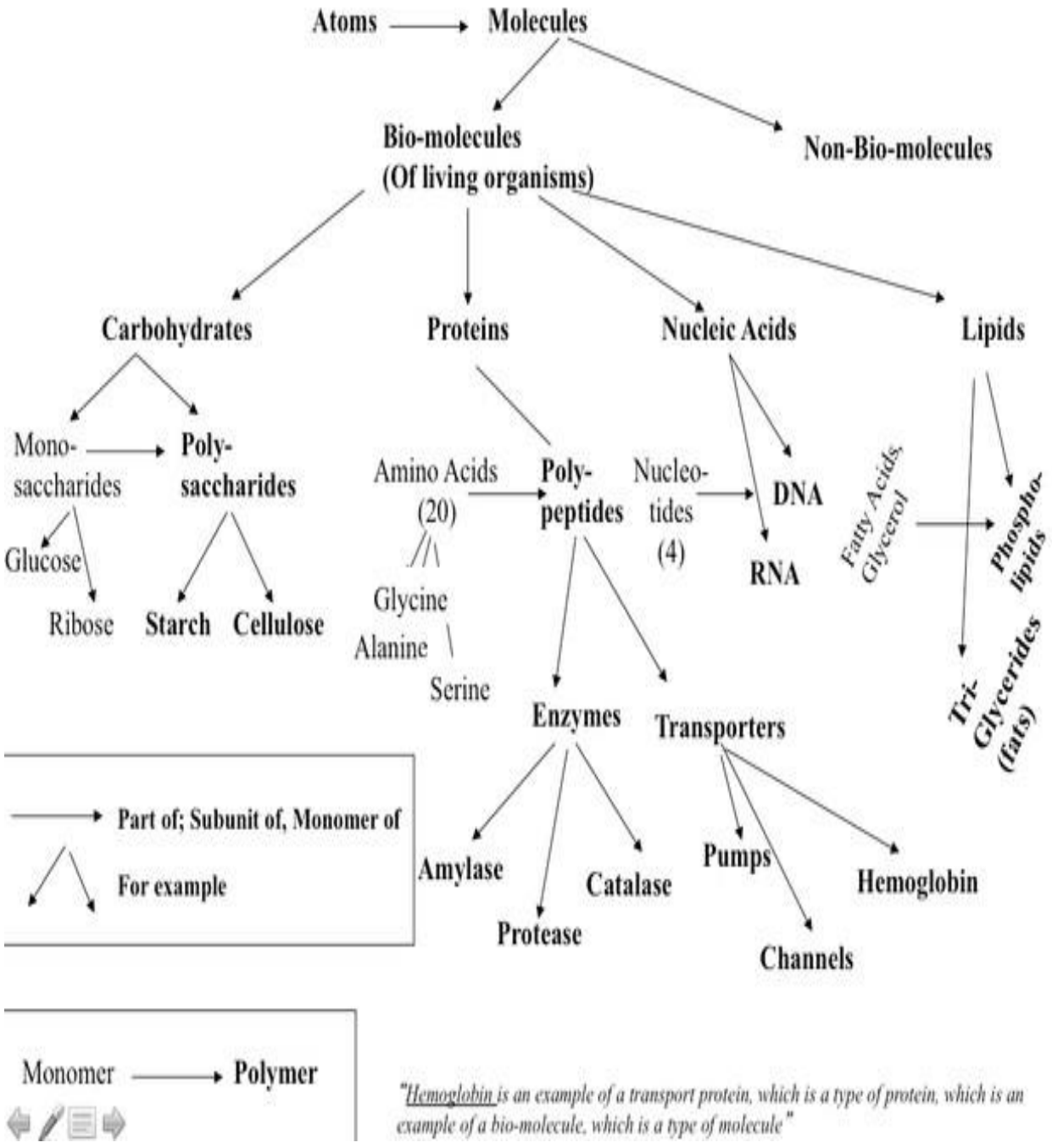
Living organisms contain water in the largest quantities. Among their organic compounds lining organisms contain proteins, carbohydrates, nucleic acids and lipids in a significant quantity. The amount of the other types of organic matter is negligible compared to the mass of these four biomolecule groups. These biomolecules (carbohydrates, lipids, proteins, nucleic acids) are well-separable structurally and functionally, though they have common properties as well. Their common feature is, that they consist of monomer units. These monomer units are connected to each other by water loss reactions (condensation) creating macromolecules. The monomer units of carbohydrates are monosaccharides that of nucleic acids are nucleotides. Proteins formed by the attachment of a lot of amino acids (monomers), while most of the lipids consist of the fatty acid monomers. They are the main characters of metabolism processes. There are wide variety of proteins and nucleic acids. Carbohydrates and lipids do not have so many variants, the number of their monomer units and the variations of the ulinks are fewer. The features of biomolecules differ from inorganic molecules.

The role of water in biological systems

- Water molecules hydrate macromolecules. Hydrogen bonds can be formed between water and macromolecules or macromolecules can possess charges thus polar water molecules surround them forming a hydration shell.
- It is solvent. Water dissolves many kinds of substances such as salts. Among non dissociating substances, water dissolves polar substances. Amphipathic molecules (containing polar and apolar parts) form micelles in water.
- It is a transfer medium, due to the fact that it is a good solvent, it takes part in mass transport between cells, tissues and organs
- It participates in many chemical reactions. It can be reactant or end product in biochemical reactions.
- It plays role in the regulation of heat balance. Due to its high specific heat and vaporization heat, water can buffer the changes of temperature in environment, thus the cells of living organisms can maintain a relatively constant temperature. Due to its high vaporization heat organisms can lose heat during sweating, protecting themselves from warming.

Molecular organizations in cells




Atoms
Molecules
Bio-molecules
(Of living organisms)
Non-Bio-molecules
Carbohydrates
Proteins
Nucleic Acids
Lipids
Mono-saccharides
Poly-saccharides
Glucose
Ribose
Starch
Cellulose
Amino Acids
(20)
Poly-peptides
Glycine
Alanine
Serine
Nucleo-tides
(4)
DNA
RNA
Fatty Acids, Glycerol
Phospho-lipids
Tri-Glycerides (fats)
Enzymes
Transporters
Amylase
Protease
Catalase
Pumps
Channels
Hemoglobin
Part of; Subunit of, Monomer of
For example
Monomer
Polymer
"Hemoglobin is an example of a transport protein, which is a type of protein, which is an example of a bio-molecule, which is a type of molecule"

Properties of water

Water (H_2O) is a polar inorganic compound that is at room temperature a tasteless and odorless liquid, which is nearly colorless apart from a hint of blue. It is by far the most studied chemical compound and is described as the "universal solvent" for its ability to dissolve many substances. This allows it to be the "solvent of life". It is the only common substance to exist as a solid, liquid, and gas on Earth's surface. Water molecules form hydrogen bonds with each other and are strongly polar. This polarity allows it to separate ions in salts and strongly bond to other polar substances such as alcohols and acids, thus dissolving them. Its hydrogen bonding causes its many unique properties, such as having a solid form less dense than its liquid form, a relatively high boiling point of 100 °C for its molar mass, and a high heat capacity. Water is amphoteric, meaning it is both an acid and a base - it produces H^+ and OH^- ions by self ionization. This means the product of the concentrations of H^+ and OH^- has to be a constant. Because water is a very good solvent, it is rarely pure, and some of the properties of impure water can vary from those of the pure substance. There are also many compounds that are essentially, if not completely, insoluble in water, such as fats, oils and other non-polar substances.

Properties

Water is the chemical substance with chemical formula H_2O; one molecule of water has two hydrogen atoms covalently bonded to a single oxygen atom. Water is a tasteless, odorless liquid at ambient temperature and pressure, and appears colorless in small quantities, although it has its own intrinsic very light blue hue. Ice also appears colorless, and water vapor is essentially invisible as a gas. Water is primarily a liquid under standard conditions, which is not predicted from its relationship to other analogous hydrides of the oxygen family in the periodic table, which are gases such as hydrogen sulfide. The elements surrounding oxygen in the periodic table, nitrogen, fluorine, phosphorus, sulfur and chlorine, all combine with hydrogen to produce gases under standard conditions. The reason that water forms liquid is that oxygen is more electronegative than all of these elements with the exception of fluorine. Oxygen attracts electrons much more strongly than hydrogen, resulting in a net positive charge on the hydrogen

atoms, and a net negative charge on the oxygen atom. These atomic charges give each water molecule a net dipole moment. Electrical attraction between water molecules due to this dipole pulls individual molecules closer together, making it more difficult to separate the molecules and therefore raising the boiling point. This attraction is known as hydrogen bonding. The molecules of water are constantly moving in relation to each other, and the hydrogen bonds are continually breaking and reforming at timescales faster than 200 femtoseconds (2×10^{-13} seconds). However, these bonds are strong enough to create many of the peculiar properties of water, some of which make it integral to life.

Density of ice and water as a function of temperature

The density of water is about 1 gram per cubic centimetre (62 lb/cu ft): this relationship was originally used to define the gram. The density varies with temperature, but not linearly: as the temperature increases, the density rises to a peak at 3.98 °C (39.16 °F) and then decreases. This unusual negative thermal expansion below 4 °C (39 °F) is also observed in molten silica. Regular, hexagonal ice is also less dense than liquid water—upon freezing, the density of water decreases by about 9%. These effects are due to the reduction of thermal motion with cooling, which allows water molecules to form more hydrogen bonds that prevent the molecules from coming close to each other. While below 4 °C the breakage of hydrogen bonds due to heating allows water molecules to pack closer despite the increase in the thermal motion (which tends to expand a liquid), above 4 °C water expands as the temperature increases. Water near the boiling point is about 4% less dense than water at 4 °C (39 °F).

Other substances that expand on freezing are acetic acid, silicon, gallium, germanium, bismuth, plutonium and also chemical compounds that form spacious crystal lattices with tetrahedral coordination. Under increasing pressure, ice undergoes a number of transitions to other allotropic forms with higher density than liquid water, such as ice II, ice III, high-density amorphous ice (HDA), and very-high-density amorphous ice (VHDA).

Melting point

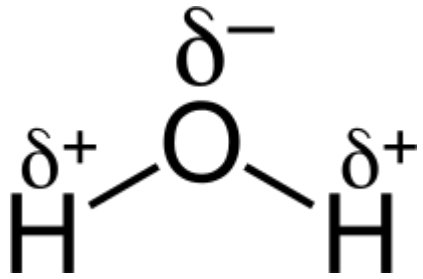
The melting point of ice is 0 °C (32 °F; 273 K) at standard pressure; however, pure liquid water can be super cooled well below that temperature without freezing if the liquid is not

mechanically disturbed. It can remain in a fluid state down to its homogeneous nucleation point of about 231 K (−42 °C; −44 °F). The melting point of ordinary hexagonal ice falls slightly under moderately high pressures, by 0.0073 °C (0.0131 °F)/atm or about 0.5 °C (0.90 °F)/70 atm as the stabilization energy of hydrogen bonding is exceeded by intermolecular repulsion, but as ice transforms into its allotropes (see crystalline states of ice) above 209.9 MPa (2,072 atm), the melting point increases markedly with pressure, i.e., reaching 355 K (82 °C) at 2.216 GPa (21,870 atm) (triple point of Ice VII).

Electrical properties

Pure water containing no exogenous ions is an excellent insulator, but not even "deionized" water is completely free of ions. Water undergoes auto-ionization in the liquid state, when two water molecules form one hydroxide anion (OH^-) and one hydronium cation (H_3O^+). Because water is such a good solvent, it almost always has some solute dissolved in it, often a salt. If water has even a tiny amount of such an impurity, then it can conduct electricity far more readily. It is known that the theoretical maximum electrical resistivity for water is approximately 18.2 MΩ·cm (182 kΩ·m) at 25 °C. This figure agrees well with what is typically seen on reverse osmosis, ultra-filtered and deionized ultra-pure water systems used, for instance, in semiconductor manufacturing plants. A salt or acid contaminant level exceeding even 100 parts per trillion (ppt) in otherwise ultra-pure water begins to noticeably lower its resistivity by up to several kΩ·m. In pure water, sensitive equipment can detect a very slight electrical conductivity of 0.05501 ± 0.0001 μS/cm at 25.00 °C. Water can also be electrolyzed into oxygen and hydrogen gases but in the absence of dissolved ions this is a very slow process, as very little current is conducted. In ice, the primary charge carriers are protons (see proton conductor).[51] Ice was previously thought to have a small but measurable conductivity of 1×10^{-10} S/cm, but this conductivity is now thought to be almost entirely from surface defects, and without those, ice is an insulator with an immeasurably small conductivity.

Polarity, hydrogen bonding and intermolecular structure



A diagram showing the partial charges on the atoms in a water molecule

An important feature of water is its polar nature. The structure has a bent molecular geometry for the two hydrogens from the oxygen vertex. The oxygen atom also has two lone pairs of electrons. One effect usually ascribed to the lone pairs is that the H–O–H gas phase bend angle is 104.48, which is smaller than the typical tetrahedral angle of 109.47°. The lone pairs are closer to the oxygen atom than the electrons sigma bonded to the hydrogens, so they require more space. The increased repulsion of the lone pairs forces the O–H bonds closer to each other. Another effect of the electronic structure is that water is a polar molecule. Due to the difference in electronegativity, there is a bond dipole moment pointing from each H to the O, making the oxygen partially negative and each hydrogen partially positive. In addition, the lone pairs of electrons on the O are in the direction opposite to the hydrogen atoms. This results in a large molecular dipole, pointing from a positive region between the two hydrogen atoms to the negative region of the oxygen atom. The charge differences cause water molecules to be attracted to each other (the relatively positive areas being attracted to the relatively negative areas) and to other polar molecules. This attraction contributes to hydrogen bonding, and explains many of the properties of water, such as solvent action. Although hydrogen bonding is a relatively weak attraction compared to the covalent bonds within the water molecule itself, it is responsible for a number of water's physical properties. These properties include its relatively high melting and boiling point temperatures: more energy is required to break the hydrogen bonds between water molecules. In contrast, hydrogen sulfide (H 2S), has much weaker hydrogen bonding due to sulfur's lower electronegativity. H_2S is a gas at room temperature, in spite of hydrogen sulfide having nearly twice the molar mass of water. The

extra bonding between water molecules also gives liquid water a large specific heat capacity. This high heat capacity makes water a good heat storage medium.

A single water molecule can participate in a maximum of four hydrogen bonds because it can accept two bonds using the lone pairs on oxygen and donate two hydrogen atoms. Other molecules like hydrogen fluoride, ammonia and methanol can also form hydrogen bonds. However, they do not show anomalous thermodynamic, kinetic or structural properties like those observed in water because none of them can form four hydrogen bonds: either they cannot donate or accept hydrogen atoms, or there are steric effects in bulky residues. In water, intermolecular tetrahedral structures form due to the four hydrogen bonds, thereby forming an open structure and a three-dimensional bonding network, resulting in the anomalous decrease in density when cooled below 4 °C. This repeated, constantly reorganizing unit defines a three-dimensional network extending throughout the liquid. This view is based upon neutron scattering studies and computer simulations, and it makes sense in the light of the unambiguously tetrahedral arrangement of water molecules in ice structures. However, there is an alternative theory for the structure of water. In 2004, a controversial paper from Stockholm University suggested that water molecules in liquid form typically bind not to four but to only two others; thus forming chains and rings. The term "string theory of water" (which is not to be confused with the string theory of physics) was coined. These observations were based upon X-ray absorption spectroscopy that probed the local environment of individual oxygen atoms. Water, the team suggests, is a muddle of the two proposed structures. They say that it is a soup flecked with "icebergs" each comprising 100 or so loosely connected molecules that are relatively open and hydrogen bonded. The soup is made of the string structure and the icebergs of the tetrahedral structure.

Water as a solvent

Presence of colloidal calcium carbonate from high concentrations of dissolved lime turns the water of Havasu Falls turquoise. Water is also a good solvent, due to its polarity. Substances that will mix well and dissolve in water (e.g. salts) are known as hydrophilic ("water-loving") substances, while those that do not mix well with water (e.g. fats and oils), are known as hydrophobic ("water-fearing") substances. The ability of a substance to dissolve in water is

determined by whether or not the substance can match or better the strong attractive forces that water molecules generate between other water molecules. If a substance has properties that do not allow it to overcome these strong intermolecular forces, the molecules are "pushed out" from the water, and do not dissolve. Contrary to the common misconception, water and hydrophobic substances do not "repel", and the hydration of a hydrophobic surface is energetically, but not entropically, favorable. When an ionic or polar compound enters water, it is surrounded by water molecules (hydration). The relatively small size of water molecules (~ 3 angstroms) allows many water molecules to surround one molecule of solute. The partially negative dipole ends of the water are attracted to positively charged components of the solute, and vice versa for the positive dipole ends. In general, ionic and polar substances such as acids, alcohols, and salts are relatively soluble in water, and non-polar substances such as fats and oils are not. Non-polar molecules stay together in water because it is energetically more favorable for the water molecules to hydrogen bond to each other than to engage in van der Waals interactions with non-polar molecules. An example of an ionic solute is table salt; the sodium chloride, NaCl, separates into Na^+ cations and Cl^- anions, each being surrounded by water molecules. The ions are then easily transported away from their crystalline lattice into solution. An example of a nonionic solute is table sugar. The water dipoles make hydrogen bonds with the polar regions of the sugar molecule (OH groups) and allow it to be carried away into solution.

Chemical properties in nature

Action of water on rock over long periods of time typically leads to weathering and water erosion, physical processes that convert solid rocks and minerals into soil and sediment, but under some conditions chemical reactions with water occur as well, resulting in metasomatism or mineral hydration, a type of chemical alteration of a rock which produces clay minerals. It also occurs when Portland cement hardens. Water ice can form clathrate compounds, known as clathrate hydrates, with a variety of small molecules that can be embedded in its spacious crystal lattice.The most notable of these is methane clathrate, $4CH_4 \cdot 23\ H_2O$, naturally found in large quantities on the ocean floor.

Acidity

Pure water has the concentration of hydroxide ions (OH^-) equal to that of the hydronium (H_3O^+) or hydrogen (H^+) ions, which gives pH of 7 at 298 K. In practice, pure water is very difficult to produce. Water left exposed to air for any length of time will dissolve carbon dioxide, forming a dilute solution of carbonic acid, with a limiting pH of about 5.7. As cloud droplets form in the atmosphere and as raindrops fall through the air minor amounts of CO_2 are absorbed, and thus most rain is slightly acidic. If high amounts of nitrogen and sulfur oxides are present in the air, they too will dissolve into the cloud and rain drops, producing acid rain.

Acid-base reactions

Water is amphoteric: it has the ability to act as either an acid or a base in chemical reactions. According to the Brønsted-Lowry definition, an acid is a proton (H^+) donor and a base is a proton acceptor. When reacting with a stronger acid, water acts as a base; when reacting with a stronger base, it acts as an acid. For instance, water receives an H^+ ion from HCl when hydrochloric acid is formed: Because the oxygen atom in water has two lone pairs, water often acts as a Lewis base, or electron pair donor, in reactions with Lewis acids, although it can also react with Lewis bases, forming hydrogen bonds between the electron pair donors and the hydrogen atoms of water. When weak acid or of a weak base is dissolved in water, water can partially hydrolyze the salt, producing the corresponding base/acid (conjugated acid/base).

Biomolecule

A representation of the 3D structure of myoglobin, showing alpha helices, represented by ribbons. This protein was the first to have its structure solved by X-ray crystallography by Max Perutz and Sir John Cowdery Kendrew in 1958, for which they received a Nobel Prize in Chemistry A biomolecule or biological molecule is molecule that is present in living organisms, including large macromolecules such as proteins, carbohydrates, lipids, and nucleic acids, as well as small molecules such as primary metabolites, secondary metabolites, and natural products. A more general name for this class of material is biological materials. Biomolecules are usually endogenous but may also be exogenous. For example, pharmaceutical drugs may be natural products or semisynthetic (biopharmaceuticals) or they may be totally synthetic.

Biology and its subsets of biochemistry and molecular biology study biomolecules and their reactions. Most biomolecules are organic compounds, and just four elements—oxygen, carbon, hydrogen, and nitrogen—make up 96% of the human body's mass. But many other elements, such as the various biometals, are present in small amounts. The uniformity of specific types of molecules (the biomolecules) and of some metabolic pathways as invariant features between the diversity of life forms is called "biochemical universals"[1] or "theory of material unity of the living beings", a unifying concept in biology, along with cell theory and evolution theory.

Amino acids

Amino acids contain both amino and carboxylic acid functional groups. (In biochemistry, the term amino acid is used when referring to those amino acids in which the amino and carboxylate functionalities are attached to the same carbon, plus proline which is not actually an amino acid). Modified amino acids are sometimes observed in proteins; this is usually the result of enzymatic modification after translation (protein synthesis). For example, phosphorylation of serine by kinases and dephosphorylation by phosphatases is an important control mechanism in the cell cycle. Only two amino acids other than the standard twenty are known to be incorporated into proteins during translation, in certain organisms:

- Selenocysteine is incorporated into some proteins at a UGA codon, which is normally a stop codon.
- Pyrrolysine is incorporated into some proteins at a UAG codon. For instance, in some methanogens in enzymes that are used to produce methane.

Besides those used in protein synthesis, other biologically important amino acids include carnitine (used in lipid transport within a cell), ornithine, GABA and taurine.

Protein structure

The particular series of amino acids that form a protein is known as that protein's primary structure. This sequence is determined by the genetic makeup of the individual. It specifies the order of side-chain groups along the linear polypeptide "backbone".

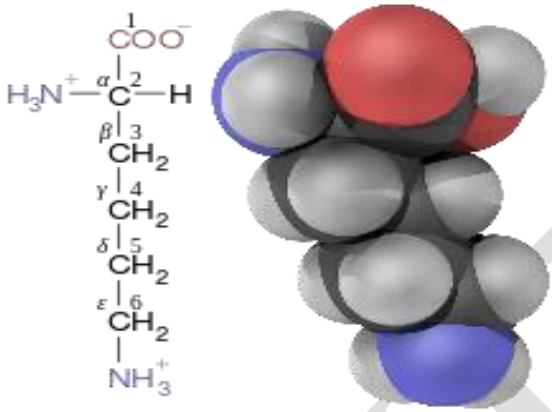
Proteins have two types of well-classified, frequently occurring elements of local structure defined by a particular pattern of hydrogen bonds along the backbone: alpha helix and

beta sheet. Their number and arrangement is called the secondary structure of the protein. Alpha helices are regular spirals stabilized by hydrogen bonds between the backbone CO group (carbonyl) of one amino acid residue and the backbone NH group (amide) of the i+4 residue. The spiral has about 3.6 amino acids per turn, and the amino acid side chains stick out from the cylinder of the helix. Beta pleated sheets are formed by backbone hydrogen bonds between individual beta strands each of which is in an "extended", or fully stretched-out, conformation. The strands may lie parallel or antiparallel to each other, and the side-chain direction alternates above and below the sheet. Hemoglobin contains only helices, natural silk is formed of beta pleated sheets, and many enzymes have a pattern of alternating helices and beta-strands. The secondary-structure elements are connected by "loop" or "coil" regions of non-repetitive conformation, which are sometimes quite mobile or disordered but usually adopt a well-defined, stable arrangement. The overall, compact, 3D structure of a protein is termed its tertiary structure or its "fold". It is formed as result of various attractive forces like hydrogen bonding, disulfide bridges, hydrophobic interactions, hydrophilic interactions, van der Waals force etc. When two or more polypeptide chains (either of identical or of different sequence) cluster to form a protein, quaternary structure of protein is formed. Quaternary structure is an attribute of polymeric

(same-sequence chains) or heteromeric (different-sequence chains) proteins like hemoglobin, which consists of two "alpha" and two "beta" polypeptide chains.

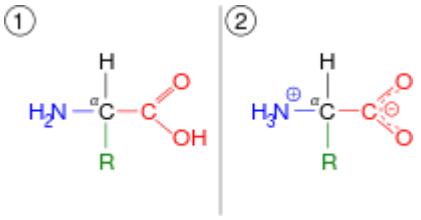
Isomerism

The alpha amino acids are the most common form found in nature, but only when occurring in the L-isomer. The alpha carbon is a chiral carbon atom, with the exception of glycine which has two indistinguishable hydrogen atoms on the alpha carbon. Therefore, all alpha amino acids but glycine can exist in either of two enantiomers, called L or D amino acids, which are mirror images of each other (*see also Chirality*). While L-amino acids represent all of the amino acids found in proteins during translation in the ribosome, D-amino acids are found in some proteins produced by enzyme posttranslational modifications after translation and translocation to the endoplasmic reticulum, as in exotic sea-dwelling organisms such as cone snails. They are also abundant components of the peptidoglycan cell walls of bacteria,[36] and D-serine may act as a neurotransmitter in the brain. D-amino acids are used in racemic crystallography to create centrosymmetric crystals, which (depending on the protein) may allow for easier and more robust protein structure determination. The L and D convention for amino acid configuration refers not to the optical activity of the amino acid itself but rather to the optical activity of the isomer of glyceraldehyde from which that amino acid can, in theory, be synthesized (D-glyceraldehyde is dextrorotatory; L-glyceraldehyde is levorotatory). In alternative fashion, the *(S)* and *(R)* designators are used to indicate the absolute stereochemistry. Almost all of the amino acids in proteins are *(S)* at the α carbon, with cysteine being *(R)* and glycine non-chiral.[39] Cysteine has its side chain in the same geometric position as the other amino acids, but the *R/S* terminology is reversed because of the higher atomic number of sulfur compared to the carboxyl oxygen gives the side chain a higher priority, whereas the atoms in most other side chains give them lower priority.

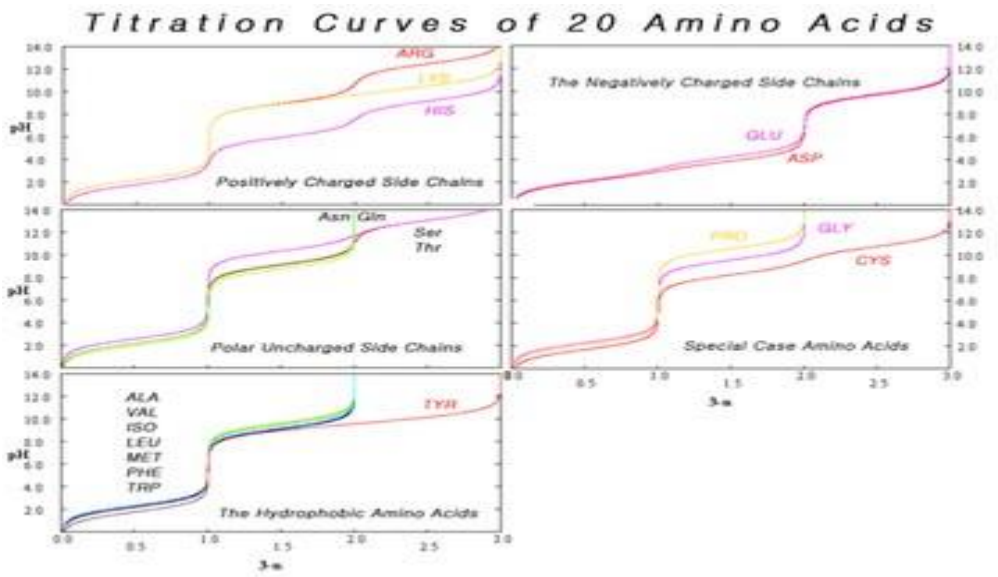


Lysine with carbon atoms labeled by position

In amino acids that have a carbon chain attached to the α–carbon (such as lysine, shown to the right) the carbons are labeled in order as α, β, γ, δ, and so on. In some amino acids, the amine group is attached to the β or γ-carbon, and these are therefore referred to as *beta* or *gamma amino acids*. Amino acids are usually classified by the properties of their side chain into four groups. The side chain can make an amino acid a weak acid or a weak base, and a hydrophile if the side chain is polar or a hydrophobe if it is nonpolar. The chemical structures of the 22 standard amino acids, along with their chemical properties, are described more fully in the article on these proteinogenic amino acids. The phrase "branched-chain amino acids" or BCAA refers to the amino acids having aliphatic side chains that are non-linear; these are leucine, isoleucine, and valine. Proline is the only proteinogenic amino acid whose side-group links to the α-amino group and, thus, is also the only proteinogenic amino acid containing a secondary amine at this position.[34] In chemical terms, proline is, therefore, an imino acid, since it lacks a primary amino group,[41] although it is still classed as an amino acid in the current biochemical nomenclature,[42] and may also be called an "N-alkylated alpha-amino acid".

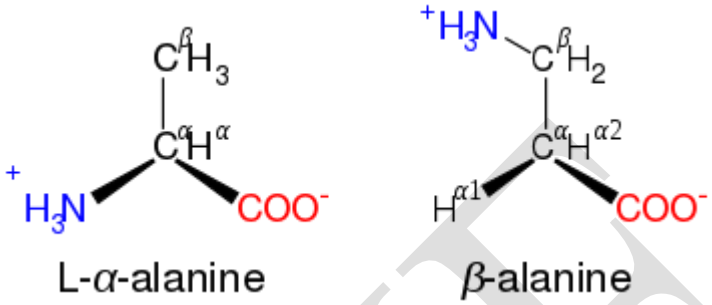


The α-carboxylic acid group of amino acids is a weak acid, meaning that it releases a hydron (such as a proton) at moderate pH values. In other words, carboxylic acid groups ($-CO_2H$) can be deprotonated to become negative carboxylates ($-CO_2^-$). The negatively charged carboxylate ion predominates at pH values greater than the pKa of the carboxylic acid group (mean for the 20 common amino acids is about 2.2, see the table of amino acid structures above). In a complementary fashion, the α-amine of amino acids is a weak base, meaning that it accepts a proton at moderate pH values. In other words, α-amino groups (NH_2-) can be protonated to become positive α-ammonium groups ($^+NH_3-$). The positively charged α-ammonium group predominates at pH values less than the pKa of the α-ammonium group (mean for the 20 common α-amino acids is about 9.4). Because all amino acids contain amine and carboxylic acid functional groups, they share amphiprotic properties. Below pH 2.2, the predominant form will have a neutral carboxylic acid group and a positive α-ammonium ion (net charge +1), and above pH 9.4, a negative carboxylate and neutral α-amino group (net charge −1). But at pH between 2.2 and 9.4, an amino acid usually contains both a negative carboxylate and a positive α-ammonium group, as shown in structure (2) on the right, so has net zero charge. This molecular state is known as a zwitterion, from the German Zwitter meaning *hermaphrodite* or *hybrid*. The fully neutral form (structure (1) on the left) is a very minor species in aqueous solution throughout the pH range (less than 1 part in 10^7). Amino acids exist as zwitterions also in the solid phase, and crystallize with salt-like properties unlike typical organic acids or amines.



Composite of titration curves of twenty proteinogenic amino acids grouped by side chain category.The variation in titration curves when the amino acids can be grouped by category. With the exception of tyrosine, using titration to distinguish among hydrophobic amino acids is problematic. At pH values between the two pKa values, the zwitterion predominates, but coexists in dynamic equilibrium with small amounts of net negative and net positive ions. At the exact midpoint between the two pKa values, the trace amount of net negative and trace of net positive ions exactly balance, so that average net charge of all forms present is zero. This pH is known as the isoelectric point pI, so pI = ½(pKa_1 + pKa_2). The individual amino acids all have slightly different pKa values, so have different isoelectric points. For amino acids with charged side chains, the pKa of the side chain is involved. Thus for Asp, Glu with negative side chains, pI = ½(pKa_1 + pKa_R), where pKa_R is the side chain pKa. Cysteine also has potentially negative side chain with pKa_R = 8.14, so pI should be calculated as for Asp and Glu, even though the side chain is not significantly charged at neutral pH. For His, Lys, and Arg with positive side chains, pI = ½(pKa_R + pKa_2). Amino acids have zero mobility in electrophoresis at their isoelectric point, although this behaviour is more usually exploited for peptides and proteins than single amino acids. Zwitterions have minimum solubility at their isoelectric point and some amino

acids (in particular, with non-polar side chains) can be isolated by precipitation from water by adjusting the pH to the required isoelectric point.



β-alanine and its α-alanine isomer

Secondary structure

Protein secondary structure is the three dimensional form of *local segments* of proteins. The two most common secondary structural elements are alpha helices and beta sheets, though beta turns and omega loops occur as well. Secondary structure elements typically spontaneously form as an intermediate before the protein folds into its three dimensional tertiary structure. Secondary structure is formally defined by the pattern of hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms in the peptide backbone. Secondary structure may alternatively be defined based on the regular pattern of backbone dihedral angles in a particular region of the Ramachandran plot regardless of whether it has the correct hydrogen bonds.

The concept of secondary structure was first introduced by Kaj Ulrik Linderstrøm-Lang at Stanford in 1952. Other types of biopolymers such as nucleic acids also possess characteristic secondary structures.

The most common secondary structures are alpha helices and beta sheets. Other helices, such as the 3_{10} helix and π helix, are calculated to have energetically favorable hydrogen-bonding patterns but are rarely observed in natural proteins except at the ends of α helices due to unfavorable backbone packing in the center of the helix. Other extended structures such as the polyproline helix and alpha sheet are rare in native state proteins but are often hypothesized as important protein folding intermediates. Tight turns and loose, flexible loops link the more "regular" secondary structure elements. The random coil is not a true secondary structure, but is

the class of conformations that indicate an absence of regular secondary structure. Amino acids vary in their ability to form the various secondary structure elements. Proline and glycine are sometimes known as "helix breakers" because they disrupt the regularity of the α helical backbone conformation; however, both have unusual conformational abilities and are commonly found in turns. Amino acids that prefer to adopt helical conformations in proteins include methionine, alanine, leucine, glutamate and lysine ("MALEK" in amino-acid 1-letter codes); by contrast, the large aromatic residues (tryptophan, tyrosine and phenylalanine) and C^{β}-branched amino acids (isoleucine, valine, and threonine) prefer to adopt β-strand conformations. However, these preferences are not strong enough to produce a reliable method of predicting secondary structure from sequence alone. Low frequency collective vibrations are thought to be sensitive to local rigidity within proteins, revealing beta structures to be generically more rigid than alpha or disordered proteins. Neutron scattering measurements have directly connected the spectral feature at ~1 THz to collective motions of the secondary structure of beta-barrel protein GFP. Hydrogen bonding patterns in secondary structures may be significantly distorted, which makes automatic determination of secondary structure difficult. There are several methods for formally defining protein secondary structure (e.g., DSSP, DEFINE, STRIDE, ScrewFit, SST).

Protein tertiary structure is the three dimensional shape of a protein. The tertiary structure will have a single polypeptide chain "backbone" with one or more protein secondary structures, the protein domains. Amino acid side chains may interact and bond in a number of ways. The interactions and bonds of side chains within a particular protein determine its tertiary structure. The protein tertiary structure is defined by its atomic coordinates. These coordinates may refer either to a protein domain or to the entire tertiary structure. A number of tertiary structures may fold into a quaternary structure.

Saccharides

Monosaccharides are the simplest form of carbohydrates with only one simple sugar. They essentially contain an aldehyde or ketone group in their structure. The presence of an aldehyde group in a monosaccharide is indicated by the prefix *aldo*-. Similarly, a ketone group is denoted by the prefix *keto*-. Examples of monosaccharides are the hexoses glucose, fructose, and

galactose and pentoses, ribose, and deoxyribose. Consumed fructose and glucose have different rates of gastric emptying, are differentially absorbed and have different metabolic fates, providing multiple opportunities for 2 different saccharides to differentially affect food intake. Most saccharides eventually provide fuel for cellular respiration.

Disaccharides are formed when two monosaccharides, or two single simple sugars, form a bond with removal of water. They can be hydrolyzed to yield their saccharin building blocks by boiling with dilute acid or reacting them with appropriate enzymes.[3] Examples of disaccharides include sucrose, maltose, and lactose.

Polysaccharides are polymerized monosaccharides, or complex carbohydrates. They have multiple simple sugars. Examples are starch, cellulose, and glycogen. They are generally large and often have a complex branched connectivity. Because of their size, polysaccharides are not water-soluble, but their many hydroxy groups become hydrated individually when exposed to water, and some polysaccharides form thick colloidal dispersions when heated in water. Shorter polysaccharides, with 3 - 10 monomers, are called oligosaccharides. A fluorescent indicator-displacement molecular imprinting sensor was developed for discriminating saccharides. It successfully discriminated three brands of orange juice beverage.[10] The change in fluorescence intensity of the sensing films resulting is directly related to the saccharide concentration.

Polysaccharide

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. They range in structure from linear to highly branched. Examples include storage polysaccharides such as starch and glycogen, and structural polysaccharides such as cellulose and chitin. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. When all the monosaccharides in a polysaccharide are the same type, the polysaccharide is called a *homopolysaccharide* or *homoglycan*, but when more than one type of monosaccharide is present they are called *heteropolysaccharides* or *heteroglycans*.

Natural saccharides are generally of simple carbohydrates called monosaccharides with general formula $(CH_2O)_n$ where *n* is three or more. Examples of monosaccharides are glucose, fructose, and glyceraldehyde. Polysaccharides, meanwhile, have a general formula of $C_x(H_2O)_y$ where *x* is usually a large number between 200 and 2500. When the repeating units in the polymer backbone are six-carbon monosaccharides, as is often the case, the general formula simplifies to $(C_6H_{10}O_5)_n$, where typically $40 \leq n \leq 3000$. As a rule of thumb, polysaccharides contain more than ten monosaccharide units, whereas oligosaccharides contain three to ten monosaccharide units; but the precise cutoff varies somewhat according to convention. Polysaccharides are an important class of biological polymers. Their function in living organisms is usually either structure- or storage-related. Starch (a polymer of glucose) is used as a storage polysaccharide in plants, being found in the form of both amylose and the branched amylopectin. In animals, the structurally similar glucose polymer is the more densely branched glycogen, sometimes called "animal starch". Glycogen's properties allow it to be metabolized more quickly, which suits the active lives of moving animals.

Cellulose and chitin are examples of structural polysaccharides. Cellulose is used in the cell walls of plants and other organisms, and is said to be the most abundant organic molecule on Earth. It has many uses such as a significant role in the paper and textile industries, and is used as a feedstock for the production of rayon (via the viscose process), cellulose acetate, celluloid, and nitrocellulose. Chitin has a similar structure, but has nitrogen-containing side branches, increasing its strength. It is found in arthropod exoskeletons and in the cell walls of some fungi. It also has multiple uses, including surgical threads. Polysaccharides also include callose or laminarin, chrysolaminarin, xylan, arabinoxylan, mannan, fucoidan and galactomannan.

Nutrition polysaccharides are common sources of energy. Many organisms can easily break down starches into glucose; however, most organisms cannot metabolize cellulose or other polysaccharides like chitin and arabinoxylans. These carbohydrate types can be metabolized by some bacteria and protists. Ruminants and termites, for example, use microorganisms to process

cellulose. Even though these complex polysaccharides are not very digestible, they provide important dietary elements for humans. Called dietary fiber, these carbohydrates enhance digestion among other benefits. The main action of dietary fiber is to change the nature of the contents of the gastrointestinal tract, and to change how other nutrients and chemicals are absorbed. Soluble fiber binds to bile acids in the small intestine, making them less likely to enter the body; this in turn lowers cholesterol levels in the blood. Soluble fiber also attenuates the absorption of sugar, reduces sugar response after eating, normalizes blood lipid levels and, once fermented in the colon, produces short-chain fatty acids as byproducts with wide-ranging physiological activities (discussion below). Although insoluble fiber is associated with reduced diabetes risk, the mechanism by which this occurs is unknown. Not yet formally proposed as an essential macronutrient (as of 2005), dietary fiber is nevertheless regarded as important for the diet, with regulatory authorities in many developed countries recommending increases in fiber intake.

Starch

Starch is a glucose polymer in which glucopyranose units are bonded by *alpha*-linkages. It is made up of a mixture of amylose (15–20%) and amylopectin (80–85%). Amylose consists of a linear chain of several hundred glucose molecules and Amylopectin is a branched molecule made of several thousand glucose units (every chain of 24–30 glucose units is one unit of Amylopectin). Starches are insoluble in water. They can be digested by breaking the *alpha*-linkages (glycosidic bonds). Both humans and animals have amylases, so they can digest starches. Potato, rice, wheat, and maize are major sources of starch in the human diet. The formations of starches are the ways that plants store glucose.

Glycogen

Glycogen serves as the secondary long-term energy storage in animal and fungal cells, with the primary energy stores being held in adipose tissue. Glycogen is made primarily by the liver and the muscles, but can also be made by glycogenesis within the brain and stomach. Glycogen is analogous to starch, a glucose polymer in plants, and is sometimes referred to as *animal starch*, having a similar structure to amylopectin but more extensively branched and

compact than starch. Glycogen is a polymer of α(1→4) glycosidic bonds linked, with α(1→6)-linked branches. Glycogen is found in the form of granules in the cytosol/cytoplasm in many cell types, and plays an important role in the glucose cycle. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose, but one that is less compact and more immediately available as an energy reserve than triglycerides (lipids). In the liver hepatocytes, glycogen can compose up to eight percent (100–120 g in an adult) of the fresh weight soon after a meal. Only the glycogen stored in the liver can be made accessible to other organs. In the muscles, glycogen is found in a low concentration of one to two percent of the muscle mass. The amount of glycogen stored in the body—especially within the muscles, liver, and red blood cells—varies with physical activity, basal metabolic rate, and eating habits such as intermittent fasting. Small amounts of glycogen are found in the kidneys, and even smaller amounts in certain glial cells in the brain and white blood cells. The uterus also stores glycogen during pregnancy, to nourish the embryo.

Lipids

Lipids (oleaginous) are chiefly fatty acid esters, and are the basic building blocks of biological membranes. Another biological role is energy storage (e.g., triglycerides). Most lipids consist of a polar or hydrophilic head (typically glycerol) and one to three nonpolar or hydrophobic fatty acid tails, and therefore they are amphiphilic. Fatty acids consist of unbranched chains of carbon atoms that are connected by single bonds alone (saturated fatty acids) or by both single and double bonds (unsaturated fatty acids). The chains are usually 14-24 carbon groups long, but it is always an even number.

For lipids present in biological membranes, the hydrophilic head is from one of three classes:

- Glycolipids, whose heads contain an oligosaccharide with 1-15 saccharide residues.
- Phospholipids, whose heads contain a positively charged group that is linked to the tail by a negatively charged phosphate group.
- Sterols, whose heads contain a planar steroid ring, for example, cholesterol.

Other lipids include prostaglandins and leukotrienes which are both 20-carbon fatty acyl units synthesized from arachidonic acid. They are also known as fatty acids

Phospholipid

Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group. The two components are joined together by a glycerol molecule. The phosphate groups can be modified with simple organic molecules such as choline. The first phospholipid identified in 1847 as such in biological tissues was lecithin, or phosphatidylcholine, in the egg yolk of chickens by the French chemist and pharmacist, Theodore Nicolas Gobley. Biological membranes in eukaryotes also contain another class of lipid, sterol, interspersed among the phospholipids and together they provide membrane fluidity and mechanical strength. Purified phospholipids are produced commercially and have found applications in nanotechnology and materials science.

Amphiphilic character

An amphiphile (from the Greek αμφις, amphis: both and φιλία, philia: love, friendship) is a term describing a chemical compound possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties. The phospholipid head contains a negatively charged phosphate group and glycerol; it is hydrophilic (attracted to water). The phospholipid tails usually consists of 2 long fatty acid chains; they are hydrophobic and avoid interactions with water. When placed in aqueous solutions, phospholipids are driven by hydrophobic interactions that result in the fatty acid tails aggregating to minimize interactions with water molecules. These specific properties allow phospholipids to play an important role in the phospholipid bilayer. In biological systems, the phospholipids often occur with other molecules (e.g., proteins, glycolipids, sterols) in a bilayer such as a cell membrane. Lipid bilayers occur when hydrophobic tails line up against one another, forming a membrane of hydrophilic heads on both sides facing the water. Such movement can be described by the fluid mosaic model, that describes the membrane as a mosaic of lipid molecules that act as a solvent for all the substances and proteins within it, so proteins and lipid molecules are then free to diffuse laterally through the lipid matrix and migrate over the membrane. Sterols contribute to membrane fluidity by hindering the

packing together of phospholipids. However, this model has now been superseded, as through the study of lipid polymorphism it is now known that the behaviour of lipids under physiological (and other) conditions is not simple.

Diacylglyceride structures

Phosphatidic acid (phosphatidate) (PA)

Phosphatidylethanolamine (cephalin) (PE)

Phosphatidylcholine (lecithin) (PC)

Phosphatidylserine (PS)

Phosphoinositides:

Phosphatidylinositol (PI)

Phosphatidylinositol phosphate (PIP)

Phosphatidylinositol bisphosphate (PIP2)

Phosphatidylinositol trisphosphate (PIP3)

DNA

Deoxyribonucleic acid (DNA) is a molecule that carries the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses. DNA and ribonucleic acid (RNA) are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), they are one of the four major types of macromolecules that are essential for all known forms of life. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. The two DNA strands are called polynucleotides since they are composed of simpler monomer units called nucleotides. Each nucleotide is composed of one of four nitrogen-containing nucleobases — cytosine (C), guanine (G), adenine (A) or thymine (T) — a sugar called deoxyribose and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA. The complementary nitrogenous base pairs are divided into two main groups, pyrimidines

and purines. The pyrimidines and purines determine the amount of hydrogen bonding which should occur between specific base pairs. Pyrimidines are known as thymine and cytosine, Purines are known adenine and guanine in a DNA molecule. The total amount of related DNA base pairs on Earth is estimated at 5.0×10^{37} and weighs 50 billion tonnes. In comparison, the total mass of the biosphere has been estimated to be as much as 4 trillion tons of carbon (TtC).

DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobases (informally, *bases*). It is the sequence of these four nucleobases along the backbone that encodes biological information. RNA strands are created using DNA strands as a template in a process called transcription. Under the genetic code, these RNA strands are translated to specify the sequence of amino acids within proteins in a process called translation. Within eukaryotic cells DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, plants, fungi and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the eukaryotic chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

DNA was first isolated by Friedrich Miescher in 1869. Its molecular structure was first identified by James Watson and Francis Crick at the Cavendish Laboratory within the University of Cambridge in 1953, whose model-building efforts were guided by X-ray diffraction data acquired by Raymond Gosling, who was a post-graduate student of Rosalind Franklin. DNA is used by researchers as a molecular tool to explore physical laws and theories, such as the ergodic

theorem and the theory of elasticity. The unique material properties of DNA have made it an attractive molecule for material scientists and engineers interested in micro- and nano-fabrication. Among notable advances in this field are DNA origami and DNA-based hybrid materials.

RNA

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand. Cellular organisms use messenger RNA (*mRNA*) to convey genetic information (using the letters G, U, A, and C to denote the nitrogenous bases guanine, uracil, adenine, and cytosine) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome. Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function where RNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (*tRNA*) molecules to deliver amino acids to the ribosome, where ribosomal RNA (*rRNA*) then links amino acids together to form proteins.

Comparison with DNA

Three-dimensional representation of the 50S ribosomal subunit. Ribosomal RNA is in ochre, proteins in blue. The active site is a small segment of rRNA, indicated in red.

The chemical structure of RNA is very similar to that of DNA, but differs in three main ways:

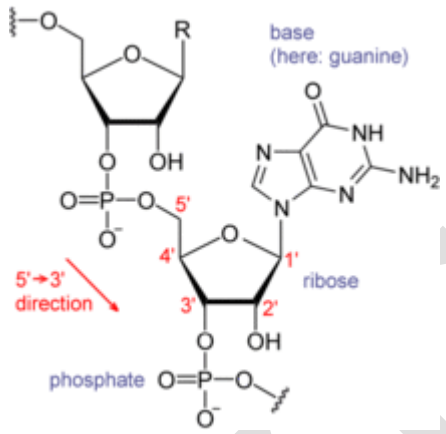
- Unlike double-stranded DNA, RNA is a single-stranded molecule in many of its biological roles and has a much shorter chain of nucleotides. However, RNA can, by complementary base pairing, form intrastrand (i.e., single-strand) double helixes, as in tRNA.

- While DNA contains *deoxyribose*, RNA contains *ribose* (in deoxyribose there is no hydroxyl group attached to the pentose ring in the 2' position). These hydroxyl groups make RNA less stable than DNA because it is more prone to hydrolysis.
- The complementary base to adenine in DNA is thymine, whereas in RNA, it is uracil, which is an unmethylated form of thymine.

Like DNA, most biologically active RNAs, including mRNA, tRNA, rRNA, snRNAs, and other non-coding RNAs, contain self-complementary sequences that allow parts of the RNA to fold and pair with itself to form double helices. Analysis of these RNAs has revealed that they are highly structured. Unlike DNA, their structures do not consist of long double helices, but rather collections of short helices packed together into structures akin to proteins. In this fashion, RNAs can achieve chemical catalysis (like enzymes). For instance, determination of the structure of the ribosome—an enzyme that catalyzes peptide bond formation—revealed that its active site is composed entirely of RNA.

Structure

Each nucleotide in RNA contains a ribose sugar, with carbons numbered 1' through 5'. A base is attached to the 1' position, in general, adenine (A), cytosine (C), guanine (G), or uracil (U). Adenine and guanine are purines, cytosine and uracil are pyrimidines. A phosphate group is attached to the 3' position of one ribose and the 5' position of the next. The phosphate groups have a negative charge each, making RNA a charged molecule (polyanion). The bases form hydrogen bonds between cytosine and guanine, between adenine and uracil and between guanine and uracil.[8] However, other interactions are possible, such as a group of adenine bases binding to each other in a bulge, or the GNRA tetraloop that has a guanine–adenine base-pair.



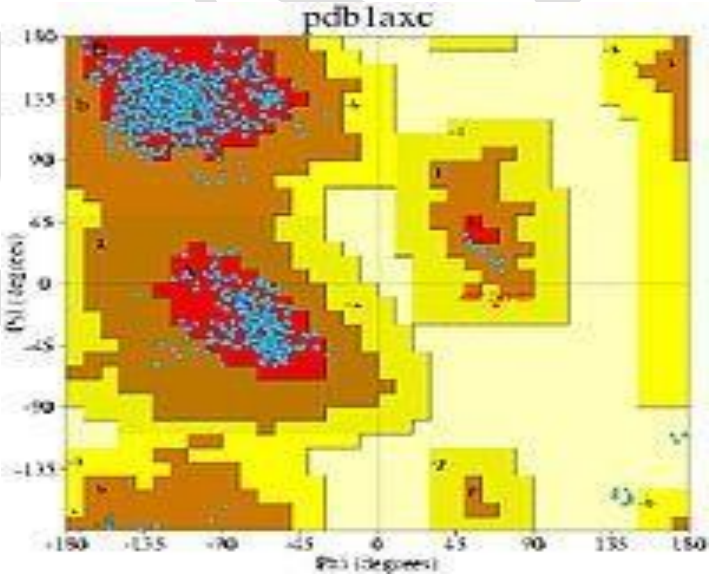
Chemical structure of RNA

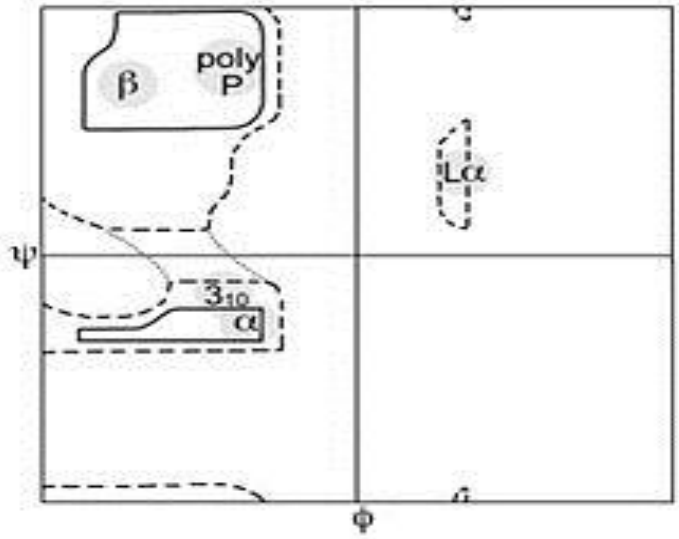
An important structural feature of RNA that distinguishes it from DNA is the presence of a hydroxyl group at the 2' position of the ribose sugar. The presence of this functional group causes the helix to mostly adopt the A-form geometry, although in single strand dinucleotide contexts, RNA can rarely also adopt the B-form most commonly observed in DNA. The A-form geometry results in a very deep and narrow major groove and a shallow and wide minor groove. A second consequence of the presence of the 2'-hydroxyl group is that in conformationally flexible regions of an RNA molecule (that is, not involved in formation of a double helix), it can chemically attack the adjacent phosphodiester bond to cleave the backbone. RNA is transcribed with only four bases (adenine, cytosine, guanine and uracil), but these bases and attached sugars can be modified in numerous ways as the RNAs mature. Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C–N bond to a C–C bond, and ribothymidine (T) are found in various places (the most notable ones being in the TΨC loop of tRNA). Another notable modified base is hypoxanthine, a deaminated adenine base whose nucleoside is called inosine (I). Inosine plays a key role in the wobble hypothesis of the genetic code. There are more than 100 other naturally occurring modified nucleosides. The greatest structural diversity of modifications can be found in tRNA, while pseudouridine and nucleosides with 2'-O-methylribose often present in rRNA are the most common. The specific roles of many of these modifications in RNA are not fully understood. However, it is notable that, in ribosomal RNA, many of the post-

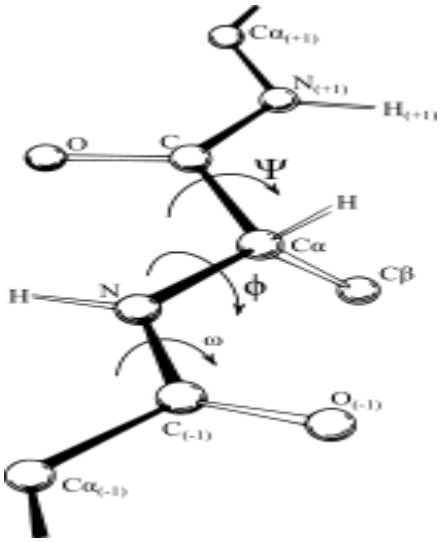
transcriptional modifications occur in highly functional regions, such as the peptidyl transferase center and the subunit interface, implying that they are important for normal function.

Ramachandran plot

A Ramachandran plot (also known as a Ramachandran diagram or a [φ,ψ] plot), originally developed in 1963 by G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, is a way to visualize energetically allowed regions for backbone dihedral angles ψ against φ of amino acid residues in protein structure. The figure at left illustrates the definition of the φ and ψ backbone dihedral angles (called φ and φ' by Ramachandran). The ω angle at the peptide bond is normally 180°, since the partial-double-bond character keeps the peptide planar. The figure at top right shows the allowed φ,ψ backbone conformational regions from the Ramachandran et al. 1963 and 1968 hard-sphere calculations: full radius in solid outline, reduced radius in dashed, and relaxed tau (N-Cα-C) angle in dotted lines. Because dihedral angle values are circular and 0° is the same as 360°, the edges of the Ramachandran plot "wrap" right-to-left and bottom-to-top. For instance, the small strip of allowed values along the lower-left edge of the plot are a continuation of the large, extended-chain region at upper left.







A Ramachandran plot generated from human PCNA, a trimeric DNA clamp protein that contains both β-sheet and α-helix (PDB ID 1AXC). The red, brown, and yellow regions represent the favored, allowed, and "generously allowed" regions as defined by ProCheck. A Ramachandran plot can be used in two somewhat different ways. One is to show in theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein (as at top right). A second is to show the empirical distribution of datapoints observed in a single structure (as at right, here) in usage for structure validation, or else in a database of many

structures (as in the lower 3 plots at left). Either case is usually shown against outlines for the theoretically favored regions.

Possible questions

Part A

1. What are amphiphilic molecules?
(a) Highly polar
(b) Highly non-polar
(c) Neutral
(d) Having both polar and non-polar groups

2. The shape of a water molecule is
(A) Linear (B) Trigonal
(C) Tetrahedron (D) Distorted Tetrahedron

3. In taxonomy, classifications of organisms are on the basis of
(a) Overall morphology
(b) Proteomics
(c) Genomics
(d) Evolution

4. In phylogeny, classifications of organisms are on the basis of
(a) Overall morphology
(b) Proteomics
(c) Genomics
(d) Evolution

5. How many H-bond a water molecule can make with neighboring water molecules?
(a) 1
(b) 2
(c) 3
(d) 4

6. At Alkalosis condition, the blood pH is
(A) < 7.4 (B) 7.4 (C) 7.5 (D) > 7.6

7. The blood pH is mainly maintained by the following components.
(a) O_2 and CO_2 (b) CO_2 and HCO_3^- (c) O_2 and CO (d) CO and CO_2

8. Biomolecules are
(A) Endogenous (B) Exogenous (C) Either endogenous or exogenous(D) Neither endogenous nor exogenous

9. The pI of 'Lysine' can be calculated by using the formula
(a) $pI = (pK_2 + pK_R)/2$(b) $pI = (pK_1 + pK_R)/2$
(c) $pI = (pK_1 + pK_2)/2$ (d) $pI = (pK_1 + pK_2 + pK_R)/3$

10. The pI of 'glutamic acid' can be calculated by using the formula
(a) $pI = (pK_2 + pK_R)/2$
(b) $pI = (pK_1 + pK_R)/2$
(c) $pI = (pK_1 + pK_2)/2$
(d) $pI = (pK_1 + pK_2 + pK_R)/3$

11. The sugar puckering effect in A-DNA is
(a) C3' endo (b) C3' exo (c) C2' endo (d) C2' exo

Part B

1. Write any two unique features on the 3D structure of a water molecule.
2. What are major elements present in all organisms?
3. How are organisms classified?
4. Explain H-bonds with an example.
5. Explain H-bonds among water molecules in ice.
6. How do amphiphilic molecules react in water?
7. How do hydrophilic molecules react in water?
1. What are globular proteins? Give an example.
2. What are fibrous proteins? Give an example.
10. Discuss on C3'-endo- and C2'-endo-sugar conformations and their significances.
11. Draw the structures of tatomers of Guanine and Thymine.

Part C

1. Enumerate any four physical properties of water molecules in detail.
2. Enumerate any four chemical properties of water molecules in detail.
3. Write an essay on chemical composition of living matter.
4. How will you calculate pI value of Alanin/Lysine/Glutamic acid using titration curve?
5. Explain the unique features and applications of 'Ramachandran Plot'.
6. Discuss on A-DNA, B-DNA and Z-DNA in terms of their structures and functions.
7. Explain the structures, properties and biological significances of triglycerides.

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UNIT : II (Metabolisms)

UNIT - II

SYLLABUS

Metabolisms: carbohydrates, lipids (fatty acid oxidation and biosynthesis), amino acids biosynthesis, nucleotides (de novo synthesis and salvage pathways). Disorders of lipid, carbohydrate, nucleic acid, amino acid metabolism. Inborn errors of metabolism. Metabolomics.

Protein metabolism

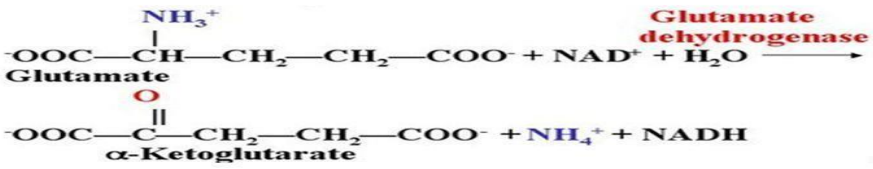
Protein metabolism denotes the various biochemical processes responsible for the synthesis of proteins and amino acids, and the breakdown of proteins (and other large molecules) by catabolism. Dietary proteins are first broken down to individual amino acids by various enzymes and hydrochloric acid present in the gastro-intestinal tract. These amino acids are further broken down to α-keto acids which can be recycled in the body for generation of energy, and production of glucose or fat or other amino acids. This break-down of amino acids to α-keto acids occurs in the liver by a process known as transamination, which follows a bimolecular ping pong mechanism.

Amino acids Metabolism

Proteins – Amino acids – Keto acids – Non-essential amino acids and Non-protein nitrogenous compounds

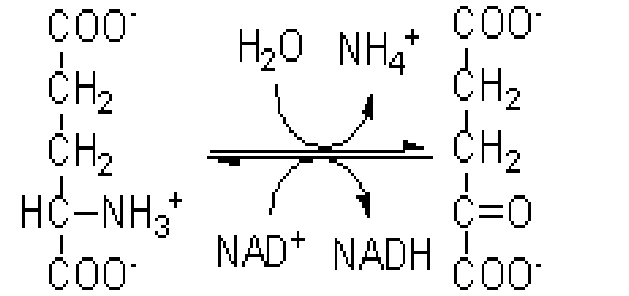
Oxidative deamination

- Removes α-amino group from Glutamate (amino-acid) which is released as inorganic ammonium ion ($^+NH_4$ is toxic-urea cycle)
- Provides α-ketoglutarate for transamination
- Catalysed by *Glutamate Dehydrogenase*



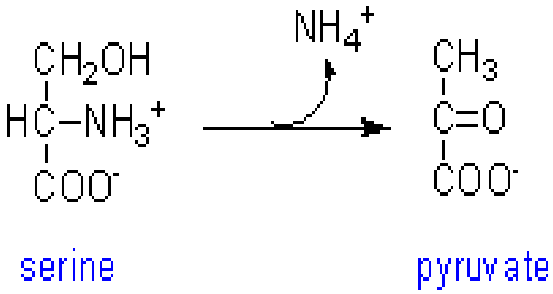
- $^+NH_4$ is toxic and so it is excreted (removed) from the body in the form of urea by urea cycle.

glutamate dehydrogenase



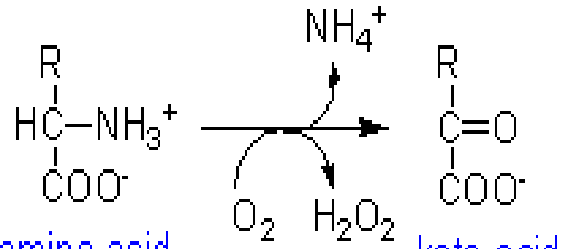
glutamate α-ketoglutarate

serine deaminase



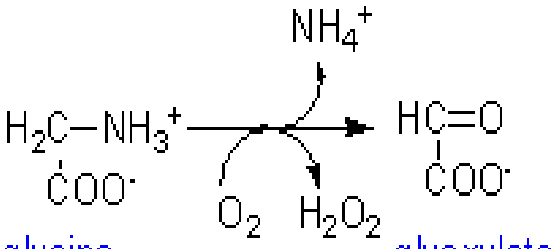
serine pyruvate

amino acid oxidase



amino acid keto-acid

glycine oxidase

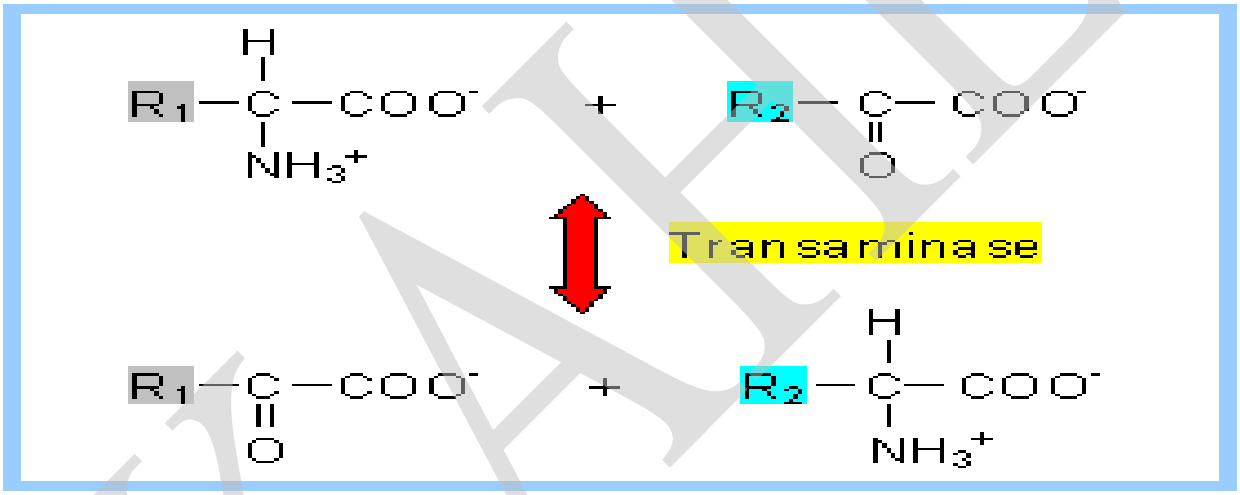


glycine glyoxylate

Transamination

Transfer of amino group between an amino acid and keto acid
Formation of a new amino acids and keto acid

Aminotransferases – Transaminases – Pyridoxal phosphate - two types - tissues
AST – Aspartate aminotransferases – GOT
ALT – Alanine aminotransferases - GPT



Decarboxylation

Removal of CO_2 from amino acids – amines – biogenic amines

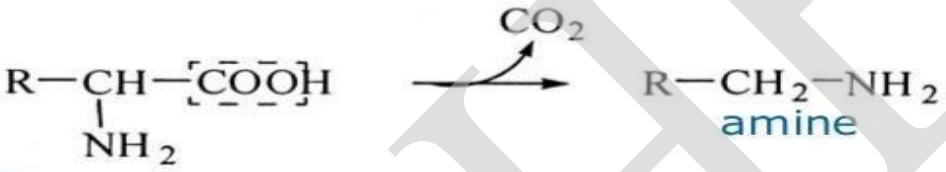
Histidine to histamine – Tryptophan to tryptamine – Tyrosine totyramine

Decarboxylase – histidine/Tryptophan/Tyrosine decarboxylase

Co-enzyme Pyridoxal phosphate – various tissues

Decarboxylation of amino acids

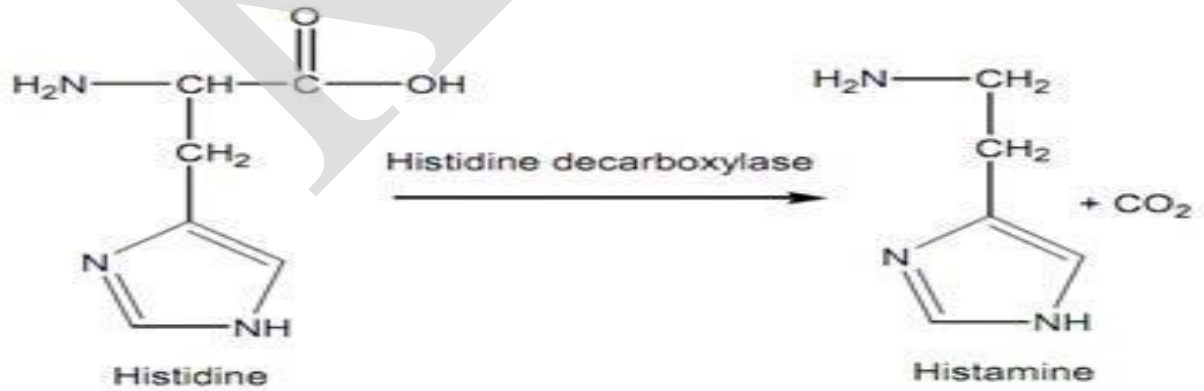
Decarboxylation – removal of *carbon dioxide* from amino acid with formation of *amines*.



Usually amines have high physiological activity (hormones, neurotransmitters etc).

Enzyme: ***decarboxylases***
Coenzyme – **pyrydoxalphosphate**

Decarboxylation



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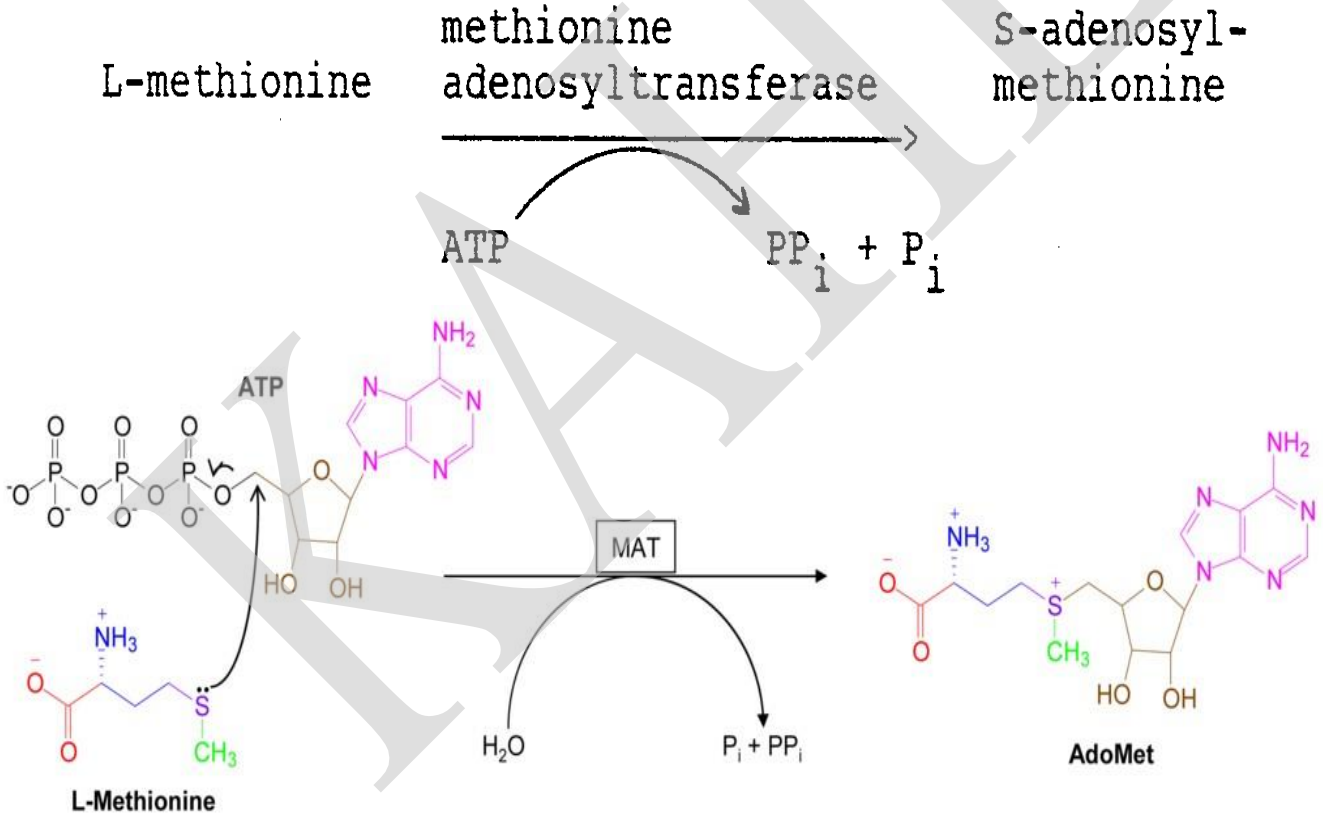
Transmethylation

Transfer of methyl groups – transmethylase – liver – physiologically active compounds – epinephrine – creatine – thymine – choline – homocysteine.



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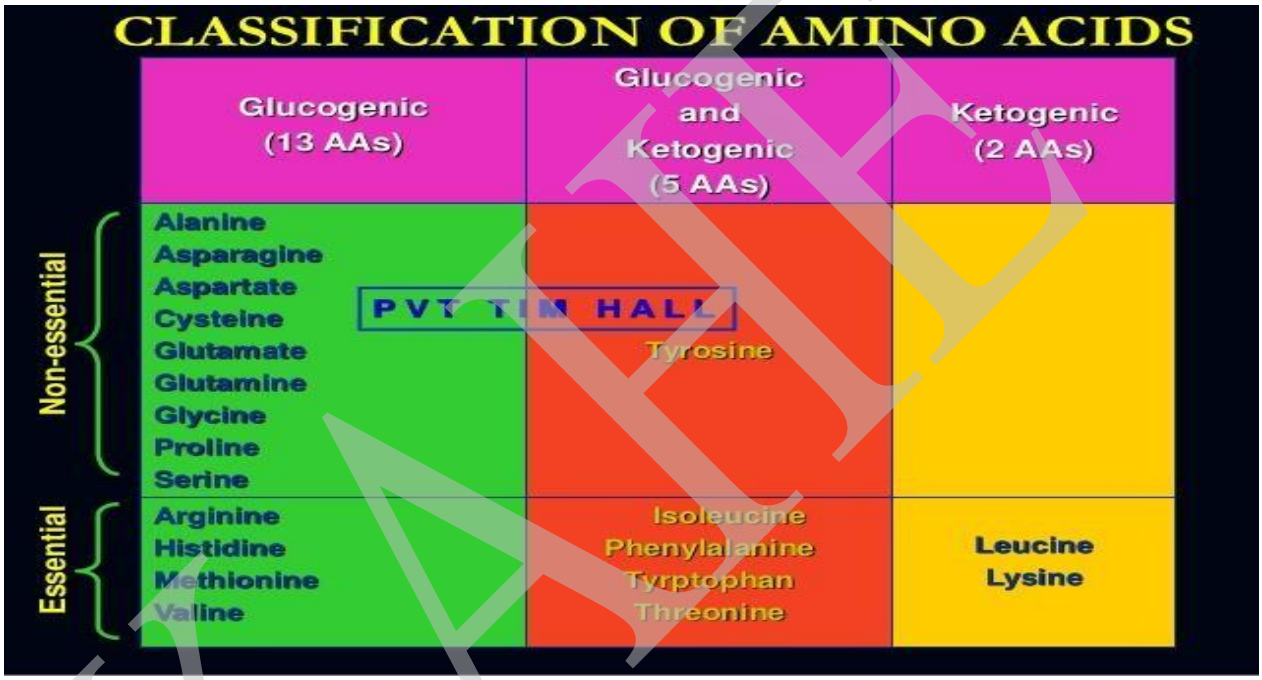

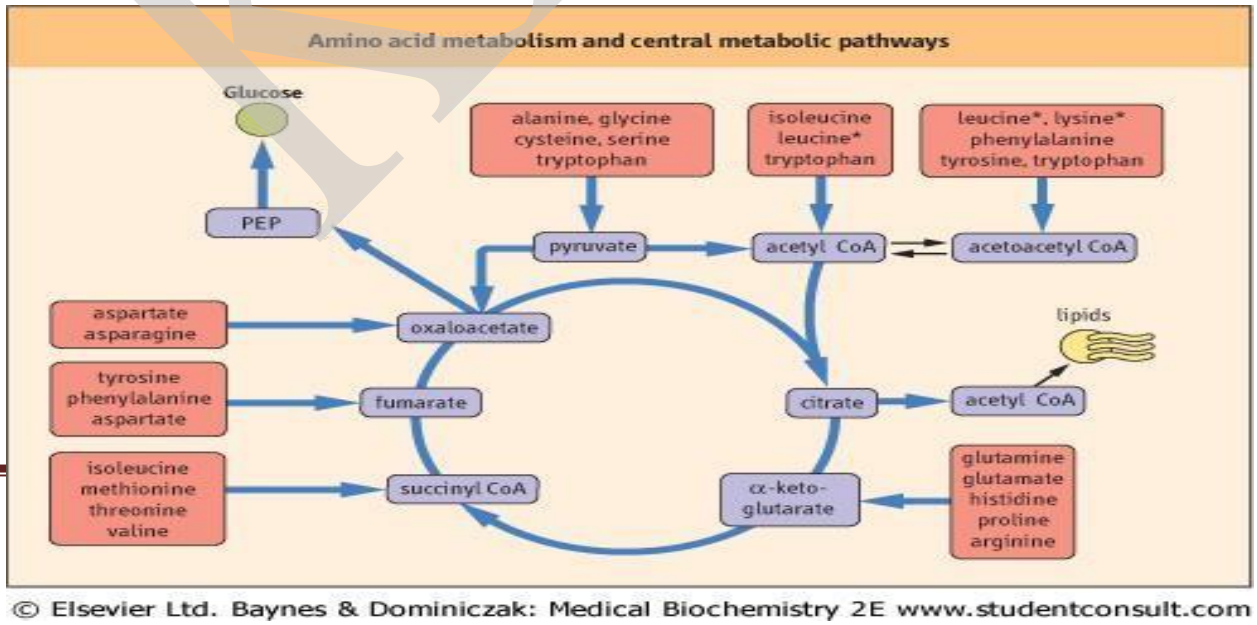

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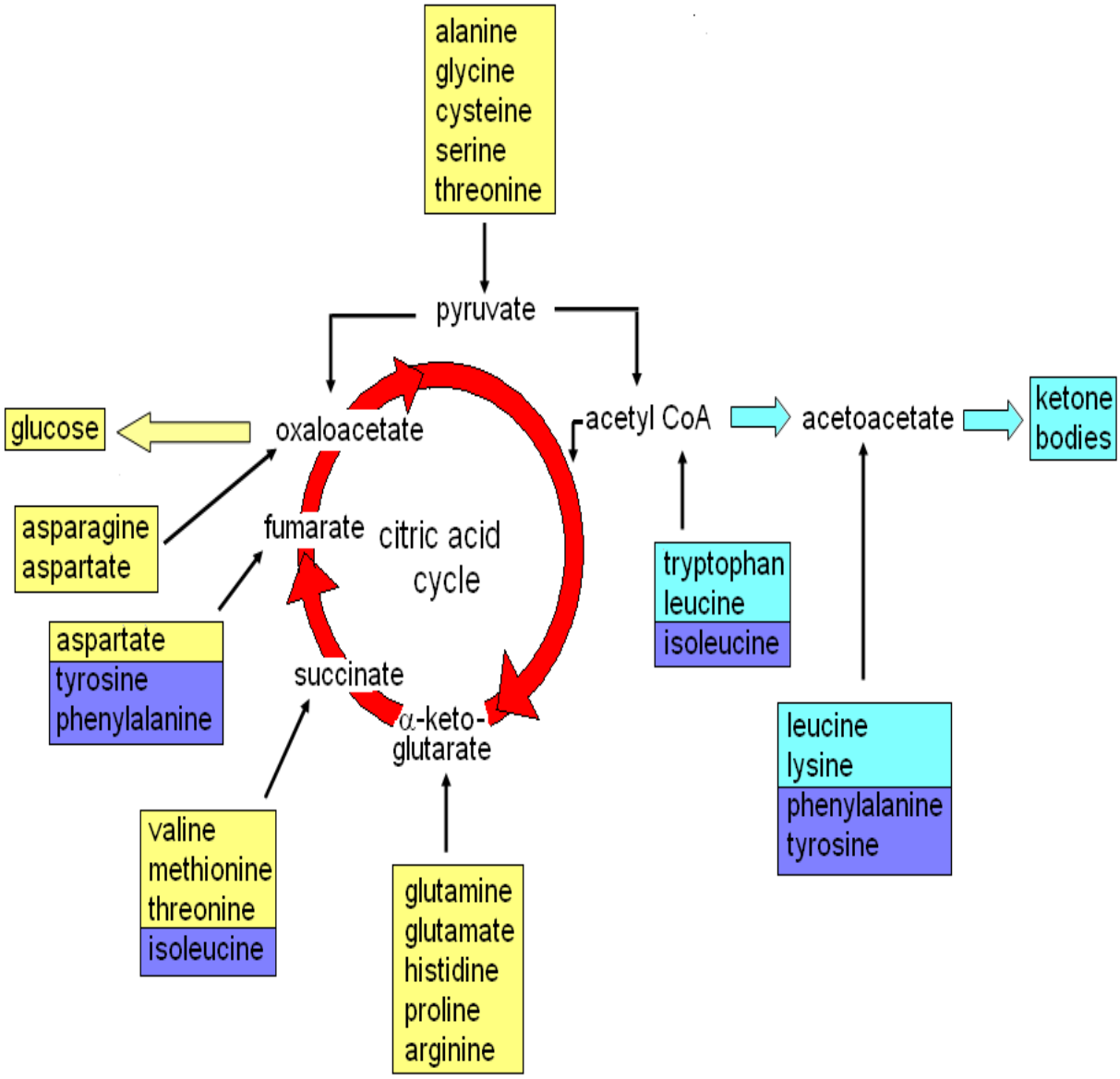
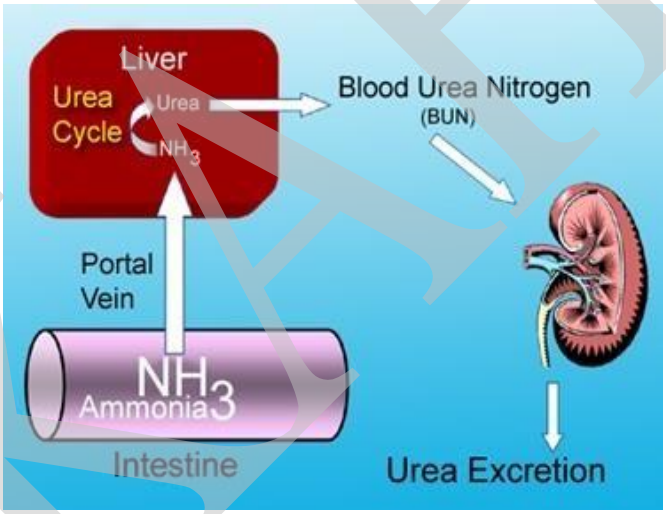


Urea cycle

Living organisms – excess nitrogen – amino acids metabolism
ammonia – urea – uric acid – Ammonotelic – urotelic – uricotelic
Krebs cycle – urea – mitochondria and cytoplasm of liver cells – arginase.



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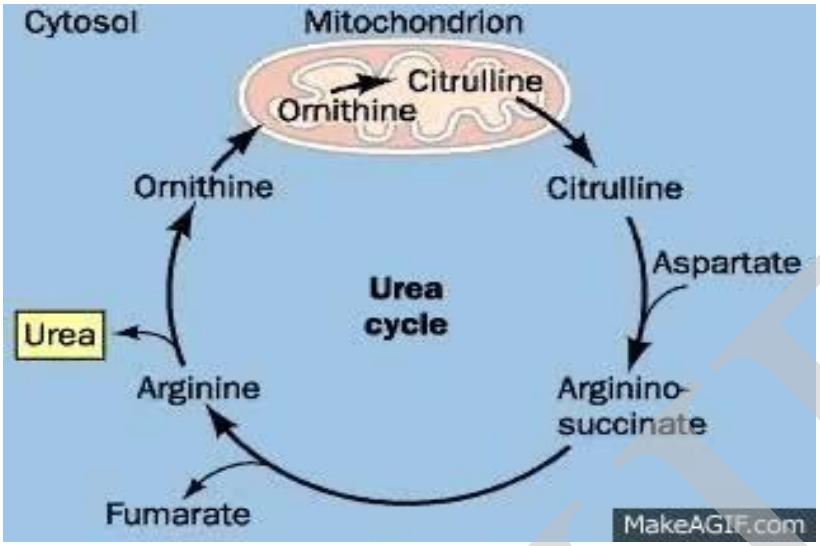

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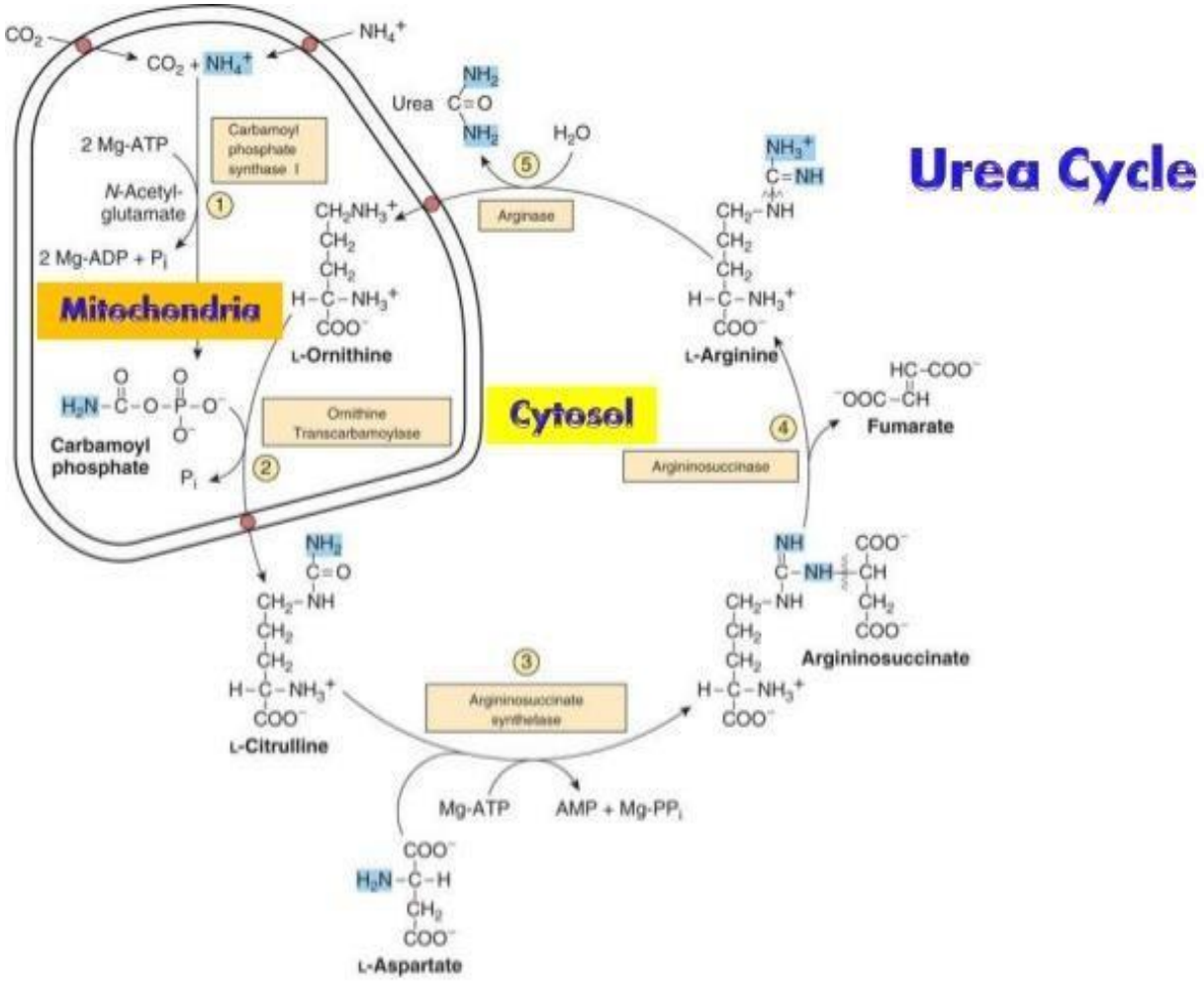
Urea Cycle
Mitochondria
Cytosol
Carbamoyl phosphate synthase I
2 Mg-ATP
N-Acetyl-glutamate
2 Mg-ADP + Pi
Carbamoyl phosphate
Ornithine Transcarbamoylase
L-Ornithine
Arginase
Urea
L-Arginine
Argininosuccinase
Fumarate
Argininosuccinate
Argininosuccinate synthetase
L-Citrulline
Mg-ATP
AMP + Mg-PPi
L-Aspartate

Biosynthesis of amino acids (AA)

Humans can synthesize only 10 of the 20 AA.

Essential AA = AA that cannot be synthesized „de novo". They must be obtained from diet.

Nonessential AA:

Ala is synthesized from pyruvate.

Cys is synthesized from Met and Ser.

Tyr is formed by hydroxylation from Phe.

Essential AA	Nonessential AA
Arg	Ala
His	Asn
Ile	Asp
Leu	Cys
Lys	Gln
Met	Glu
Phe	Gly
Thr	Pro
Trp	Ser
Val	Tyr

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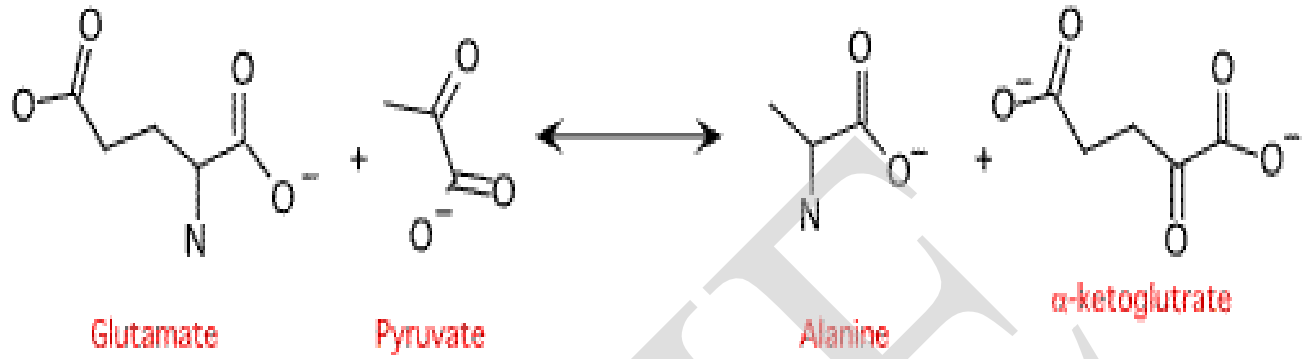

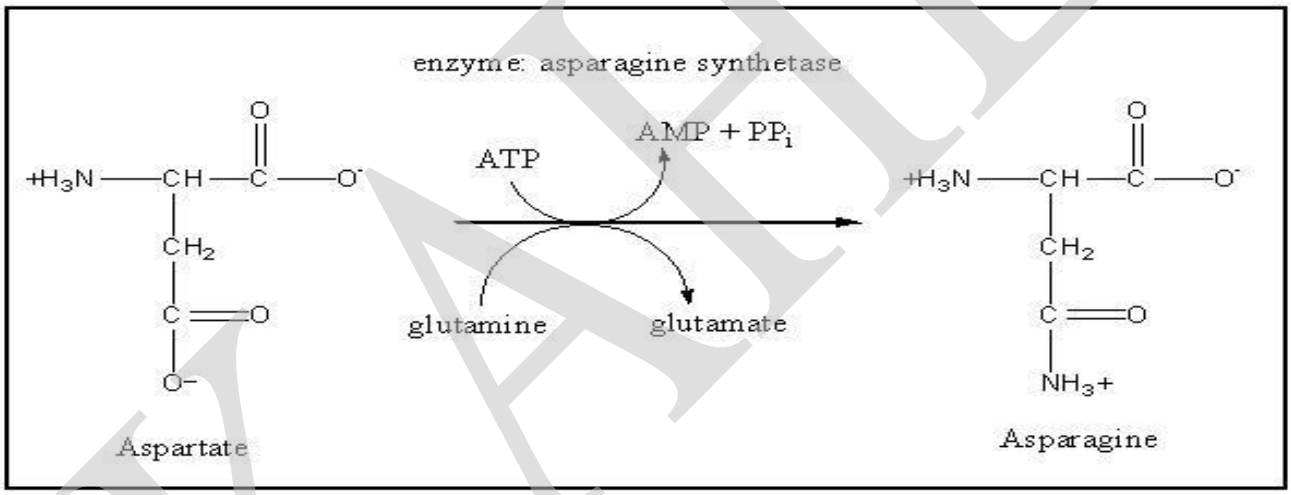

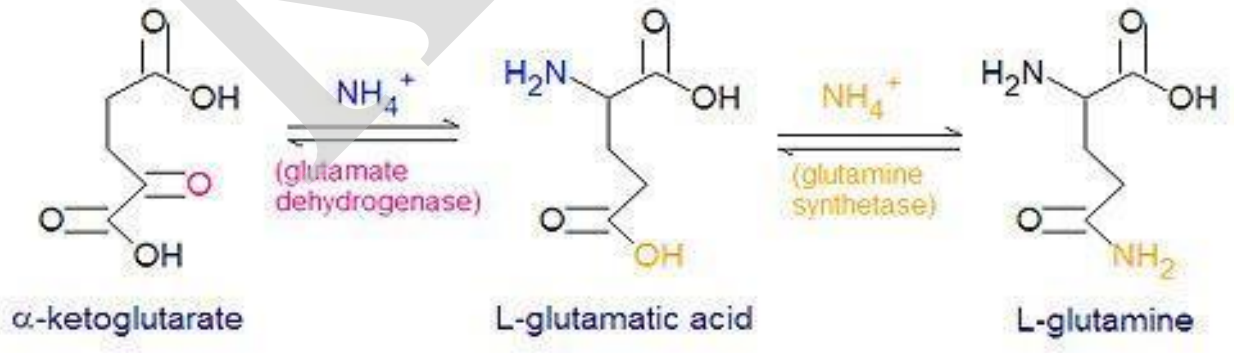

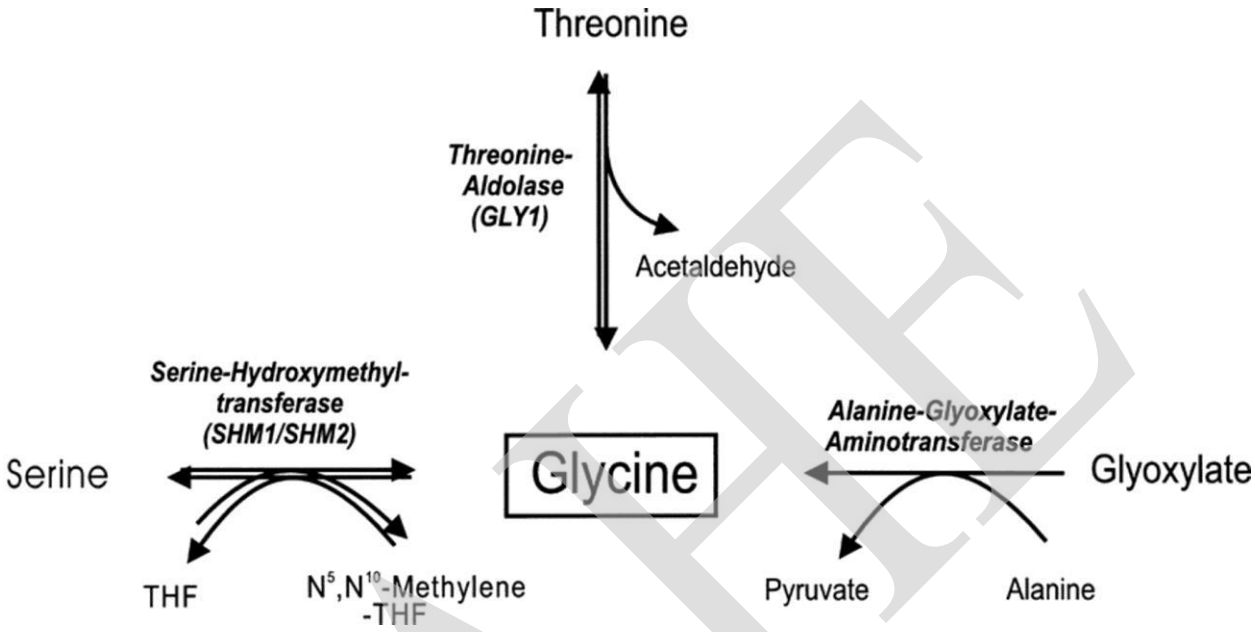
Threonine
Threonine-Aldolase (GLY1)
Acetaldehyde
Serine-Hydroxymethyl-transferase (SHM1/SHM2)
Serine
Glycine
Alanine-Glyoxylate-Aminotransferase
Glyoxylate
THF
N5,N10-Methylene-THF
Pyruvate
Alanine

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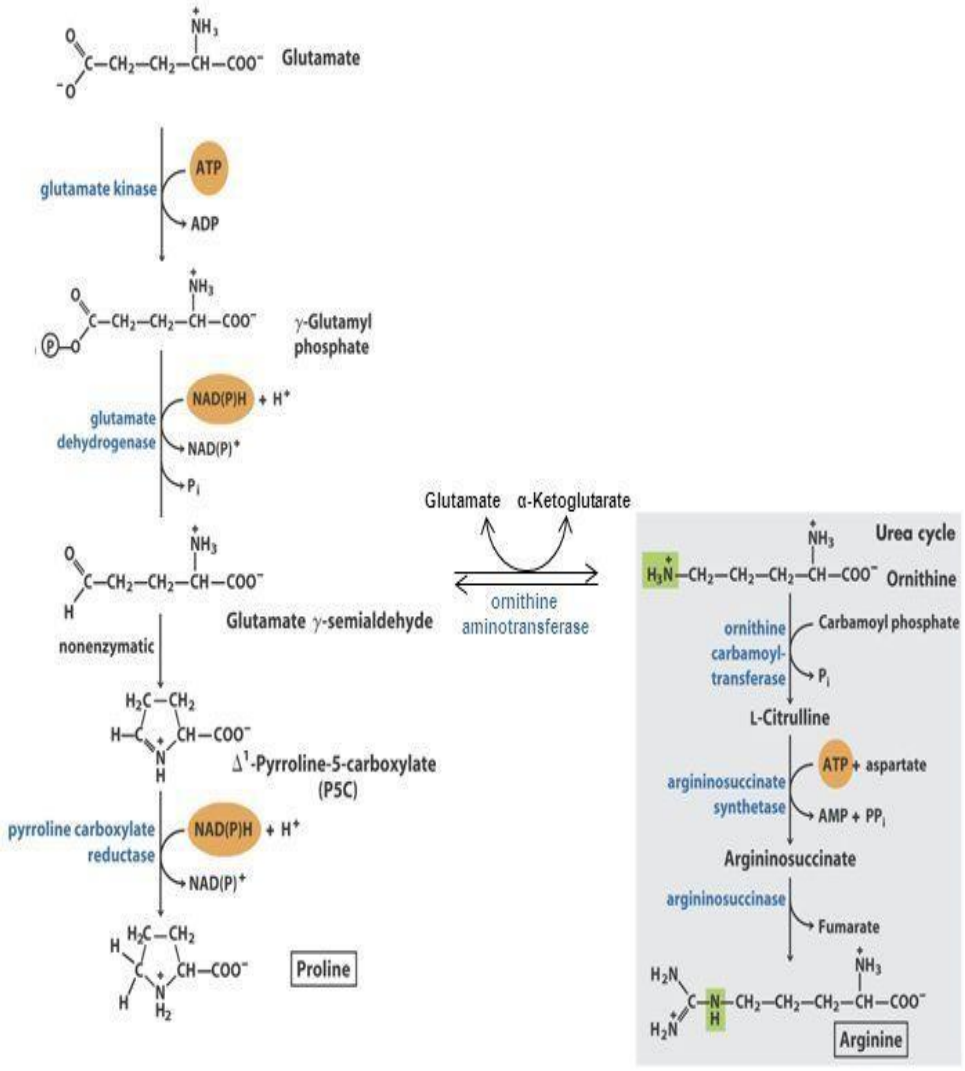
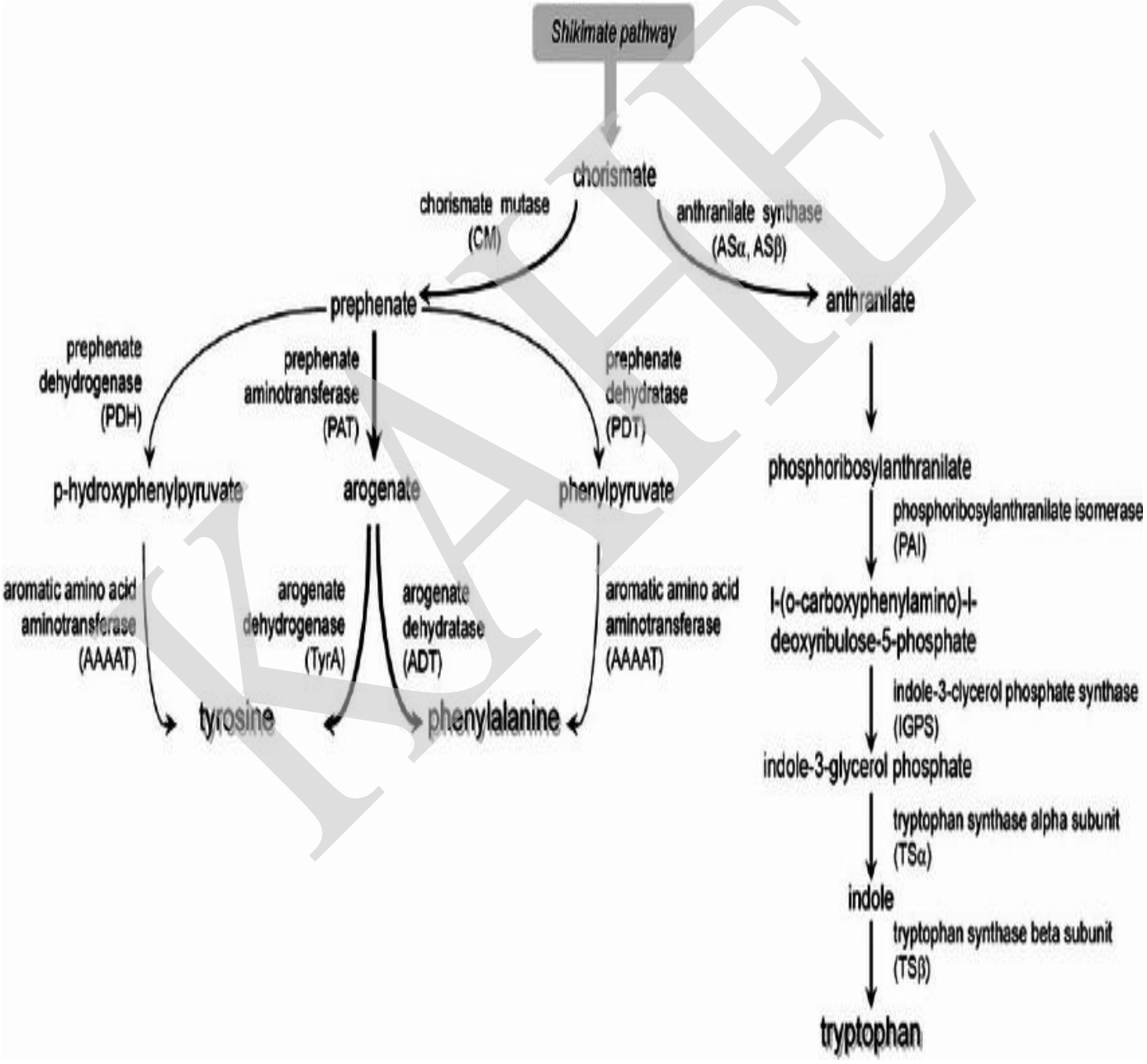
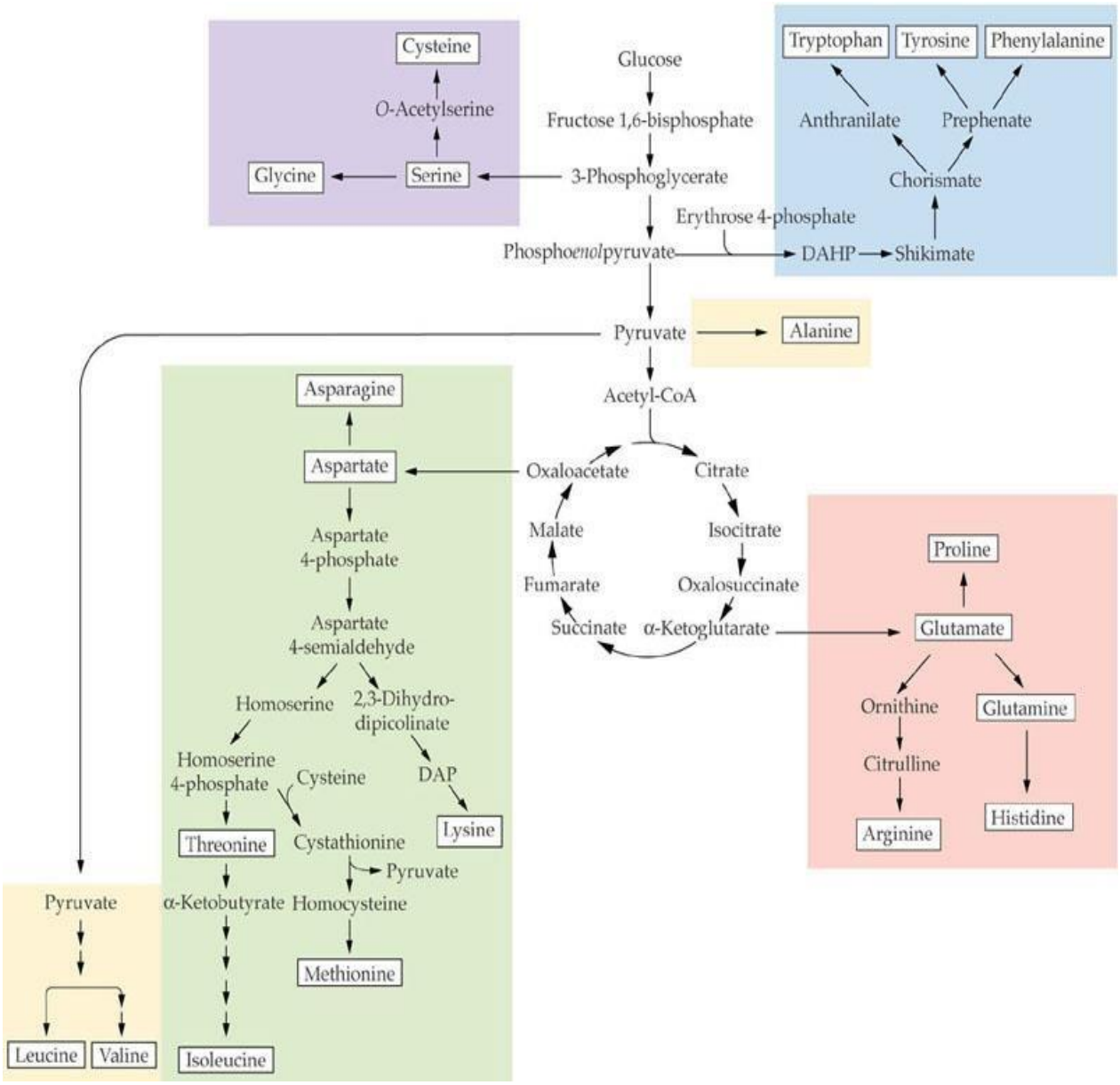
Biosynthesis of Proline and Arginine



Biosynthesis of aromatic amino acids





DAP: Diaminopimelate
DAHP: Deoxy Arabino Heptulosonate Phosphate

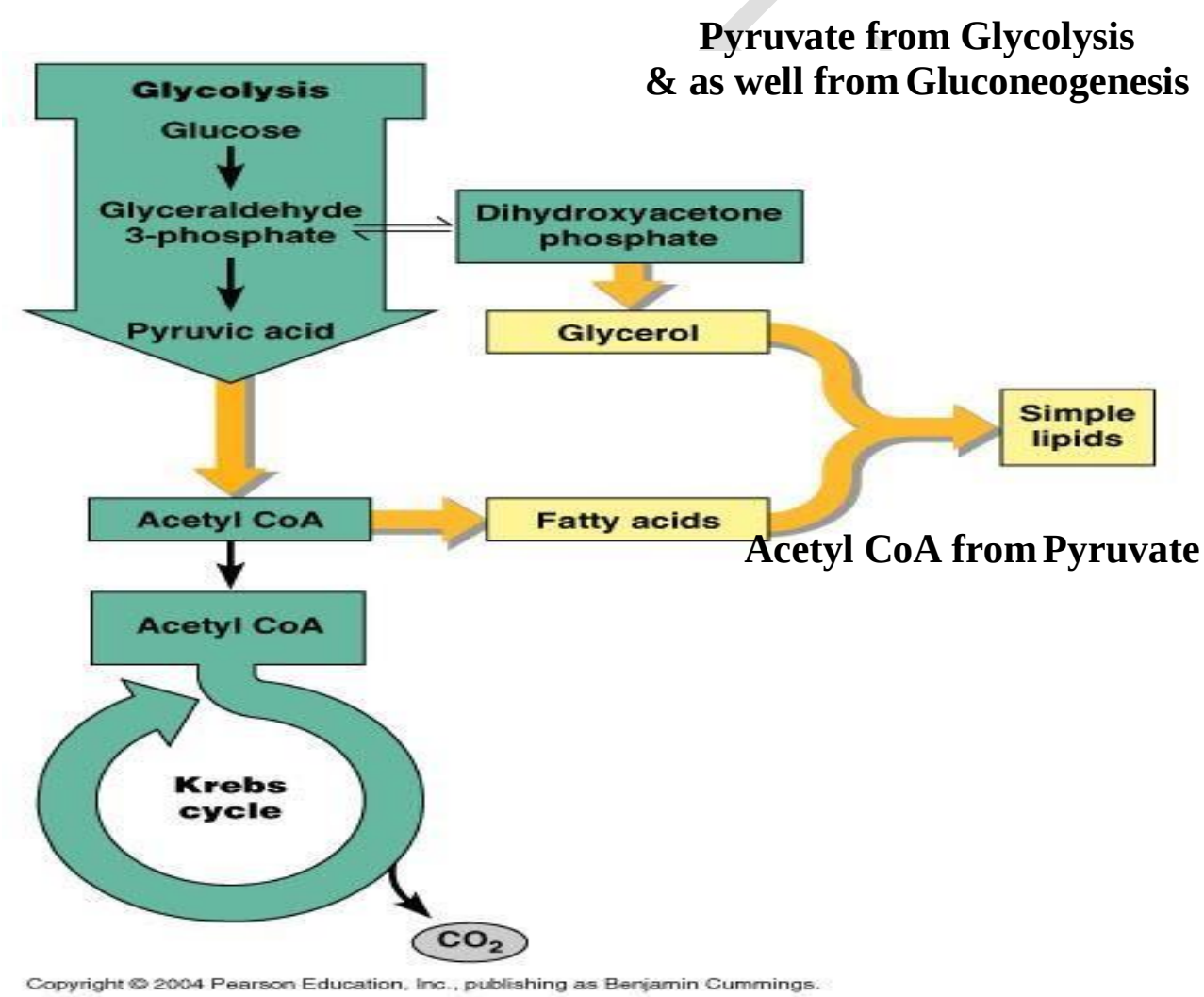
Lipid metabolism

Lipid metabolism is the synthesis and degradation of lipids in cells. Lipid metabolism is the break down or storage of fats for energy; these fats are obtained from consuming food and absorbing them or they are synthesized by an animal's liver. The majority of lipids found in the human body from ingesting food are Triglycerides, also known as fats. Since lipids are fats, lipid metabolism is often considered the digestion and absorption processes of dietary fats. Break down of lipids in the body begins in the mouth through chemical digestion. Lipids then continue to the stomach where chemical digestion continues, and mechanical digestion begins (Peristalsis). The majority of lipid digestion and absorption, however, occurs once the fats reach the small intestines. Chemicals from both the pancreas and liver are transported to the small intestines to help breakdown the fats, along with further mechanical digestion, until they are able to be absorbed into the small intestines.

Lipid metabolism does exist in plants, though the processes differ in some ways when compared to animals. Lipogenesis is the process of synthesizing these fats. Lipid metabolism often begins with hydrolysis, which occurs when a chemical breaks down as a reaction to coming in contact with water. Since lipids (fats) are hydrophobic, hydrolysis in lipid metabolism occurs in the cytoplasm which ends up creating glycerol and fatty acids. Due to the hydrophobic nature of lipids they require special transport proteins known as lipoproteins, which are hydrophilic. Lipoproteins are categorized by their density levels. The varying densities between the types of lipoproteins are characteristic to what type of fats they transport. A number of these lipoproteins are synthesized in the liver, but not all of them originate from this organ.

Lipid Metabolism Disorders are illnesses where trouble occurs in breaking down or synthesizing fats (or fat-like substances). A good deal of the time these disorders are hereditary, meaning it's a condition that is passed along from parent to child through their genes. Gaucher's

Disease (Type I, Type II, and Type III), Neimann-Pick Disease, Tay-Sachs Disease, and Fabry's Disease are all diseases where those afflicted can have a disorder of their body's lipid metabolism. Rarer diseases concerning a disorder of the lipid metabolism are Sitosterolemia, Wolman's Disease, Refsum's Disease, and Cerebrotendinous Xanthomatosis.



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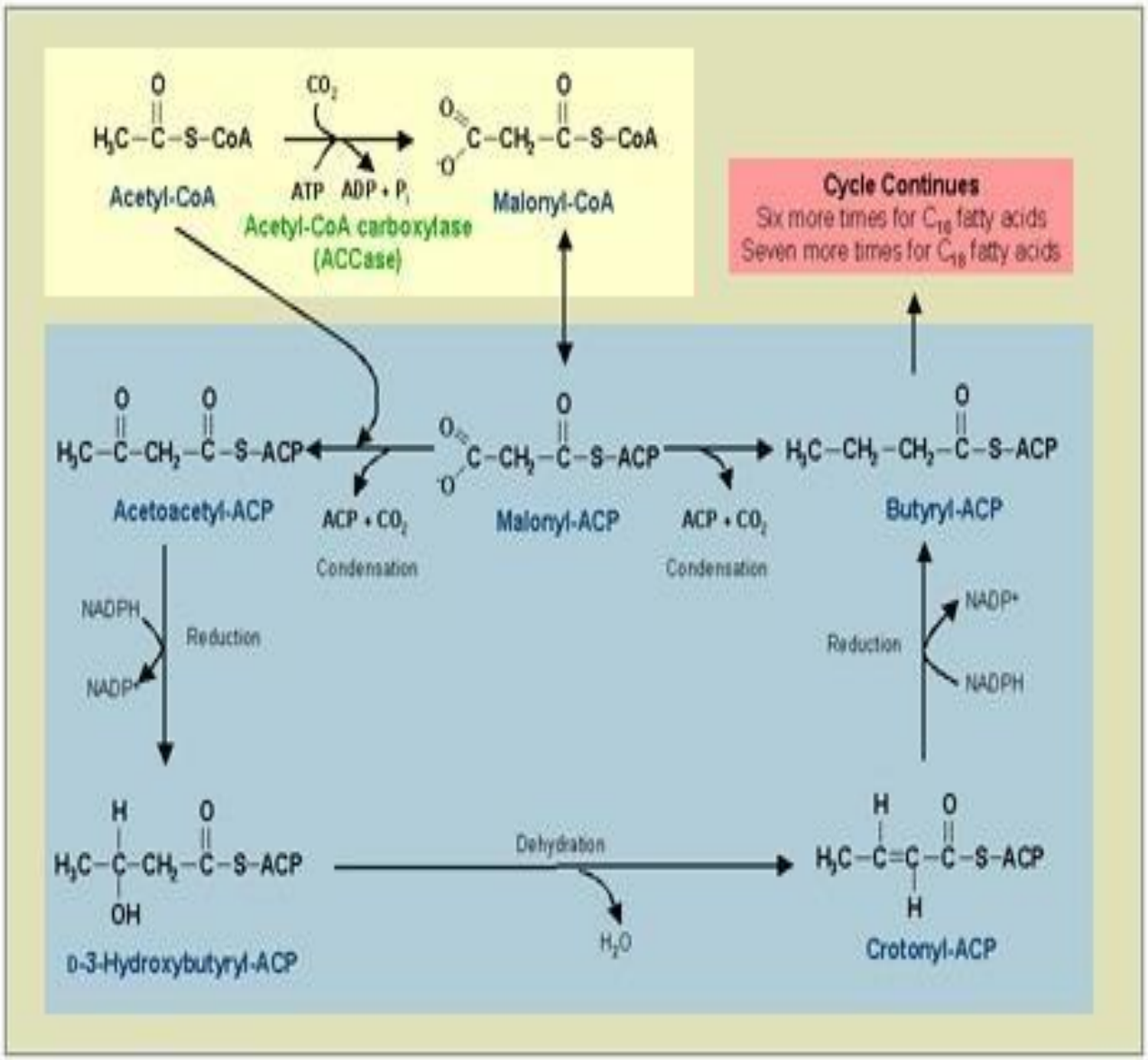
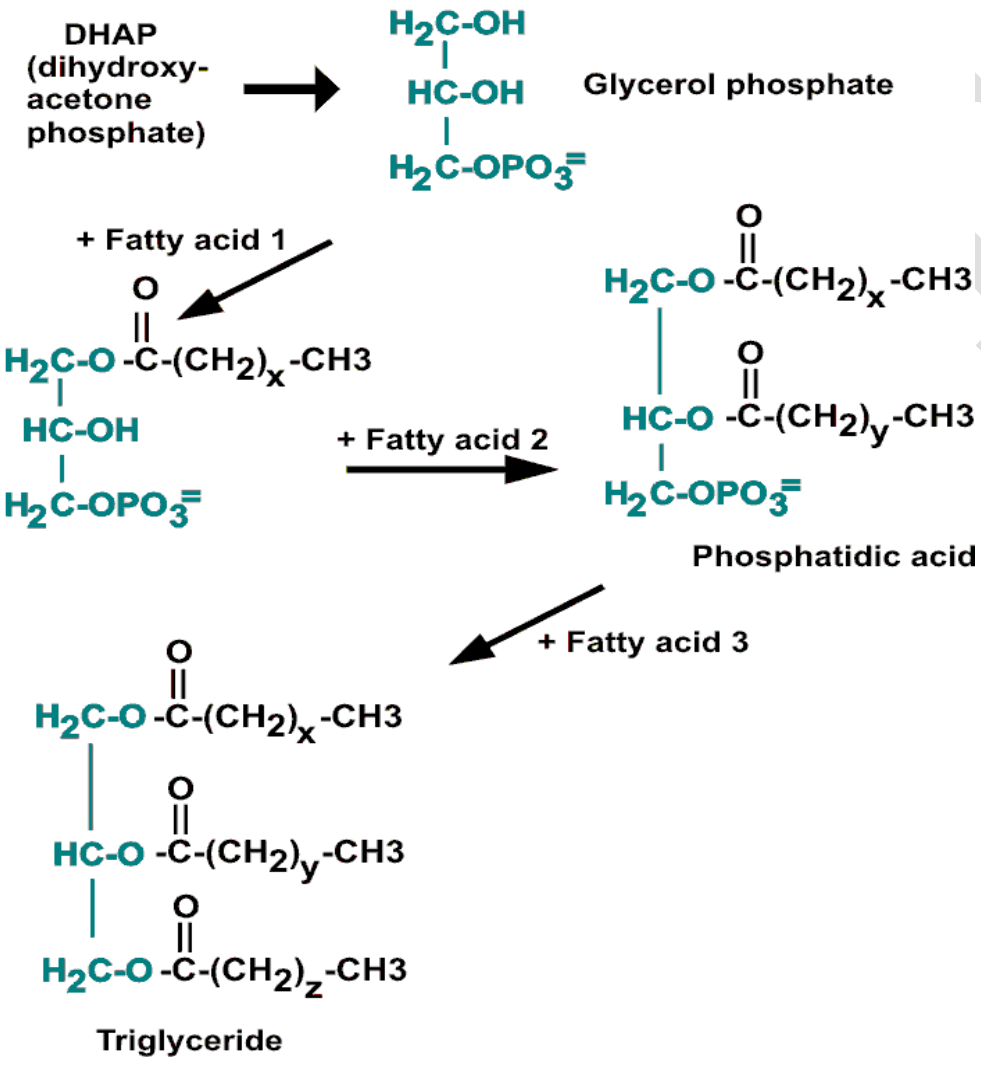
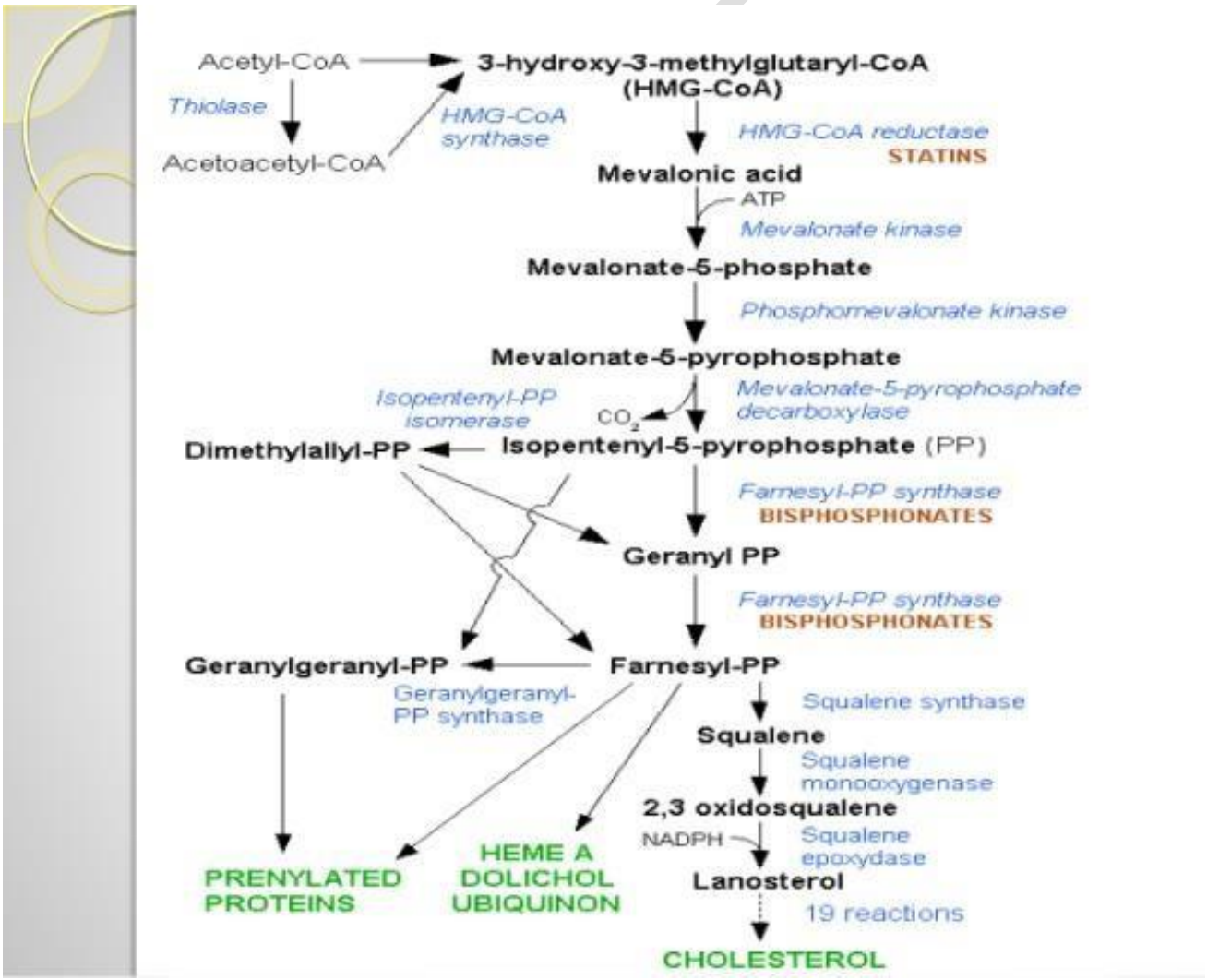
Fatty Acid Synthesis

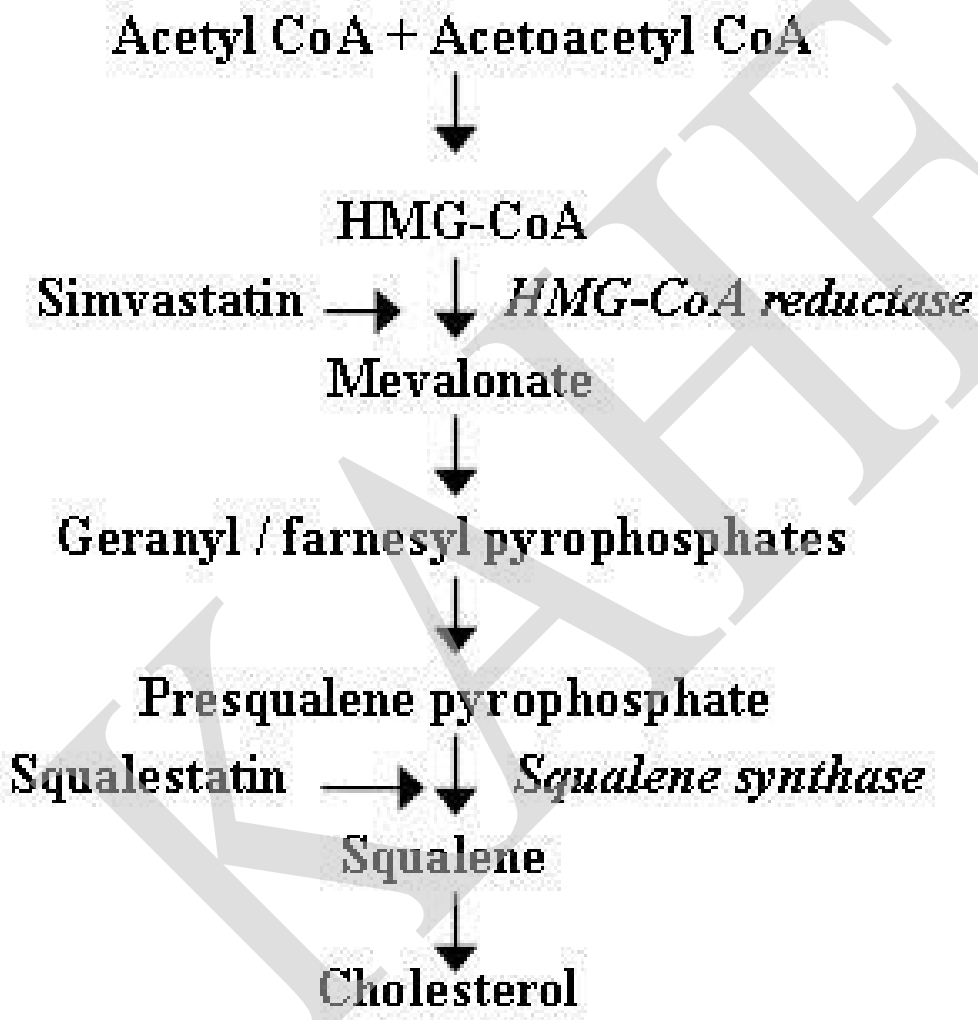


Triglyceride (fat) biosynthesis



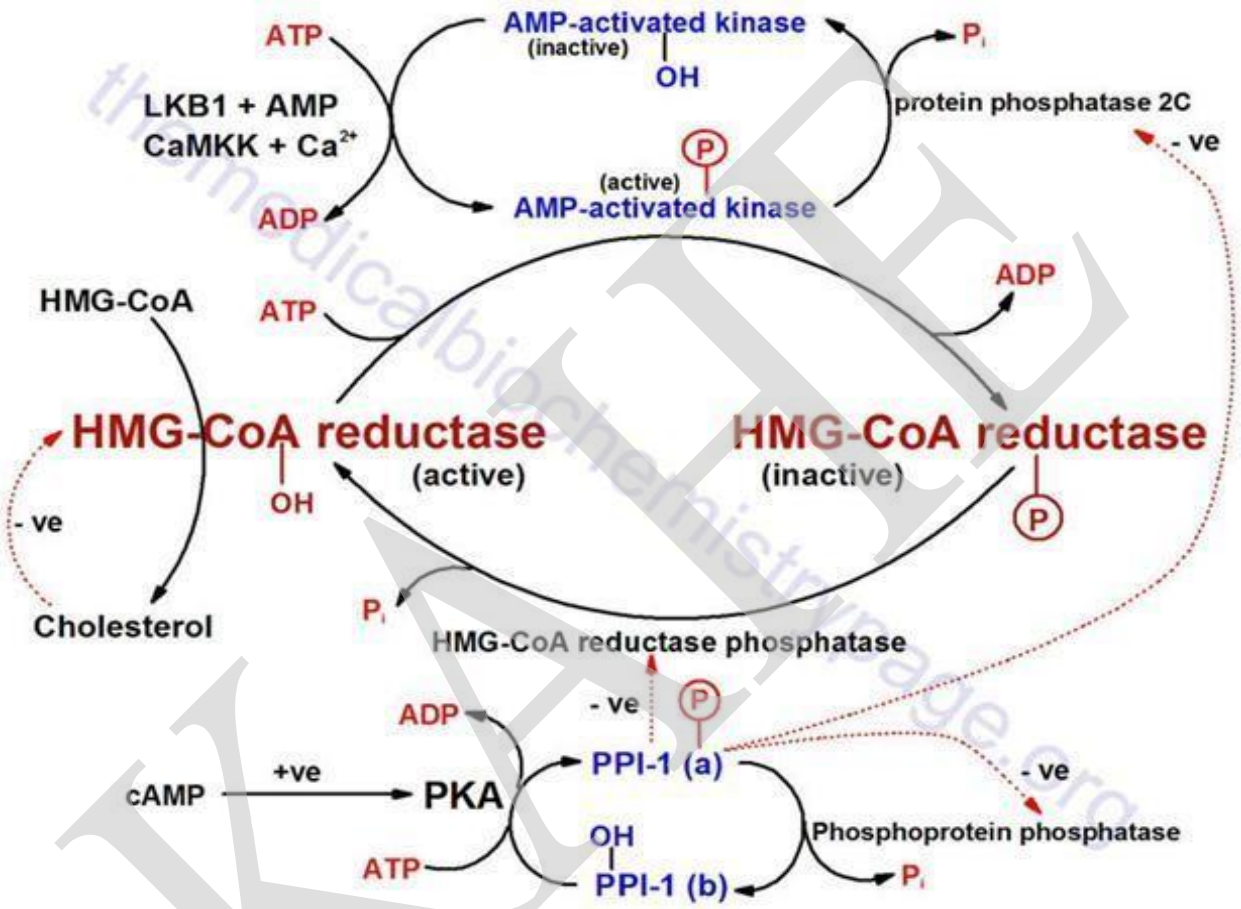
Biosynthesis of Cholesterol

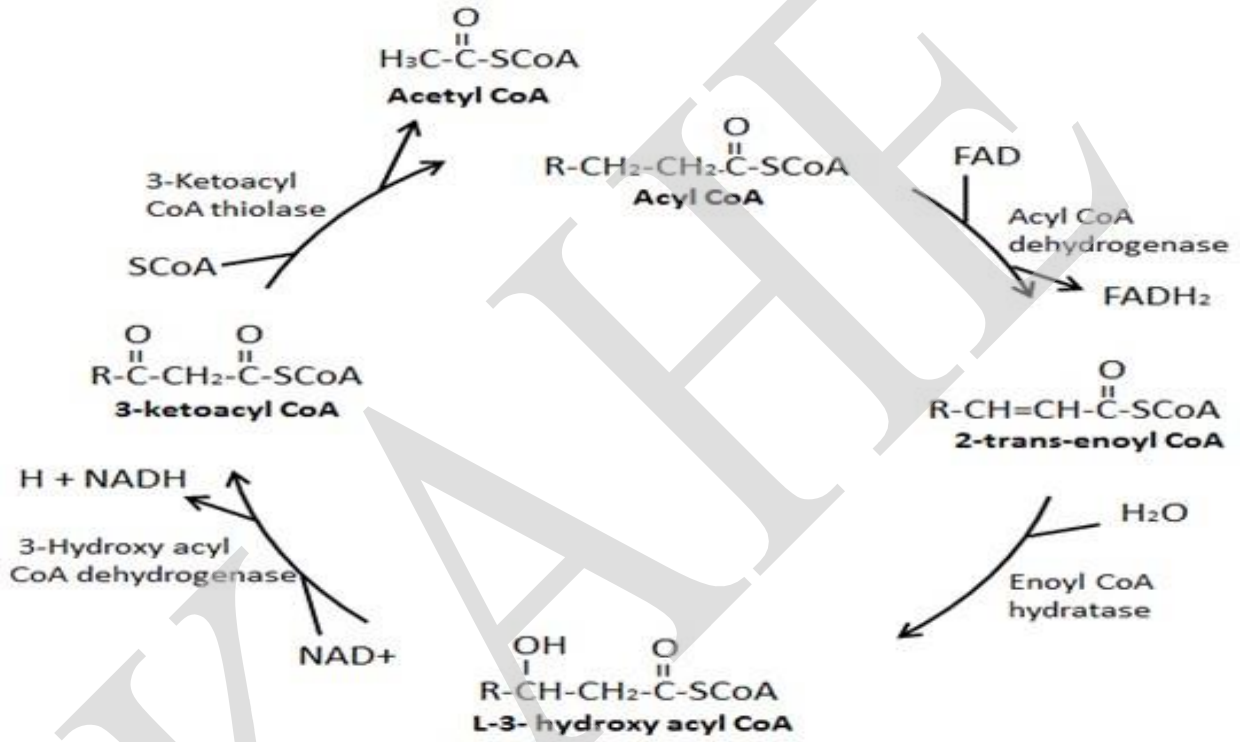




Lipid-lowering medications – statins – atorvastatin – fluvastatin – lovastatin – pitavastatin – pravastatin - rosuvastatin and simvastatin – Target – HMG-CoA reductase

Lapaquistat – TAK-473 – Target – Squalene synthase


ATP
AMP-activated kinase
(inactive)
OH
Pi
LKB1 + AMP
CaMKK + Ca2+
protein phosphatase 2C
- ve
P
(active)
AMP-activated kinase
ADP
ADP
HMG-CoA
ATP
HMG-CoA reductase
(active)
OH
HMG-CoA reductase
(inactive)
P
- ve
Cholesterol
Pi
HMG-CoA reductase phosphatase
ADP
- ve
P
PPI-1 (a)
- ve
cAMP
+ve
PKA
OH
Phosphoprotein phosphatase
ATP
PPI-1 (b)
Pi



Oxidation of fatty acids

Intestines absorbs fats – emulsified by bile acids – hydrolysed by pancrease lipases – FAs – combine with Glycerol (glycolysis) – TG – combine with proteins – lipoproteins – enter into circulation to performs various biological functions.

Types of oxidations – beta oxidation – FA – inert molecule to active molecules – cytosol

FA + CoA, ATP, Acyl CoA synthetase – Fatty acyl CoA – carnitine, carnitine acyl transferase – into mitochondria.

In Mitochondria - Fatty acyl CoA, FAD, NAD – converted into acetyl CoA (C_2) and Acyl CoA (C_{n-2})

Calculate the yield of ATP when one mole of stearic acid is completely oxidized to CO_2 and H_2O.

Process	Reduced co-enzymes formed	ATPs formed
Fatty acid activation Stearoyl-CoA	---	-2
Beta-Oxidations	8 NADH	24
	8 $FADH_2$	16
Citric acid cycle	27 NADH	81
	09 $FADH_2$	18
	---	09 (GTP)

Total ATPs 146

Linoleic acid – 9,12 – octadecadienoic acid – oxidized to CO_2 and H_2O – first double bond does not need an FAD to reduce it and second double bond consumes an NADPH – total ATP produced 146-2-3 = 141.

Nucleic acid metabolism

Nucleic acid metabolism is the process by which nucleic acids (DNA and RNA) are synthesized and degraded. Nucleic acids are polymers of nucleotides. Nucleotide synthesis is an anabolic mechanism generally involving the chemical reaction of phosphate, pentose sugar, and a nitrogenous base. Destruction of nucleic acid is a catabolic reaction. Additionally, parts of the nucleotides or nucleobases can be salvaged to recreate new nucleotides. Both synthesis and degradation reactions require enzymes to facilitate the event. Defects or deficiencies in these enzymes can lead to a variety of diseases. Nucleotides can be separated into purines and pyrimidines. They are both primarily produced in the liver. They both contain a sugar and a phosphate, but have nitrogenous bases that are different sizes. Because of this, the two different groups are synthesized in different ways. However, all nucleotide synthesis requires the use of phosphoribosyl pyrophosphate (PRPP) which donates the ribose and phosphate necessary to create a nucleotide.

Purine Synthesis

Adenine and guanine are the two nucleotides classified as purines. In purine synthesis, PRPP is turned into inosine monophosphate, or IMP. Production of IMP from PRPP requires glutamine, glycine, aspartate, and 6 ATP, among other things.[1] IMP is then converted to AMP (adenosine monophosphate) using GTP and aspartate, which is converted into fumarate. While IMP can be directly converted to AMP, synthesis of GMP (guanosine monophosphate) requires an intermediate step, in which NAD+ is used to form the intermediate xanthosine monophosphate, or XMP. XMP is then converted into GMP by using the hydrolysis of 1 ATP and the conversion of glutamine to glutamate. AMP and GMP can then be converted into ATP and GTP, respectively, by kinases that add additional phosphates.

ATP stimulates production of GTP, while GTP stimulates production of ATP. This cross regulation keeps the relative amounts of ATP and GTP the same. Excess of either nucleotide could increase the likelihood of DNA mutations, where the wrong purine nucleotide is inserted.

Lesch-Nyhan syndrome is caused by a deficiency in hypoxanthine-guanine phosphoribosyltransferase or HGPRT, the enzyme that catalyzes the reversible reaction of producing guanine from GMP. This is a sex-linked congenital defect that causes overproduction of uric acid along with mental retardation, spasticity, and an urge to self-mutilate.[1][2][3] Pyrimidine synthesis. Uridine-triphosphate (UTP), at left, reacts with glutamine and other chemicals to form cytidine-triphosphate (CTP), on the right.

Pyrimidine nucleotides include cytidine, uridine, and thymidine. The synthesis of any pyrimidine nucleotide begins with the formation of uridine. This reaction requires aspartate, glutamine, bicarbonate, and 2 ATP molecules (to provide energy), as well as PRPP which provides the ribose-monophosphate. Unlike in purine synthesis, the sugar/phosphate group from PRPP is not added to the nitrogenous base until towards the end of the process. After uridine-monophosphate is synthesized, it can react with 2 ATP to form uridine-triphosphate or UTP. UTP can be converted to CTP (cytidine-triphosphate) in a reaction catalyzed by CTP synthetase. Thymidine synthesis first requires reduction of the uridine to deoxyuridine (see next section), before the base can be methylated to produce thymidine. ATP, a purine nucleotide, is an activator of pyrimidine synthesis, while CTP, a pyrimidine nucleotide, is an inhibitor of pyrimidine synthesis. This regulation helps to keep the purine/pyrimidine amounts similar, which is beneficial because equal amounts of purines and pyrimidines are required for DNA synthesis. Deficiencies of enzymes involved in pyrimidine synthesis can lead to the genetic disease Orotic aciduria which causes excessive excretion of orotic acid in the urine.

Converting nucleotides to deoxynucleotides

Nucleotides are initially made with ribose as the sugar component, which is a feature of RNA. DNA, however, requires *deoxy*ribose, which is missing the 2'-hydroxyl (-OH group) on the ribose. The reaction to remove this -OH is catalyzed by ribonucleotide reductase. This enzyme converts NDPs (nucleoside-diphosphate) to dNDPs (deoxynucleoside-diphosphate). The nucleotides must be in the diphosphate form for the reaction to occur. In order to synthesize thymidine, a component of DNA which only exists in the deoxy form, uridine is converted to

deoxyuridine (by ribonucleotide reductase), and then is methylated by thymidylate synthase to create thymidine.

Degradation of nucleic acids

The breakdown of DNA and RNA is occurring continuously in the cell. Purine and pyrimidine nucleosides can either be degraded to waste products and excreted or can be salvaged as nucleotide components.

Pyrimidine catabolism

Cytosine and uracil are converted into beta-alanine and later to malonyl-CoA which is needed for fatty acid synthesis, among other things. Thymine, on the other hand, is converted into β-aminoisobutyric acid which is then used to form methylmalonyl-CoA. The leftover carbon skeletons such as acetyl-CoA and Succinyl-CoA can then be oxidized by the citric acid cycle. Pyrimidine degradation ultimately ends in the formation of ammonium, water, and carbon dioxide. The ammonium can then enter the urea cycle which occurs in the cytosol and the mitochondria of cells.

Pyrimidine bases can also be salvaged. For example, the uracil base can be combined with ribose-1-phosphate to create uridine monophosphate or UMP. A similar reaction can also be done with thymine and deoxyribose-1-phosphate. Deficiencies in enzymes involved in pyrimidine catabolism can lead to diseases such as Dihydropyrimidine dehydrogenase deficiency which has negative neurological effects.

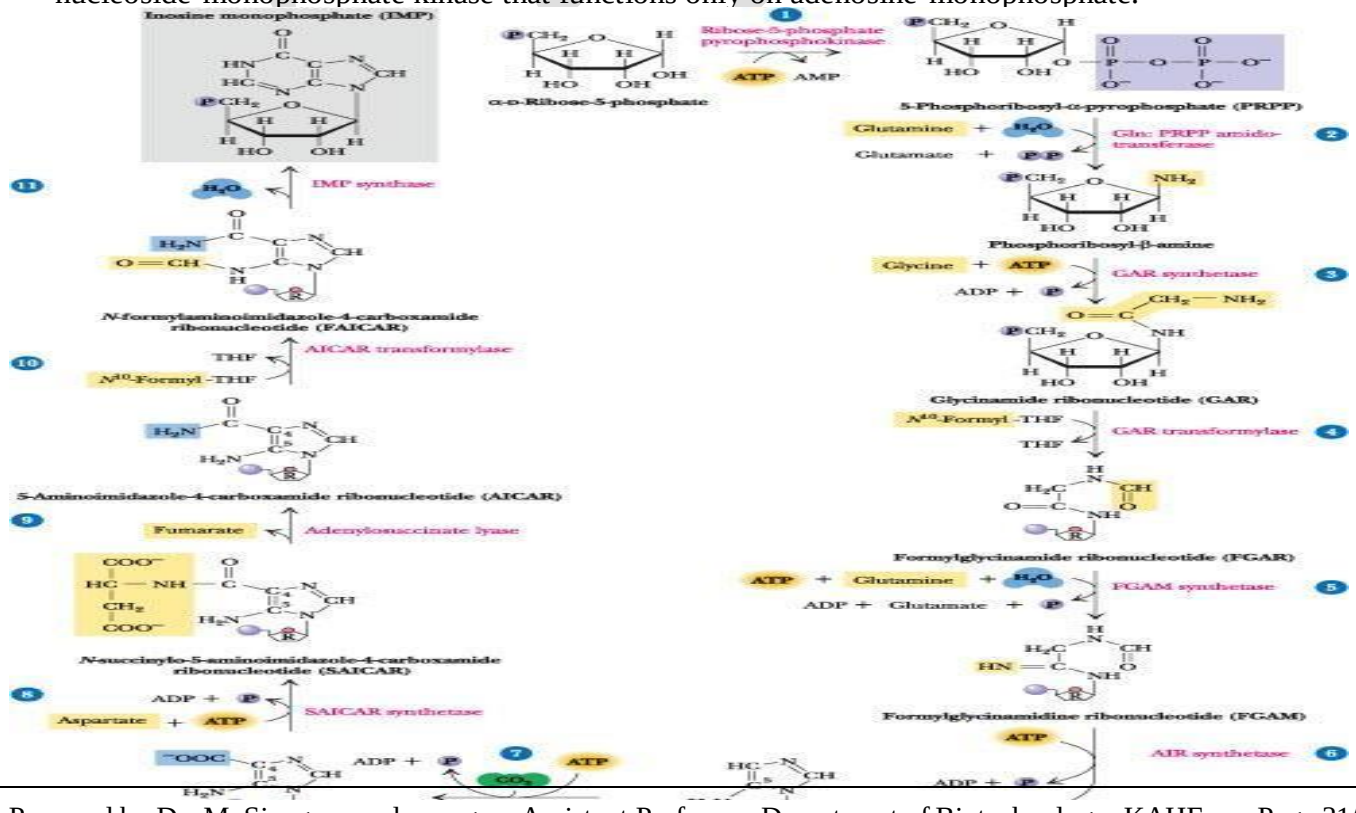
Purine catabolism

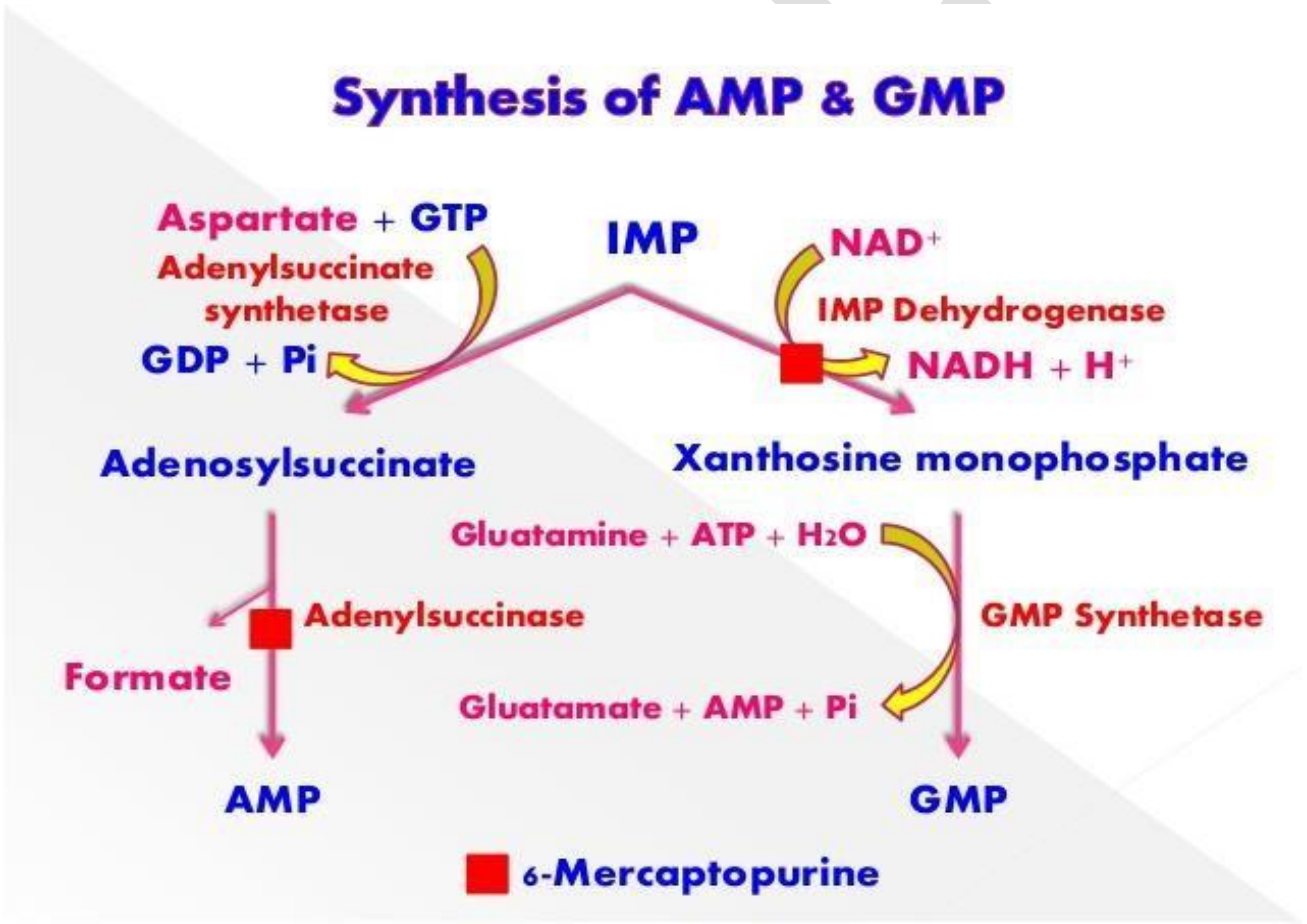
Purine degradation takes place mainly in the liver of humans and requires an assortment of enzymes to degrade purines to uric acid. First, the nucleotide will lose its phosphate through 5'-nucleotidase. The nucleoside, adenosine, is then deaminated and hydrolyzed to form hypoxanthine via adenosine deaminase and nucleosidase respectively. Hypoxanthine is then oxidized to form xanthine and then uric acid through the action of xanthine oxidase. The other purine nucleoside, guanosine, is cleaved to form guanine. Guanine is then deaminated via

guanine deaminase to form xanthine which is then converted to uric acid. Oxygen is the final electron acceptor in the degradation of both purines. Uric acid is then excreted from the body in different forms depending on the animal. Free purine and pyrimidine bases that are released into the cell are typically transported intercellularly across membranes and salvaged to create more nucleotides via nucleotide salvage. For example, adenine + PRPP --> AMP + PPi. This reaction requires the enzyme adenine phosphoribosyltransferase. Free guanine is salvaged in the same way except it requires hypoxanthine-guanine phosphoribosyltransferase. Defects in purine catabolism can result in a variety of diseases including gout, which stems from an accumulation of uric acid crystals in various joints, and adenosine deaminase deficiency, which causes immunodeficiency.

Interconversion of nucleotides

Once the nucleotides are synthesized they can exchange phosphates among one another in order to create mono-, di-, and tri-phosphate molecules. The conversion of a nucleoside-diphosphate (NDP) to a nucleoside-triphosphate (NTP) is catalyzed by nucleoside diphosphate kinase, which uses ATP as the phosphate donor. Similarly, nucleoside-monophosphate kinase carries out the phosphorylation of nucleside-monophosphates. Adenylate kinase is a specific nucleoside-monophosphate kinase that functions only on adenosine-monophosphate.




Synthesis of AMP & GMP
Aspartate + GTP
Adenylsuccinate synthetase
GDP + Pi
IMP
NAD+
IMP Dehydrogenase
NADH + H+
Adenosylsuccinate
Xanthosine monophosphate
Gluatamine + ATP + H2O
Adenylsuccinase
GMP Synthetase
Formate
Gluatamate + AMP + Pi
AMP
GMP
6-Mercaptopurine

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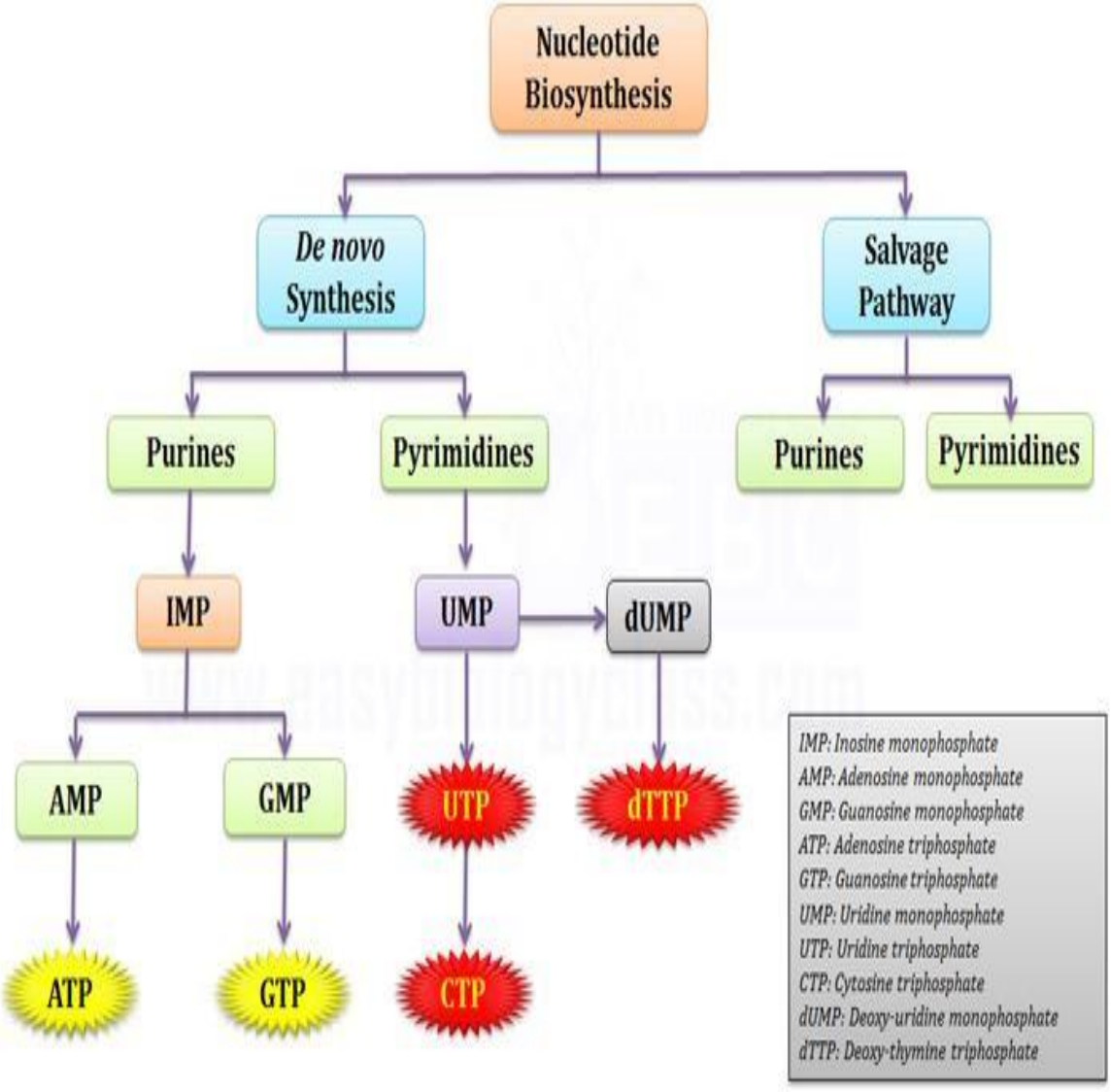
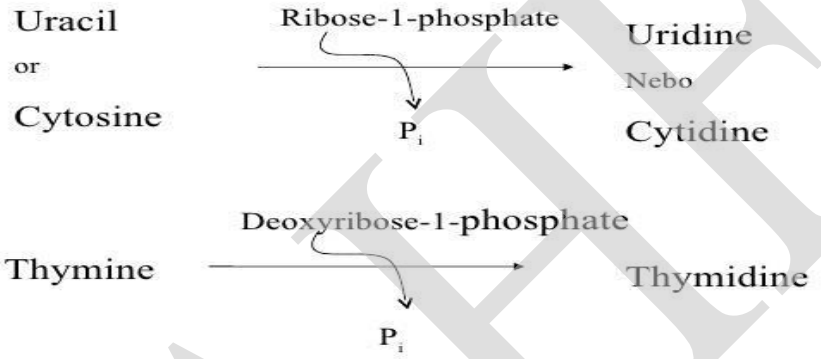


Formation of pyrimidine nucleotides by *salvage pathway (using of free bases for the synthesis)*

1. Relatively non-specific pyrimidine nucleoside phosphorylase converts the pyrimidine bases to their nucleosides



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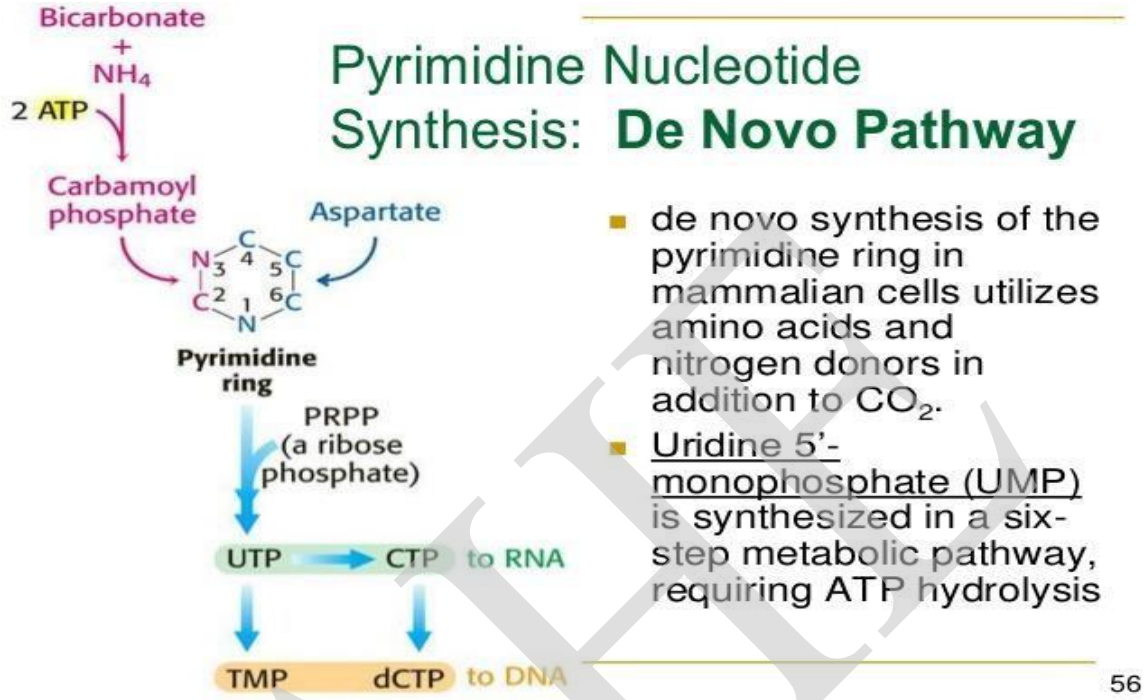

Pyrimidine Nucleotide Synthesis: **De Novo Pathway**

- de novo synthesis of the pyrimidine ring in mammalian cells utilizes amino acids and nitrogen donors in addition to CO_2.
- Uridine 5'-monophosphate (UMP) is synthesized in a six-step metabolic pathway, requiring ATP hydrolysis

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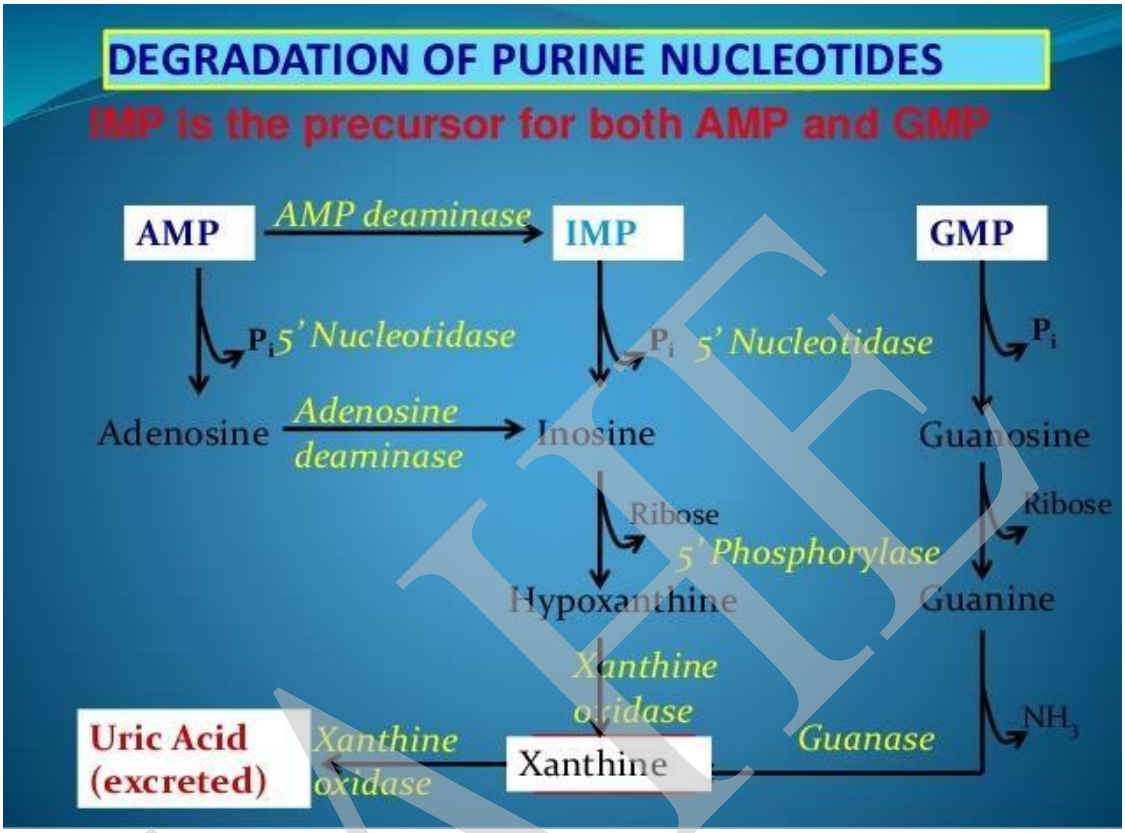

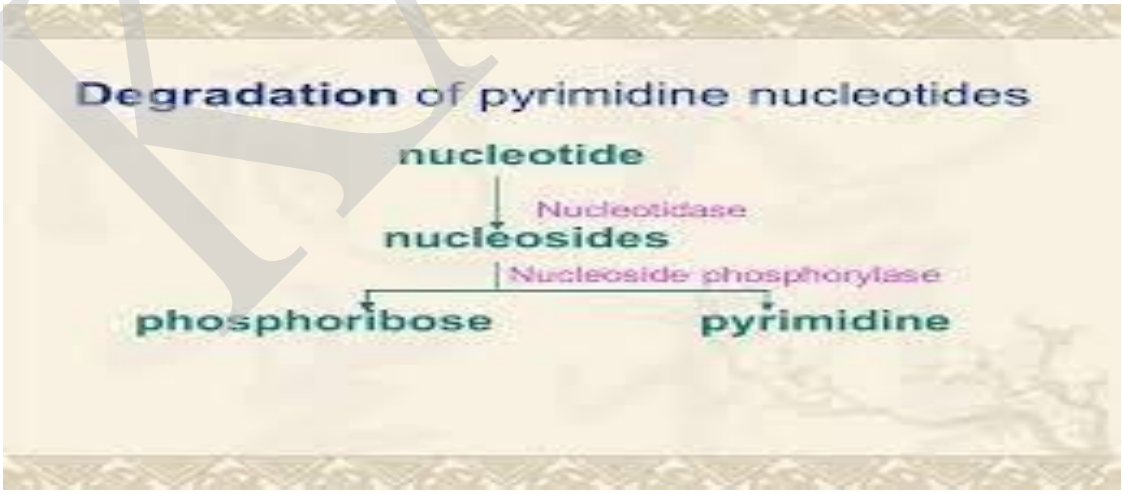

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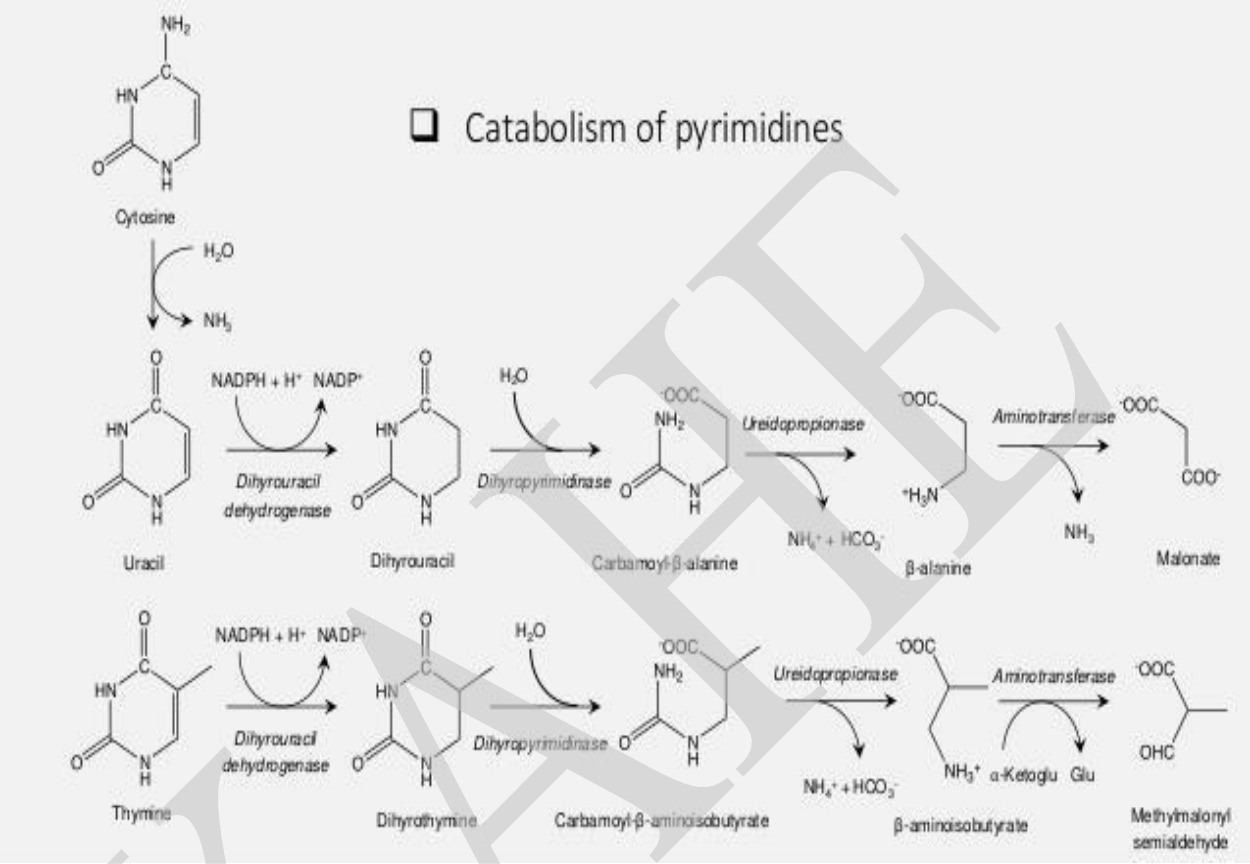
Metabolism of Nucleotides



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Possible questions

Part A

1. Oxidation of fatty acids occurs
(A) In the cytosol (B) In the matrix of mitochondria
(C) On inner mitochondrial membrane (D) On the microsomes

2. In humans, a dietary essential fatty acid is
(A) Palmitic acid (B) Stearic acid (C) Oleic acid (D) Linoleic acid

3. A purine nucleotide is
(A) AMP (B) UMP (C) CMP (D) TMP

4. In a DNA molecule the 'TTP' concentration is 30%, the 'GTP' concentration will be
(A) 10% (B) 20% (C) 30% (D) 40%

5. UTP is converted to CTP by
(A) Methylation (B) Isomerisation (C) Amination (D) Reduction

6. Carnitine is required for the transport of
(A) Triglycerides out of liver (B) Triglycerides into mitochondria
(C) Short chain fatty acids into mitochondria (D) Long chain fatty acids into mitochondria

7. An increased melting temperature of duplex DNA results from a high content of
(A) Adenine + Guanine (B) Thymine + Cytosine
(C) Cytosine + Guanine (D) Cytosine + Adenine

Part B

1. Write a short note on deamination/transdeamination/decarboxylation of amino acids.
2. Write a short note on GOT/ GPT/ AST/ ALT/ glucogenic/ ketogeic amino acids.
3. Write a short note on ammonotelic/urotelic/uricotelic.
4. Briefly write about 'Familial hypercholesterolemia'
5. How does phospholipase A1/A2/C/D act on phospholipids?
6. Write a short note on 'salvage pathway' of purine biosynthesis.
7. Write a short note on 'Allopurinol'.

Part C

1. Write an essay the 'amino acid catabolism'.
2. Discuss on the following topics:
a) Transdeamination
b) Transmethylation
c) Urea cycle.
3. Enumerate the various steps involved in the biosynthesis of any eight essential/ non-essential amino acids.
4. Enumerate the various steps involved in the biosynthesis and biodegradation of fatty acids
5. Discuss on the biosynthesis of phospholipids and their degradation processes.
6. Explain in detail the biosynthesis of cholesterol and its control reactions.
7. Calculate the yield of ATP when one mole of stearic acid is completely oxidized to CO_2 and H_2O.

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UNIT : III (Bioenergetics)

UNIT - III

SYLLABUS

Bioenergetics: TCA Cycle, glycolysis, gluconeogenesis, Pentose phosphate shunt, Embden-Meyerhof pathway, urea cycle, interconnection of pathways, Metabolic regulation, Bioenergetics: Respiratory chain, ATP cycle, energy-rich compounds.

Carbohydrate metabolism

Carbohydrate metabolism denotes the various biochemical processes responsible for the formation, breakdown, and interconversion of carbohydrates in living organisms. Carbohydrates are central to many essential metabolic pathways. Plants synthesize carbohydrates from carbon dioxide and water through photosynthesis, storing the absorbed energy internally. Plants are consumed by animals and fungi, and the stored carbohydrates are broken down during cellular respiration to make energy available to cells. This energy is temporarily stored in form of high energy molecules, such as ATP, for use in various cellular processes. Humans, and other organisms capable of aerobic respiration, metabolize glucose and oxygen to release energy, with carbon dioxide and water as byproducts.

Although humans consume a variety of carbohydrates, digestion breaks down complex carbohydrates into a few simple monomers for metabolism: glucose, fructose, and galactose. Glucose constitutes about 80% of the products, and is the primary structure that is distributed to cells in the tissues. Most of the fructose and galactose travel to the liver, where they can be converted to glucose. Ultimately, glucose is distributed to cells to be broken down or stored as glycogen.

Metabolic pathways

Glycolysis

Glycolysis is the process of breaking down a glucose molecule into two pyruvate molecules, while storing energy released during this process as ATP and NADH.[2] Nearly all organisms that break down glucose utilize glycolysis. Glucose regulation and product use are the primary categories in which these pathways differ between organisms. In some tissues and organisms, glycolysis is the sole method of energy production. This pathway is anaerobic,

because it doesn't require oxygen. Glycolysis consists of ten steps, split into two phases. During the first phase, it requires the breakdown of two ATP molecules. During the second phase, chemical energy from the intermediates is transferred into ATP and NADH. The breakdown of one molecule of glucose results in two molecules of pyruvate, which can be further oxidized to access more energy in later processes.

Glycogenolysis

Glycogenolysis refers to the breakdown of glycogen. In the liver, muscles, and the kidney, this process occurs to provide glucose when necessary. A single glucose molecule is cleaved from a branch of glycogen, and is transformed into glucose-1-phosphate during this process. This molecule can then be converted to glucose-6-phosphate, an intermediate in the glycolysis pathway. Glucose-6-phosphate can then progress through glycolysis. Glycolysis only requires the input of one molecule of ATP when the glucose originates in glycogen. Alternatively, glucose-6-phosphate can be converted back into glucose in the liver and the kidneys, allowing it to raise blood glucose levels if necessary. Glucagon in the liver stimulates glycogenolysis when the blood glucose is lowered, known as hypoglycemia. The glycogen in the liver can function as a backup source of glucose between meals. Adrenaline stimulates the breakdown of glycogen in the skeletal muscle during exercise. In the muscles, glycogen ensures a rapidly accessible energy source for movement.

Glycogenesis

Glycogenesis refers to the process of synthesizing glycogen. In humans, excess glucose is converted glycogen via this process. Glycogen is a highly branched structure, consisting of glucose molecules linked together. The branching of glycogen increases its solubility, and allows for a higher number of glucose molecules to be accessible for breakdown. Although glycogenesis occurs in nearly all human tissues, its primary location is the liver and skeletal muscles.[2] A variety of enzymes link together glucose, in the form of glucose-6-phosphate.

Gluconeogenesis

Gluconeogenesis is the reverse process of glycolysis. It involves the conversion of non-carbohydrate molecules into glucose. The non-carbohydrate molecules that are converted in this

pathway include pyruvate, lactate, glycerol, alanine, and glutamine. This process occurs when there are lowered amounts of glucose. The production of glucose by this pathway is important to tissues that cannot use any other fuels, such as the brain. The liver is the primary location of gluconeogenesis, but some also occurs in the kidney. This pathway is regulated by multiple different molecules. Glucagon, adrenocorticotropic hormone, and ATP encourage gluconeogenesis. Gluconeogenesis is inhibited by AMP, ADP, and insulin.

Pentose phosphate pathway

The pentose phosphate pathway is an alternative method of oxidizing glucose. It occurs in the liver, adipose tissue, adrenal cortex, testis, milk glands, phagocyte cells, and red blood cells. It produces products that are used in other cell processes, while reducing NADP.

Fructose metabolism

Fructose must undergo certain extra steps in order to enter the glycolysis pathway. Enzymes located in certain tissues can add a phosphate group to fructose. This phosphorylation creates fructose-6-phosphate, an intermediate in the glycolysis pathway that can be broken down directly in those tissues. This pathway occurs in the muscles, adipose tissue, and kidney.[In the liver, enzymes cleave fructose into glyceraldehyde and dihydroxyacetone phosphate, both of which are later intermediates in the glycolysis pathway.

Galactose metabolism

Lactose, or milk sugar, consists of one molecule of glucose and one molecule of galactose. After separation from glucose, galactose travels to the liver for conversion to glucose. Galactokinase uses one molecule of ATP to phosphorylate galactose. The phosphorylated galactose is then converted to glucose-1-phosphate, and then eventually glucose-6-phosphate, which can be broken down in glycolysis.

Energy production

Typically, a breakdown of one molecule of glucose by aerobic respiration (i.e. involving both glycolysis and Krebs cycle) is about 33-35 ATP. This is categorized as:

- Anaerobic breakdown by glycolysis - yielding 8-10 ATP
- Aerobic respiration by Krebs cycle - yielding 25 ATP

Oxidation of one gram of carbohydrate yields approximately 4 kcal of energy

Glucoregulation

Glucoregulation is the maintenance of steady levels of glucose in the body; it is part of homeostasis, and so keeps a constant internal environment around cells in the body. Hormones released from the pancreas regulate the metabolism of glucose. Insulin and glucagon are the primary hormones involved in maintaining a steady level of glucose in the blood, and the release of each is controlled by the amount of nutrients currently available. The amount of insulin released in the blood and sensitivity of the cells to the insulin both determine the amount of glucose that cells break down.

The hormone insulin is the primary regulatory signal in animals, suggesting that the basic mechanism is very old and very central to animal life. When present, it causes many tissue cells to take up glucose from the circulation, causes some cells to store glucose internally in the form of glycogen, causes some cells to take in and hold lipids, and in many cases controls cellular electrolyte balances and amino acid uptake as well. Its absence turns off glucose uptake into cells, reverses electrolyte adjustments, begins glycogen breakdown and glucose release into the circulation by some cells, begins lipid release *from* lipid storage cells, etc. The level of circulatory glucose (known informally as "blood sugar") is the most important signal to the insulin-producing cells. Because the level of circulatory glucose is largely determined by the intake of dietary carbohydrates, diet controls major aspects of metabolism via insulin. In humans, insulin is made by beta cells in the pancreas, fat is stored in adipose tissue cells, and glycogen is both stored and released as needed by liver cells. Regardless of insulin levels, no glucose is released to the blood from internal glycogen stores from muscle cells.

The hormone glucagon, on the other hand, has an effect opposite to that of insulin, forcing the conversion of glycogen in liver cells to glucose, which is then released into the blood. Muscle cells, however, lack the ability to export glucose into the blood. The release of glucagon is precipitated by low levels of blood glucose. Other hormones, notably growth hormone, cortisol, and certain catecholamines (such as epinepherine) have glucoregulatory actions similar to glucagon.

Carbohydrates as fuel

Carbohydrates are a superior short-term fuel for organisms because they are simpler to metabolize than fats or those amino acids (components of proteins) that can be used for fuel. In animals, the most important carbohydrate is glucose. The concentration of glucose in the blood is used as the main control for the central metabolic hormone, insulin. Starch, and cellulose in a few organisms (e.g., some animals (such as termites) and some microorganisms (such as protists and bacteria)), both being glucose polymers, are disassembled during digestion and absorbed as glucose. Some simple carbohydrates have their own enzymatic oxidation pathways, as do only a few of the more complex carbohydrates. The disaccharide lactose, for instance, requires the enzyme lactase to be broken into its monosaccharide components; many animals lack this enzyme in adulthood.

Carbohydrates as storage

Carbohydrates are typically stored as long polymers of glucose molecules with glycosidic bonds for structural support (e.g. chitin, cellulose) or for energy storage (e.g. glycogen, starch). However, the strong affinity of most carbohydrates for water makes storage of large quantities of carbohydrates inefficient due to the large molecular weight of the solvated water-carbohydrate complex. In most organisms, excess carbohydrates are regularly catabolised to form acetyl-CoA, which is a feed stock for the fatty acid synthesis pathway; fatty acids, triglycerides, and other lipids are commonly used for long-term energy storage. The hydrophobic character of lipids makes them a much more compact form of energy storage than hydrophilic carbohydrates. However, animals, including humans, lack the necessary enzymatic machinery and so do not synthesize glucose from lipids (with a few exceptions, e.g. glycerol).

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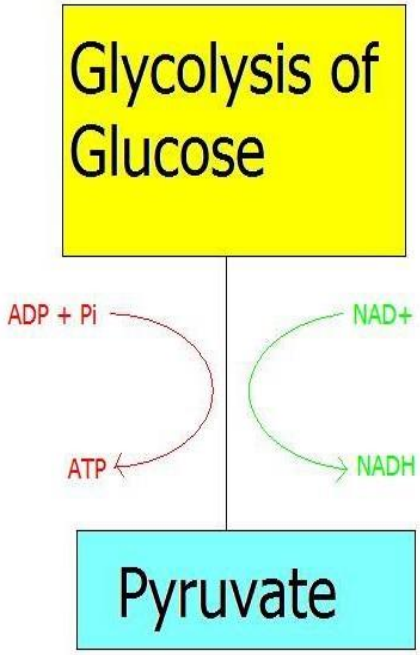

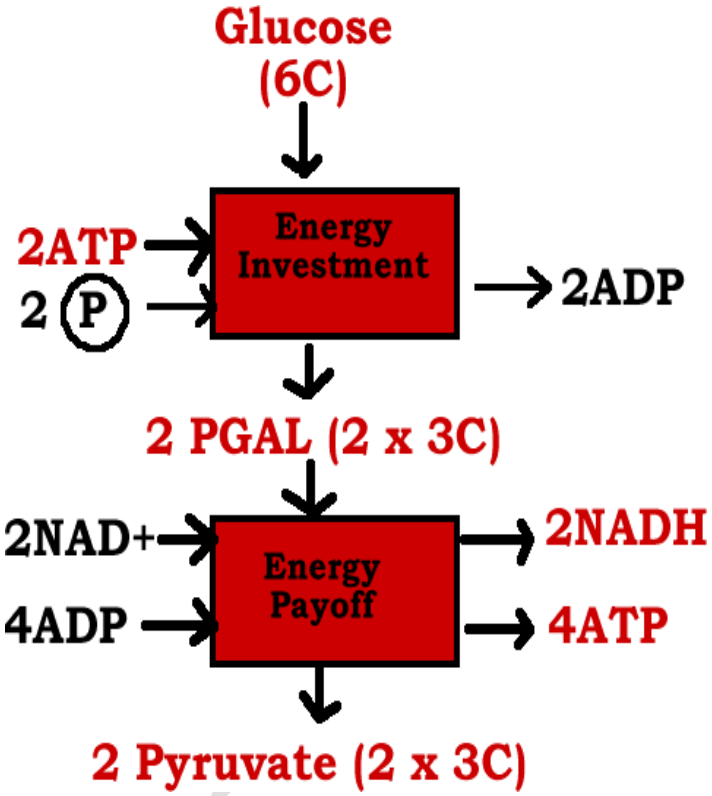

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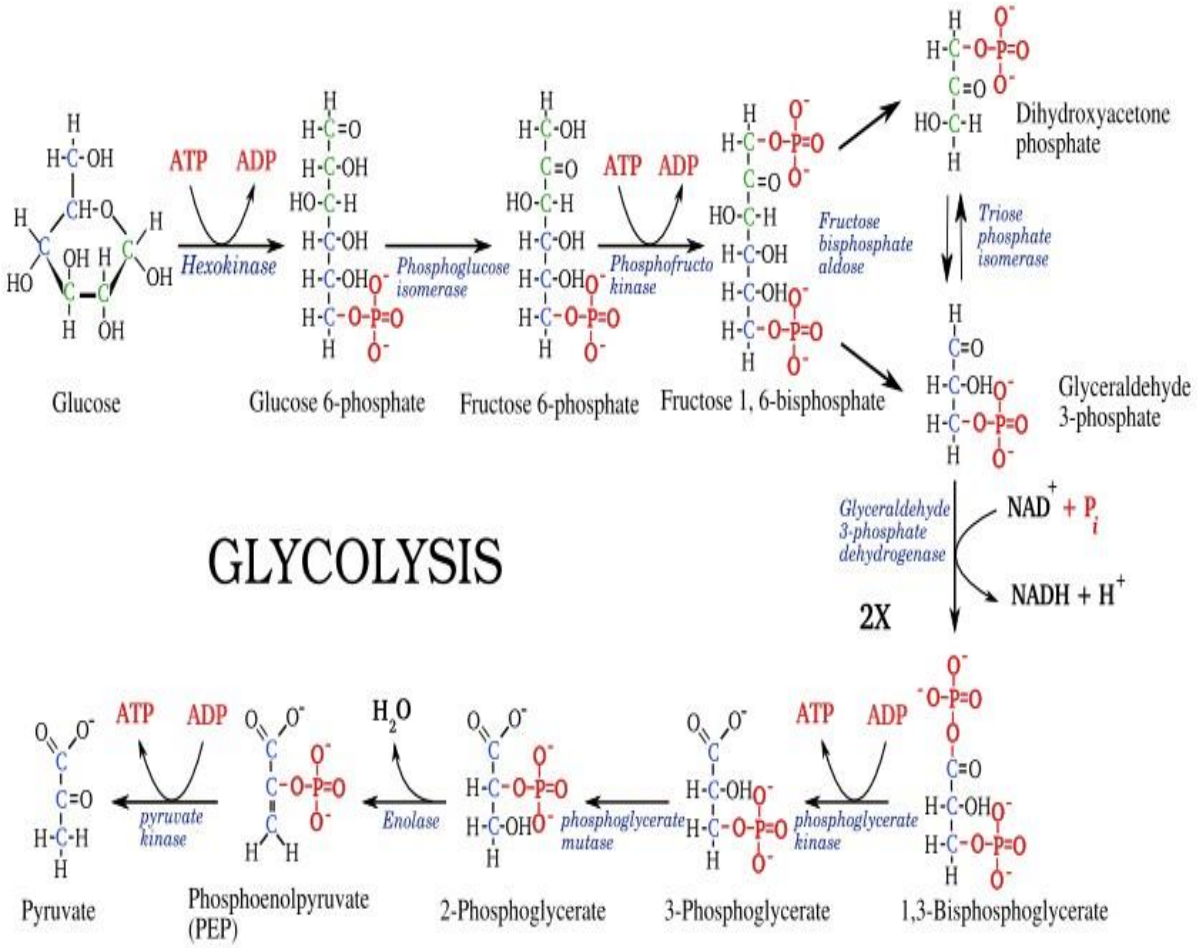
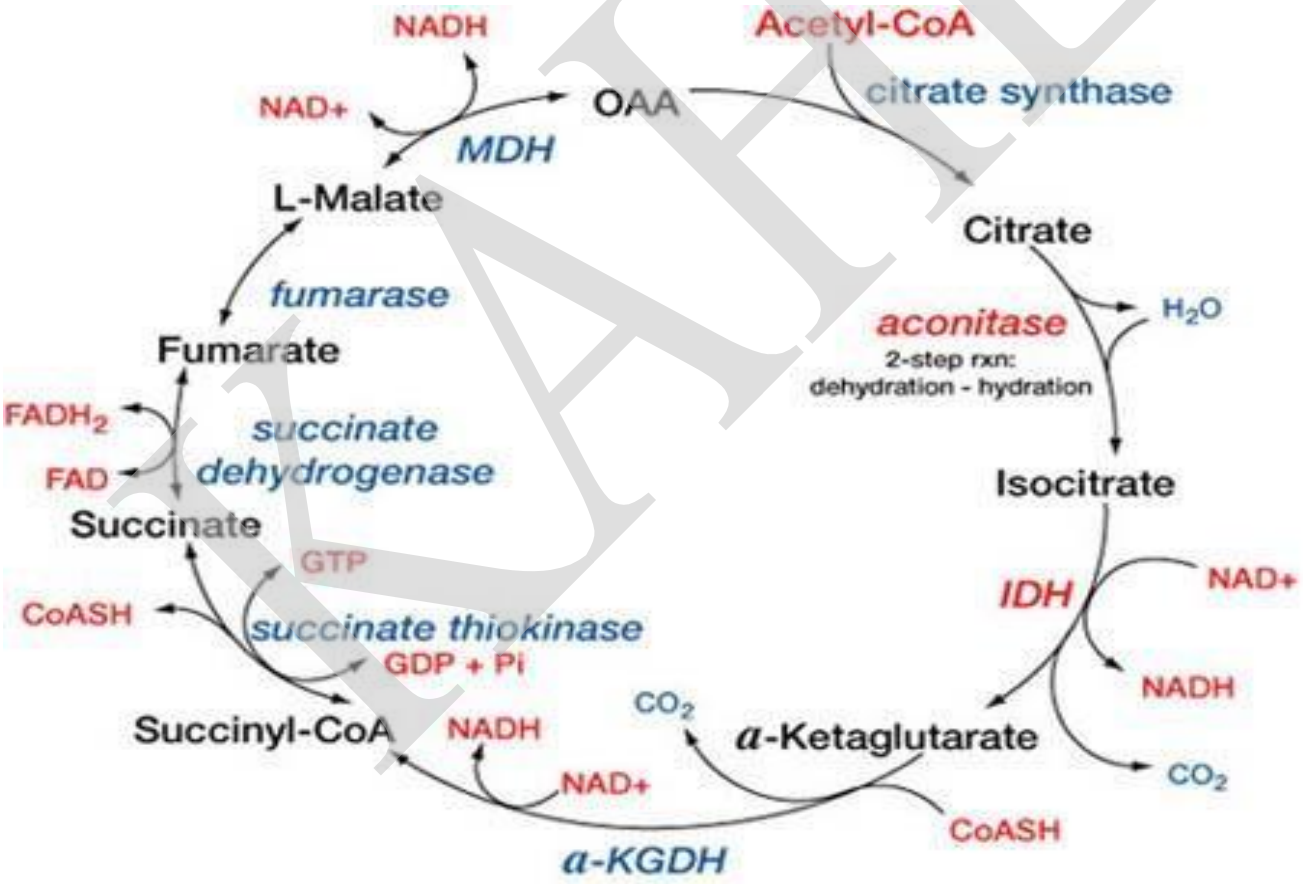
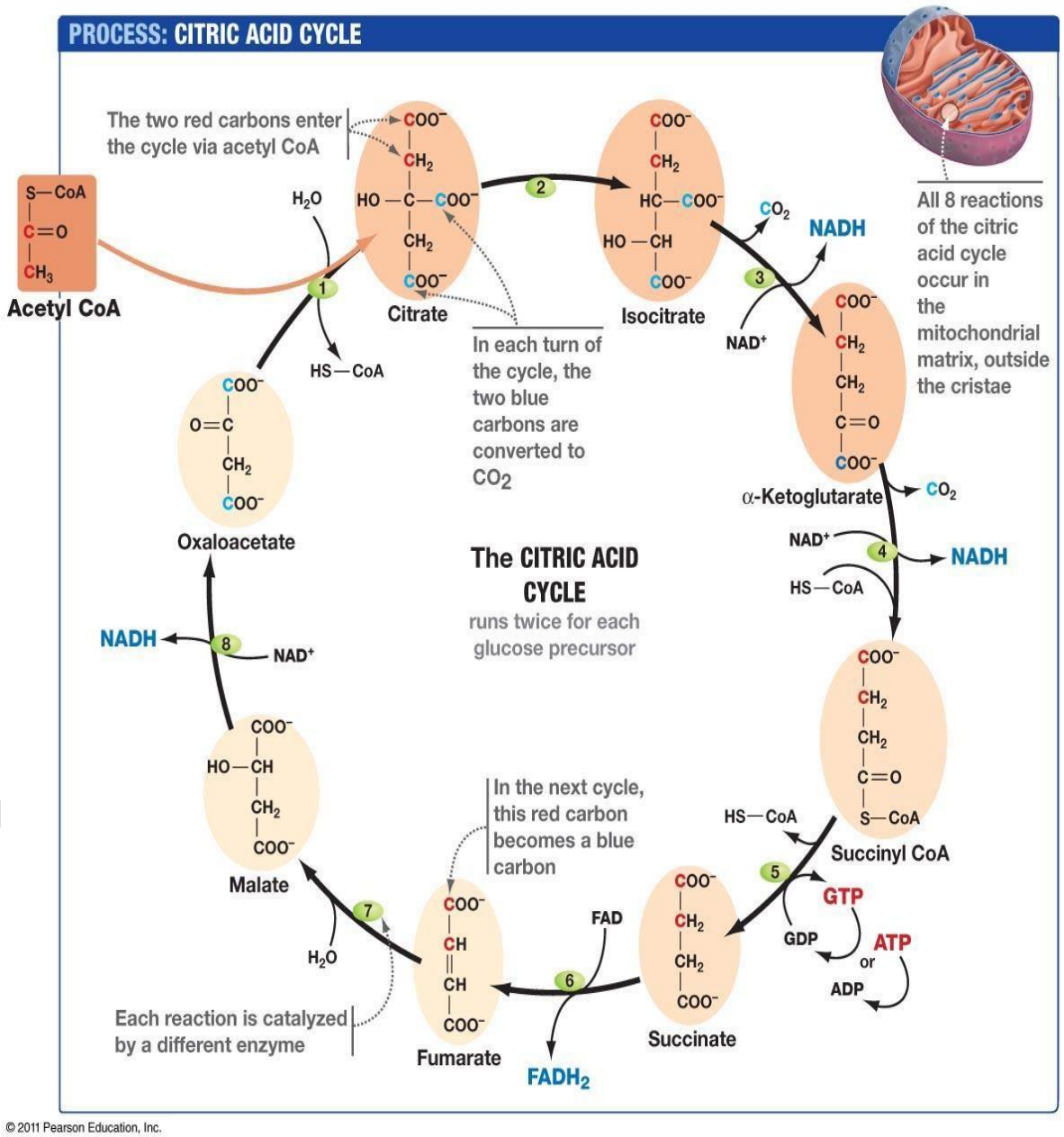


TCA cycle/ Citric acid cycle/ Krebs cycle

Aerobic phase – Acetyl CoA - from pyruvate of glycolysis
24 (12+12) ATP formed




PROCESS: CITRIC ACID CYCLE
The two red carbons enter the cycle via acetyl CoA
Acetyl CoA
Citrate
Isocitrate
α-Ketoglutarate
Succinyl CoA
Succinate
Fumarate
Malate
Oxaloacetate
NADH
GTP
ATP
FADH2
In each turn of the cycle, the two blue carbons are converted to CO2
All 8 reactions of the citric acid cycle occur in the mitochondrial matrix, outside the cristae
The CITRIC ACID CYCLE
runs twice for each glucose precursor
In the next cycle, this red carbon becomes a blue carbon
Each reaction is catalyzed by a different enzyme
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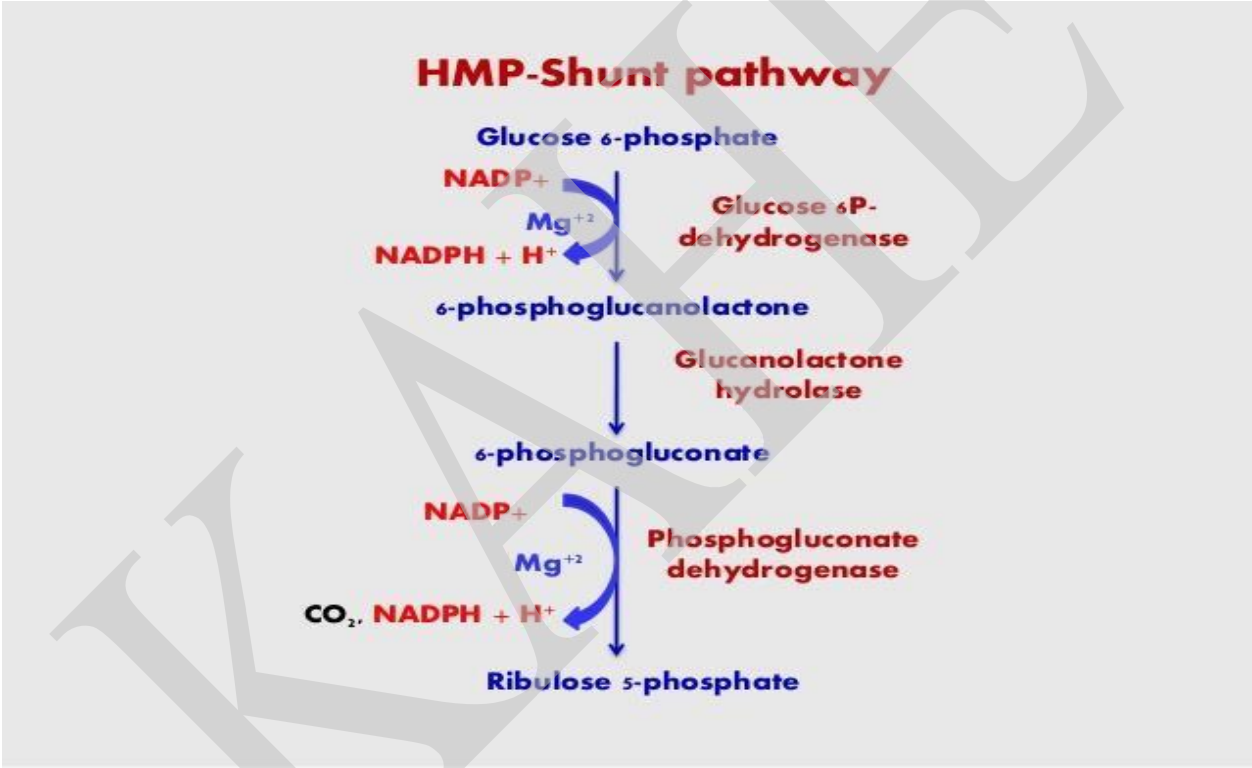
Glucose degradation – Glycolysis and TCA
Alternative pathways – Hexose Monophosphate (HMP) Shunt Pathway
Pentose phosphate pathway



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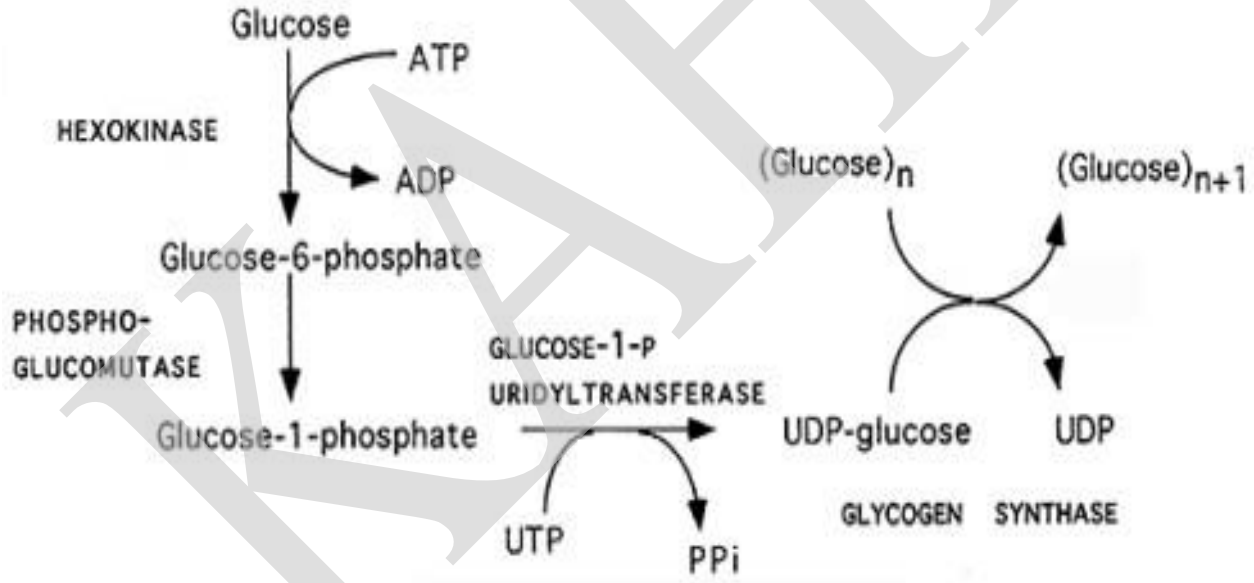
Glucose-6-phosphate + $2NADP^+$ + H_2O → Ribulose-5-phosphate + CO_2 + $2NADPH + 2H^+$

NADH vs. NADPH

ATP – H+/electron donor - biosynthesis of fatty acids / nucleotides

Glycogenesis



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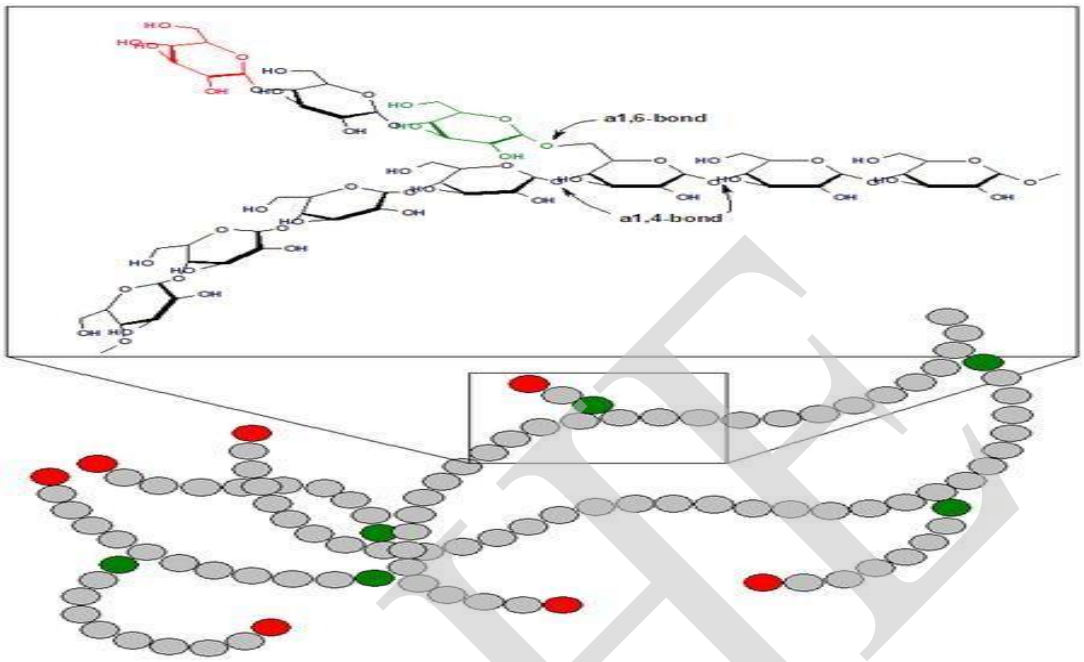
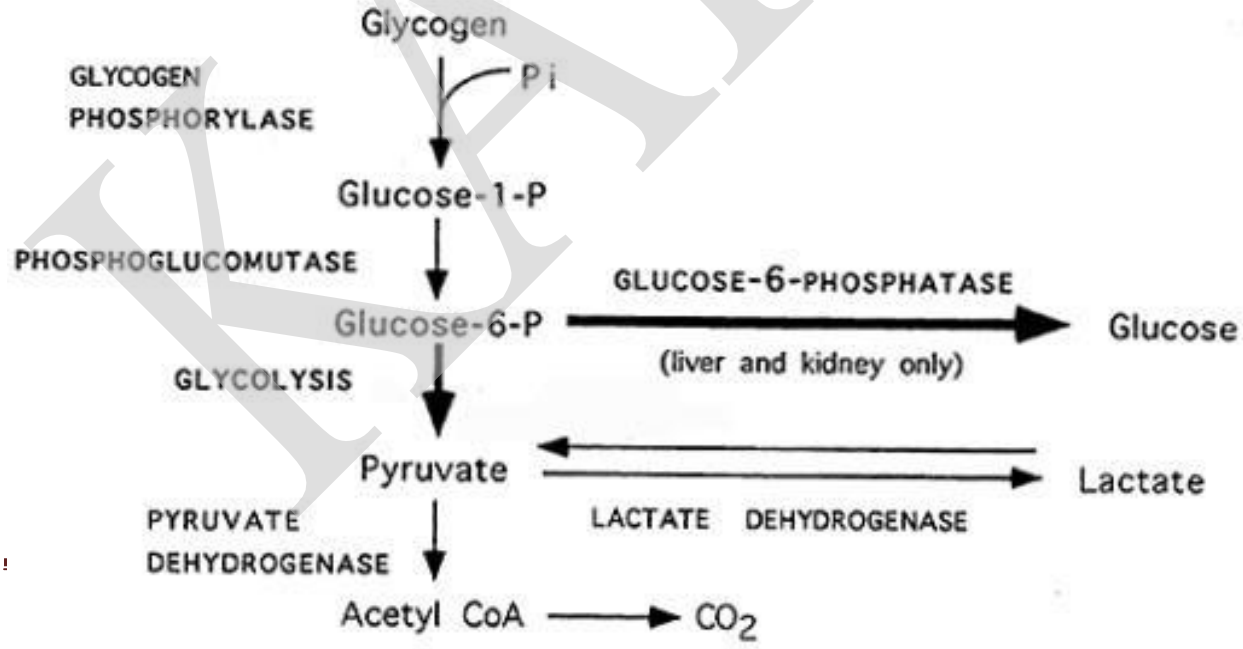


Glycogenolysis



Gluconeogenesis

- **Definition:** It is the formation of glucose from non-carbohydrate sources
- Site: Only in Liver & Kidney
- It occurs partly in cytoplasm & partly in mitochondria

• Importance of Gluconeogenesis:

1. It is the chief source of blood glucose after the first 18 hours-fasting
2. It removes blood lactate produced by RBCs & muscles and blood glycerol produced by adipose tissue or absorbed by intestine

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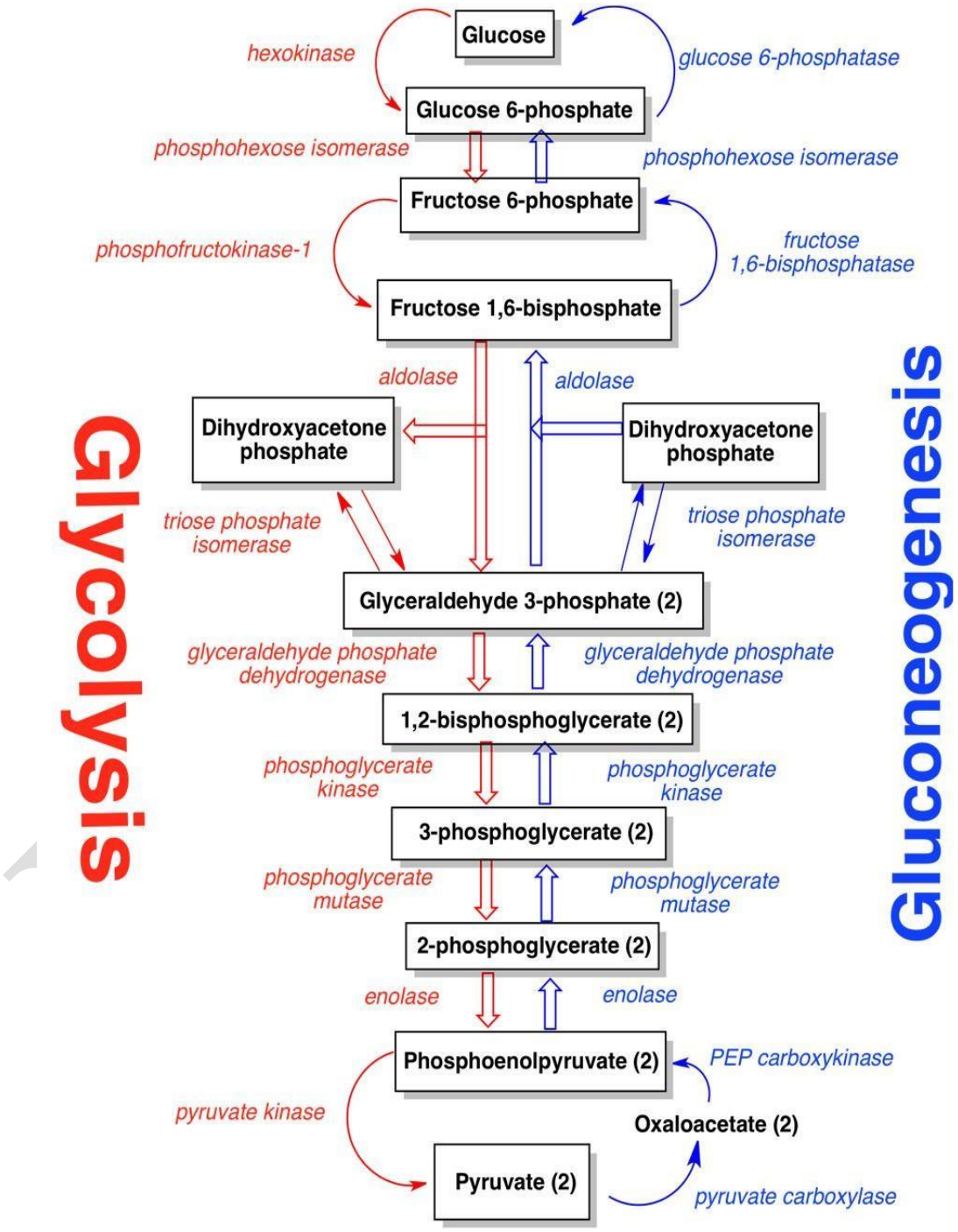
Glycolysis
Gluconeogenesis
Glucose
hexokinase
glucose 6-phosphatase
Glucose 6-phosphate
phosphohexose isomerase
phosphohexose isomerase
Fructose 6-phosphate
phosphofructokinase-1
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
aldolase
aldolase
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
triose phosphate isomerase
triose phosphate isomerase
Glyceraldehyde 3-phosphate (2)
glyceraldehyde phosphate dehydrogenase
glyceraldehyde phosphate dehydrogenase
1,2-bisphosphoglycerate (2)
phosphoglycerate kinase
phosphoglycerate kinase
3-phosphoglycerate (2)
phosphoglycerate mutase
phosphoglycerate mutase
2-phosphoglycerate (2)
enolase
enolase
Phosphoenolpyruvate (2)
PEP carboxykinase
pyruvate kinase
Oxaloacetate (2)
Pyruvate (2)
pyruvate carboxylase

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Gluconeogenesis – from amino acids – glucogenic amino acids – converted to the intermediates of TCA cycle – transamination/deamination – pyruvate – glucose formation

Gluconeogenesis – from glycerol of TG – adipose tissues (lacks glycerol kinase) – passes to liver – phosphorylation to glycerol 3-phosphate – glycolysis – pyruvate – glucose formation

Gluconeogenesis – from lactic acid – muscles – glycogen to lactate due to heavy muscular work – lactate diffuses out – enter blood stream – liver – lactate is oxidized to pyruvate – gluconeogenesis – glucose formation – glycogenesis – glycogen formation – blood glucose – Muscles – glycogenolysis – lactact accumulation – repeat the processes – Cori cycle

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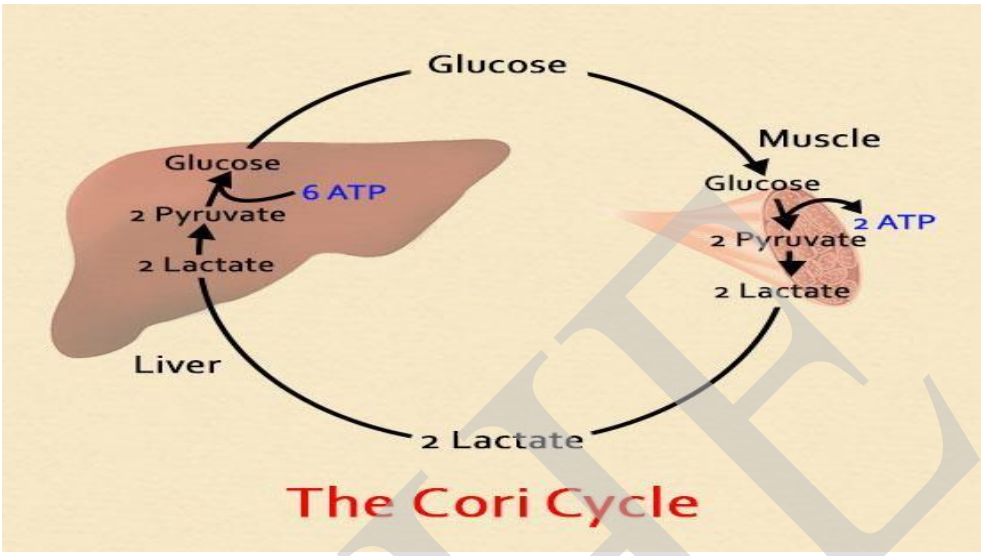

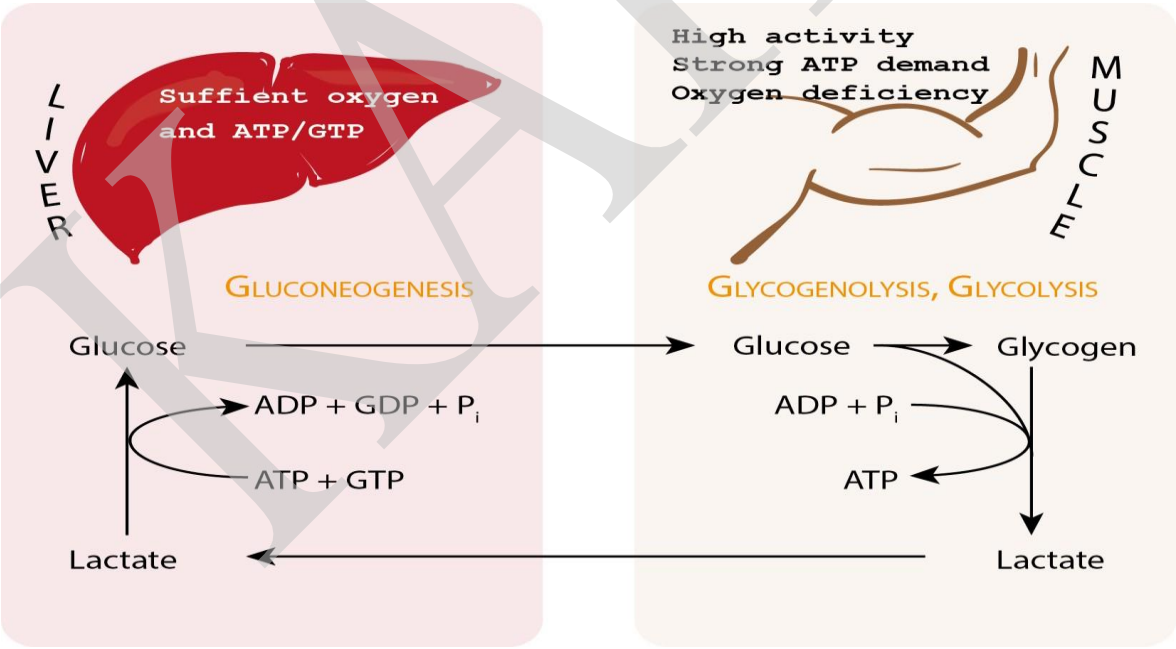

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Diabetes Mellitus

Excretion of large volume of urine containing glucose – group of metabolic disorder – Insulin deficiency – IDDM – life-long insulin therapy

Insulin defective – NIDDM

Hyperglycemia – high blood glucose level – decreased permeability of cell membrane

Polyuria – excretion of increased quantity of urine

Polydipsia – excessive thirst – to replace excreted water

Polyphagia – excessive appetite – to replace the lost nourishment

Ketosis and acidosis – not enough glucose for energy production – fat from adipose tissues – metabolism – ketone bodies – ketosis – acidosis – coma

Atherosclerosis – deposition of lipids in the walls of the blood vessels

Diagnosis – GTT – overnight fasting – 75 gm glucose water – 300 ml – within 15 minutes blood glucose level at 30 minutes intervals for several hours.

Normal individual assimilation - 80 – 120 mg/100 ml

Diabetic individual assimilation - >180 mg/100 ml (glucose in urine)

Diabetes Mellitus

GTT or OGTT

75 g glucose was given at time 0.
Venous plasma glucose (mmole/l) levels are given
at different time intervals (minutes post glucose level)

Case No.	Clinical Details	0	30	60	90	120
1	Male, 65 yrs, obese	8.8	13.8	17.5	16.8	16.7
2	Female, 62 yrs, BMS	6.0	11.7	15.2	16.4	17.0
3	Male, 41 yrs, glycosuria	7.4	9.5	10.8	10.1	9.5
4	Female, 75 yrs	5.0	8.6	10.7	11.0	10.2

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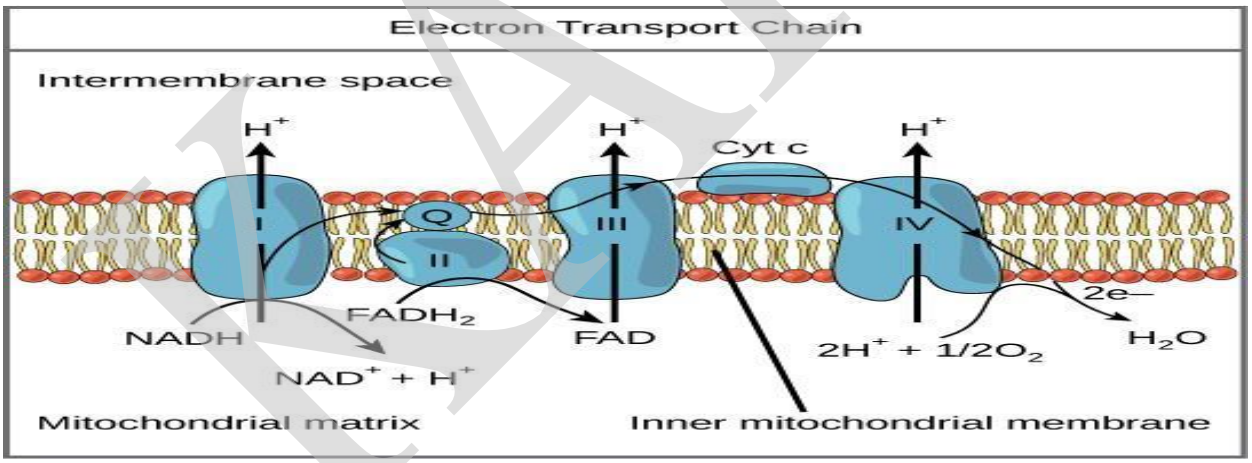
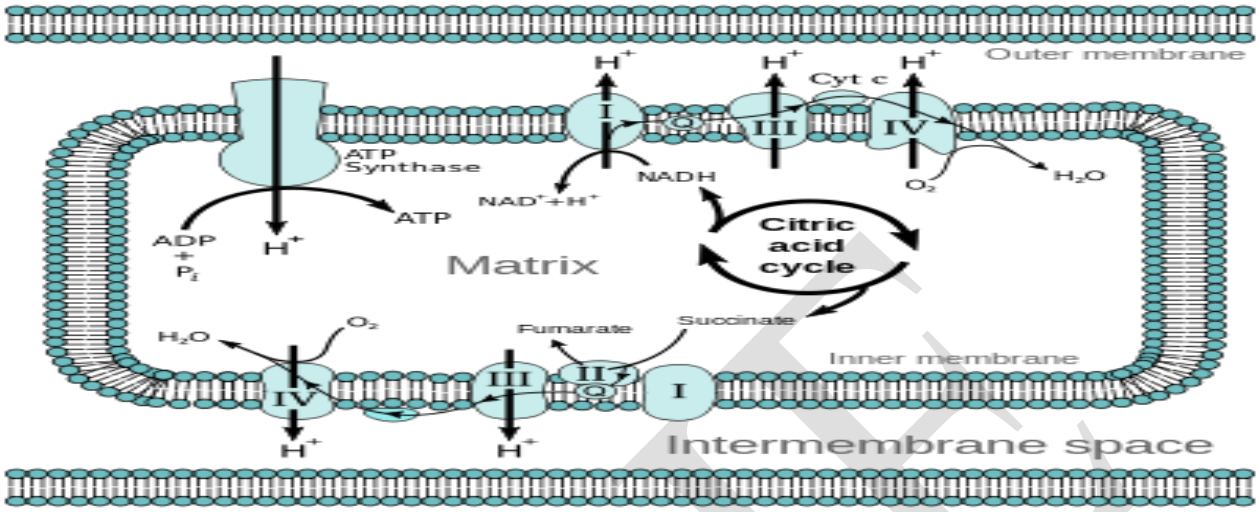
UNIT : III (Bioenergetics)

Electron transport chain

ETC – transfer of electrons – redox reactions – series of compounds – reductants – reducing agents – electron donor – oxidants – oxidizing agents – electron acceptor; Final acceptor of electrons – molecular oxygen in aerobic conditions – other than oxygen exist in anaerobic condition.

ETC in mitochondria – most eukaryotic cells have mitochondria – ATP by ATP-synthase from ADP – oxidative phosphorylation; series of compounds in the redox reactions – each electron donor passes electrons to more electronegative acceptors (coupled with proton gradient in IMM) – until electrons are passed to molecular oxygen – converted to water.





Complex I: NADH and Ubiquinone oxidoreductase – NADH is oxidized to NAD^+ - by reducing FMN to $FMNH_2$ – 2 electrons from the reactions – transfer to ubiquinone – first electron results semiquinone and second electron results ubiquinol – 4 protons translocated from the mitochondrial matrix to the intermenbrane space.

Complex II: 4 protein subunits – succinate dehydrogenase, succinate dehydrogenase iron-sulphur subunit – succinate dehydrogenase complex subunits C & D – transfer electrons *via* FAD – parallel to Complex I – but no proton transfer – hence contributes less energy.

Complex III: CoQH2 – Cytochrome c reductase – ubiquinol is converted into quinol – two electrons transferred to Cyt c - Fe (III) of Cyt c to Fe (II) – 4 protons translocated.

Complex IV: Cytochrome c oxidase – 4 electrons are removed from 4 Cyt c - transferred to molecualr oxygen – 2 molecules of water – translocating 4 protons from matrix to the proton gradient in intermembrane space.

ATP Synthase – Complex V – F_0 & F_1 – F_0 act as ion channel – facilitates protons flux back into the mitochondrial matrix – producing free energy generation – drive F_1 to synthesize ATP from ADP.

Inhibitors to ETC

Inhibitors of respiratory chain - inhibit Cytochrome oxidase.

Inhibitors - Amobarbital, Carbon monoxide & Cyanide.

Inhibitors to oxidative phosphorylation – Oligomycin & Atrctyloside.

Uncouplers – by dissipating the protons – electro chemical gradient

Inhibitors - 2,4-Dinitro phenol, Dinitrocresol.

Ionophores – ion carriers – carrying specific ions through the membrane –

including protons (H^+)

Inhibitors - Valiomycin and Gramicidin

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Ketone bodies

Produced by liver from fatty acids – carbohydrate restricted diet, fasting, starvation, prolonged intense exercise and untreated Type 1 DM.

Three general ketone bodies - acetoacetate, bata hydroxy butyric acid and acetone – acetyl CoA – CoA – dimer of the CoA is acetoacetate – reduced form of the acetoacetate is beta hydroxy butyric acid – decarboxylated acetoacetate is acetone.

The acetoacetate and bata hydroxy butyric acid can be converted to acetyl CoA by most tissues of the body other than liver (lacks the enzyme beta-ketoacyl-CoA transferase, also called as thiophorase) – the acetone to lactic acid by liver (detoxification) – oxidized to pyruvate and then to acetyl CoA – lipid metabolism.

Endogenous Ketone bodies – fruity smell; other ketone bodies like beta-ketopentanoate and beta-hydroxypentanoate from synthetic TG.

Ketone bodies

Blood glucose level low – energy requirements – Glucagon from pancreas – Epinephrine from adrenal medulla – promotes gluconeogenesis and glycogenolysis – fatty acids from adipose tissues – high energy fuel for cells – cells have mitochondria – fatty acids metabolized in mitochondria only – RBC do not contain mitochondria – entirely dependent on glycolysis.

Fatty acids – beta oxidation - Fatty acyl CoA – acetyl CoA – citric acid cycle – 12 ATPs (11 ATPs + 1GTP) per acetyl CoA.

Under starvation – oxaloacetate to malate (hydrogenation) – moved to liver cells from mitochondria – glucose – release to blood stream.

Under the circumstances – oxaloacetate is unavailable for condensation with acetyl-CoA – diverted – formation of ketone bodies – released by liver into blood stream – all cells with mitochondria take up the ketone bodies and convert them into acetyl-CoA – used as a fuel through TCA cycle - Unlike free fatty acids, ketone bodies can cross the BBB and are therefore available as fuel for the cells of the CNS, acting as a substitute for glucose, on which these cells normally survive.

The occurrence of high levels of ketone bodies in the blood during starvation, a low carbohydrate diet, prolonged heavy exercise and uncontrolled type 1 diabetes mellitus is known as ketosis and in its extreme form in out-of-control type 1 diabetes mellitus, as ketoacidosis.

Healthy conditions, ketone bodies in blood is maintained around 1 mg/dl (about 0.1 mM).

Synthesis of ketone bodies exceed the rate of utilization – concentration in blood increases – Ketonemia; Excretion of ketone bodies in urine – Ketonuria.

The 'Ketonemia' and 'Ketouria' are commonly referred as 'Ketosis'.

Extreme form of the 'Ketosis' is refereed as 'Ketoacidosis' (acidic blood pH).

Ketosis, 0.5 – 5 mM
Ketoacidosis , 15 – 25 mM.

<u>Hyperglycemic Ketoacidosis</u>

Low insulin – high glucagon – induce liver to produce at an inappropriately increased rate – resulting excess acetyl CoA – ketone bodies – lower blood pH – passively into the urine – osmotic diuresis due to the glucose and ketone bodies – removal of water and electrolytes from the blood – potentially fatel dehydration.

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UNIT : III (Bioenergetics)

Possible questions

Part A

1. The total number of irreversible steps in the glycolysis is
(A) 1 (B) 2 (C) 3 (D) 4

2. The net numbers of ATP production in a TCA cycle is
(A) 2 (B) 4 (C) 8 (D) 24

3. Polyphagia is
(A) excessive thirst (B) excessive appetite
(C) excessive excretion of urine (D) glucose in urine

4. The important reducing power produced in the HMP shunt pathway is
(A) NADH (B) FADH (C) NADPH (D) $FADH_2$

5. Lactate is converted into glucose in
(A) Liver (B) Muscle (C) Kidney (D) Lung

6. The enzyme playing important role in linking the glycolysis and TCA cycle is
(A) Pyruvate carboxylase (B) Pyruvate kinase
(C) Phosphoenol pyruvate kinase (D) Pyruvate dehydrogenase

7. Pyruvate is converted to oxaloacetate by
(A) Pyruvate carboxylase (B) Pyruvate kinase
(C) Phosphoenol pyruvate kinase (D) Pyruvate dehydrogenase

8. Glucagon stimulates synthesizing of
(A) Glucose from glycogen (B) Glucose from pyruvate
(C) Glycogen from glucose (D) Glycogen from pyruvate

9. Epinephrine stimulates synthesizing of
(A) Glucose from glycogen (B) Glucose from pyruvate
(C) Glycogen from glucose (D) Glycogen from pyruvate

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Part B

1. Write the net biological reaction of the glycolysis process.
2. Write a short note on TCA cycle.
3. List out the enzymes involved in the preparatory phase/payoff phase of glycolysis.
4. Write a short note on HMP shunt pathway.
5. Write the biological significance of HMP shunt pathway.

Part C

1. Explain the glycolysis process and its biological significances in detail.
2. Explain the glycolysis process and its control reaction by 'phosphofructo kinase'.
3. Enumerate the various steps in tricarboxylic acid cycle in detail.
4. Discuss in detail the 'glycogenesis' and 'glycogenolysis' processes.
5. Discuss about various regulatory mechanisms of glycogen metabolism.

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CLASS : I M. Sc., BT **COURSE NAME: Biochemistry and Microbiology**
BATCH : 2019 – 2021
COURSE CODE : 19BTP101
UNIT : IV (Microbial Diversity and Techniques)

UNIT - IV

SYLLABUS

Microbial Diversity and Techniques: Diversity- Bacteria, fungi, algae - distribution, reproduction, characteristics, nutrition. Techniques - staining, Microscopy - Principle, types, applications. Microbial growth - nutrients, media, isolation, maintenance, preservation, curve, measurements, factors, regulation.

Microbiology is the study of microorganisms, those being unicellular (single cell), multicellular (cell colony), or acellular (lacking cells). Microbiology encompasses numerous sub-disciplines including virology, parasitology, mycology and bacteriology.

Eukaryotic microorganisms possess membrane-bound cell organelles and include fungi and protists, whereas prokaryotic organisms—all of which are microorganisms—are conventionally classified as lacking membrane-bound organelles and include eubacteria and archaebacteria. Microbiologists traditionally relied on culture, staining, and microscopy. However, less than 1% of the microorganisms present in common environments can be cultured in isolation using current means. Microbiologists often rely on molecular biology tools such as DNA sequence based identification, example 16s rRNA gene sequence used for bacteria identification.

Viruses have been variably classified as organisms, as they have been considered either as very simple microorganisms or very complex molecules. Prions, never considered as microorganisms, have been investigated by virologists, however, as the clinical effects traced to them were originally presumed due to chronic viral infections, and virologists took search—discovering "infectious proteins".

The existence of microorganisms was predicted many centuries before they were first observed, for example by the Jains in India and by Marcus Terentius Varro in ancient Rome. The first recorded microscope observation was of the fruiting bodies of moulds, by Robert Hooke in 1666, but the Jesuit priest Athanasius Kircher was likely the first to see microbes, which he mentioned observing in milk and putrid material in 1658. Antonie van Leeuwenhoek is

considered a father of microbiology as he observed and experimented with microscopic organisms in 1676, using simple microscopes of his own design. Scientific microbiology developed in the 19th century through the work of Louis Pasteur and in medical microbiology Robert Koch.

Modern microbiology. Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

Two Australians, **Barry J. Marshall** and **Robin Warren** won the 2005 Nobel Prize for showing that bacterial infections of *Helicobacter pylori* (= *Campylobacter pylori*) and not the stress, is responsible for painful ulcers in the stomach and intestine. The 1982 discovery transformed **peptic ulcer disease** from a chronic, frequently disabling condition to one that can be cured by a short regimen of antibiotics and medicines. At the same time, nucleic acid sequencing methods were developed which left its impact in all the areas of biology. Sequencing technology helped microbiologists to reveal phylogenetic evolutionary) relationships among prokaryotes, which led to evolutionary new concepts in the field biological classification. The field of **Genomics** is also a contribution of sequencing technology, in which the **comparative analysis of the genes of different organisms** is carried out. The huge amounts genomic information now in hand are leading to major advances in medicine, microbial ecology, industrial microbiology, and many

other areas of biology. The genomics era has given birth to a new subdiscipline, **Proteomics.** The proteomics is defined as **the study of protein expression in cells.** The significance of such developments in molecular biology to all of biology is understood by the fact that numerous Nobel Prizes have been awarded to researchers for their work in this field

Microbial Diversity

Bacteria

Bacteria are microscopic unicellular, prokaryotic organisms. The study of bacteria is called Bacteriology. Ehrenberg (1829) established the genus bacterium. Bacteria are present everywhere, in the water, in the soil, in the air, on our body and in our body. Eg. E.coli, lactobacillus, streptococcus, etc.

Major Features of Bacteria

The following are the major features of bacteria:

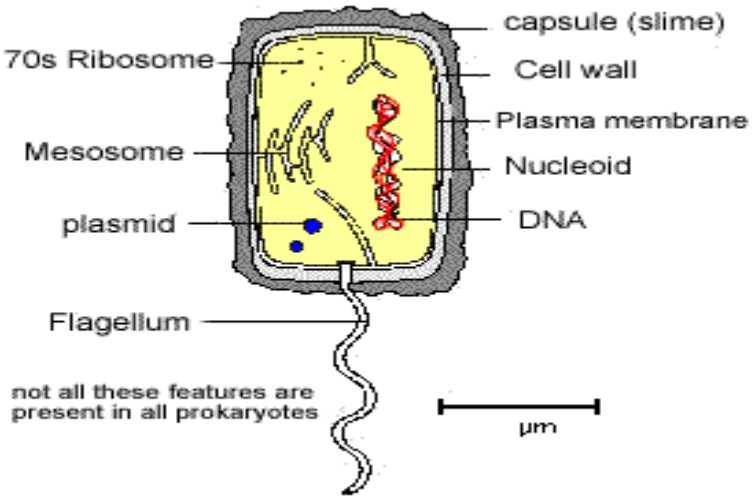
1. They exist everywhere.
2. They are unicellular. some exist as colonies.
3. They are prokaryotic.
4. They range in size from 0.5micron to 3micron.
5. They are in the form of rods, spheres, spirals or filaments.
6. The cell is enclosed in a cell envelope made up of a capsule, a cell wall and a plasma membrane.
7. Nuclear material is represented by a nucleoid without nuclear membrane.
8. An extra chromosomal DNA called plasmid is usually present in the cytoplasm.
9. Cell organelles include 70S ribosomes and mesosomes others organelles such as mitochondria, lysosomes, golgi body, endoplasmic reticulum, centrioles, etc. are absent.
10. Appendages like flagella, pili are present.
11. They are either Gram positive or Gram negative.
12. They show absorptive mode of nutrition.
13. They multiply by binary fission.
14. Some produce endospores.

Structure of Bacteria

- Bacteria are unicellular, microscopic, prokarvotic organisms lacking chlorophyll.
- Bacteria were omnipresent. They range in size from 0.5micro meter to 600 micro meter.
- The bacteria are either spherical or rod shaped or spiral or curved,
- The spherical bacterium is called coccus, coccus means a berry.
- The individual spherical bacterium is called micrococcus. Some spherical bacteria are arranged in pairs and they are called diplococci (sl. Diplococcus).
- When the cocci are arranged in chains, they are called tetracocci. When the cocci are arranged in chains , they are called streptococci. When the cocci are arranged in clusters like a bunch of grapes, they are called staphylococci.
- The rod shaped bacteria are called bacilli (sl. bacillus). The bacillus may be found individually or in pairs or in the form of chains or in the form of a bunch of grapes.
- A chain of is called streptobacillus. A bunch of bacilli is called *Staphylo bacillus.*
- The spiral bacteria are spirally curved. They may be slightly curved like a comma eg. *Vibrio* or spirally coiled eg. *Spirllum*. In addition there are filamentous bacteria and fungus like bacteria. They are multicellular.
- There are two types of bacteria. They are Gram positive bacteria and Gram negative bacteria. Gram positive bacteria retain violet colour on Gram staining Gram negative bacteria appear in red colour.
- The bacteria are motile or non-motile. They may are may not contain flagella.
- When the flagellum is absent, the bacterium is called atrichous. When the bacterium contains only one flagellum at the end, it is called monotrichous. When the flagellum is present at both the ends, the bacterium is called amphitrichous. When there is a bunch of flagella at one end, the bacterium is called lophotrichous. In some bacteria, the flagella are present all over the cell, these bacteria are called peritrichous.

- The flagella are present in bacilli and spiral bacteria. They are absent from cocci. They are whip-like. Each flagellum has three parts, namely basal body, a hook and a shaft. The flagella are used for locomotion.
- The bacteria may be motile or non-motile. The bacilli and spiralla are motile. The cocci are non-motile. Motility is brought about by a flagella.
- Some hair-like structures are present on the bacteria. These are called pili or fimbriae. They are used for attachment. Some pili are longer in some bacteria and they are called sex pili.
- A bacterial cells protected by a cell envelope. The cell envelope is made up of a capsule, a cell wall and a plasma membrane.
- In some cells a slimy cover is present in stead of a capsule.
- In some other cells an outer plasma membrane is present between the capsule and the cell wall.
- The bacteria covered by a capsule are called capsulated bacteria. The bacteria which do not contain a capsule are called non-capsulated bacteria.
- The cell envelope encloses the cytoplasm. It is colloidal in nature. It does not exhibit streaming movement. It contains ribosomes and mesosomes. Golgi bodies, endoplasmic reticulum and mitochondria are absent. The ribosomes are 70S type.
- Mesosomes are pocket-like structures formed by the invagination of plasma membrane.
- The general size of a prokaryotic cell is about 1-2 um.
- Note the absence of membrane bound organelles
- There is no true nucleus with a nuclear membrane
- The ribosome's are smaller than eukaryotic cells
- The slime capsule is used as a means of attachment to a surface
- Only flagellate bacteria have the flagellum

Plasmids are very small circular pieces of DNA that maybe transferred from one bacteria to another



Structure and function of bacteria

Structure	**Function of bacteria**
Cell Wall	• Made of murein (not cellulose), which is a glycoprotein or peptidoglycan (i.e. a protein/carbohydrate complex).There are two kinds of bacterial cell wall, which are identified by the Gram Stain technique when observed under the microscope. Gram positive bacteria stain purple, while Gram negative bacteria stain pink. The technique is still used today to identify and classify bacteria. We now know that the different staining is due to two types of cell wall
Plasma membrane	• Controls the entry and exit of substances, pumping some of them in by active transport • .

Mesosome	• A tightly-folded region of the cell membrane containing all the membrane-bound proteins required for respiration and photosynthesis. • Can also be associated with the nucleoid. • This is now thought to be an artifact of the electron microscope and not a real structure.
Cytoplasm	• Contains all the enzymes needed for all metabolic reactions, since there are no organelles
Ribosome's	• The smaller (70 S) type are all free in the cytoplasm, not attached to membranes (like RER). They are used in protein synthesis which is part of gene expression.
Naked DNA	• Nucleoid is the region of the cytoplasm that contains DNA. It is not surrounded by a nuclear membrane. DNA is always circular (i.e. a closed loop), and not associated with any proteins to form chromatin. Sometimes confusingly referred to as the bacterial chromosome
Slime Capsule	• A thick polysaccharide layer outside of the cell wall, like the glycocalyx of eukaryotes. Used for sticking cells together, as a food reserve, as protection against desiccation and chemicals, and as protection against phagocytosis. In some species the capsules of many cells in a colony fuse together forming a mass of sticky cells called a biofilm. Dental plaque is an example of a biofilm.

4.1. 4 Classification of bacteria

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Bacteria have a large range of different metabolic reactions at their disposal, far more than in the eukaryotes, confined to just respiration or photosynthesis.

Fermentation:

- sometimes called organotrophs these bacteria oxidise organic molecules like glucose. In many instances they metabolise as far as lactic acid or alcohol molecules making them useful to fermentation industry.

Photosynthesis

- sometimes called Phototrophs obtain their energy from sunlight. Many bacteria are photosynthetic and use the same process of photosynthesis as plants. These phototrophic bacteria were some of the earliest forms of life on the planet, and their metabolic reactions increased the oxygen content of the atmosphere from 1% to 20%.

Nitrogen Fixing

- Lithotrophs obtain their energy by oxidising inorganic compounds like ammonia, nitrite, methane or hydrogen sulphide. These bacteria use a variety of unusual metabolic reactions and many are able to synthesise carbohydrates from carbon dioxide – the chemosynthetic bacteria. There are whole eco-systems on the deep ocean floor with no light, based on lithotrophic bacteria as producers. Although rare, lithotrophic species are enormously important in ecology, as they are responsible for much of the cycling of matter (e.g. the nitrifying bacteria). They could also be useful in biotechnology as they can synthesise useful organic compounds from waste inorganic ones (e.g. methylomonas can make carbohydrates from methane).

Fungi

Fungus is a Latin word which means Mushrooms. The study of mushroom is known as Mycology. Fungi are Non-vascular plants without cholorophyll. Their mode of nutrition is heterotrophic.

They live as saprophytes or parasites or symbionts.They are found in soil,water,air and in our food stuffs.

Characteristics:

- Fungi are eukaryotes(i.e, their cell posses a true nucleus)
- They are non-green plants.
- The body of fungus is known as thallus.It consist of a single cell as in yeast or it consists of filaments (Mould)
- They donot possesss stems,roots,leaves or vascular system.
- They do not show division of labour.

- In fungus the growth take place at the tip or apex of the filament.This type of growth is known as apical growth or terminal growth.
- Fungi are chemoorganotrophic microorganism.
- They reproduce by means of spores.spores are sexual or asexual.At the same time any part of the filament sufficient to start a new individual organism.
- They reproductive structure are differentiated from somatic structure.
- The reproductive structure are important in classification and identification of fungi.
- The somatic structure of any fungus resemble those any other fungi.
- The optimum temperature for the growth of fungus between 20ºC and 30ºC
- Fungi can withstand extremely low temperature as low as 195ºC for at least few hours.
- Fungi prefer an acid medium for growth (pH 6)
- Although light is not essential for growth, some light is essential for sporulation in many species.

General structure

Fungi are non-vascular plants lacking chlorophyll. They are eukaryotic prostists.It includes yeast, moulds, and mushrooms. The body of fungus is not differentiated into roots, stem and leaves.

The body of fungus is called thallus.Hence it include under the group Thallophyta, which also include algae.The study of fungus is called mycology and the scientists who are studying fungi are called Mycologists.

The fungi of microbiological importance are yeast, penicillium, Agaricus, Rhizopus, Puccina etc. Fungi are either unicellular or multicellular forms.The yeast are unicellular.The moulds and mushrooms are multicellular.

The multicellular in the form of filament. The filaments body is called mycelium. Each filament of the mycelium is called a hypha.

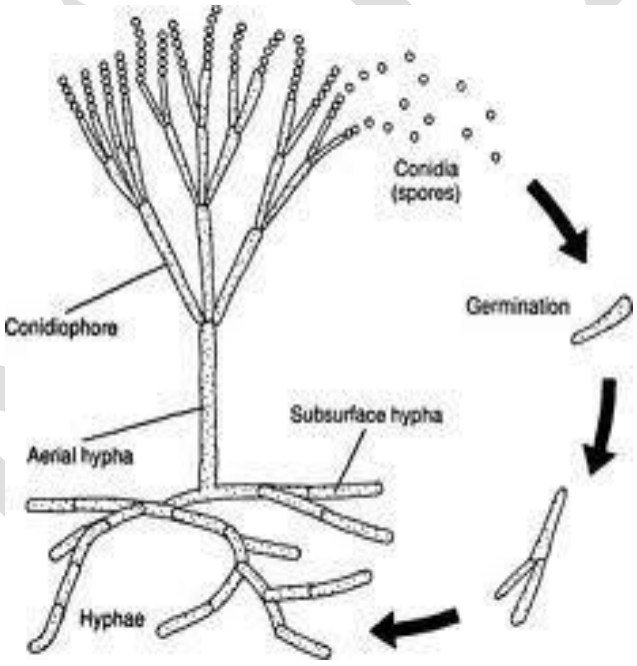
Mycelium It can be divided into vegetarian mycelium which grows into the medium and the aerial mycelium which projects from the surface.

Hypha They are tubular in nature and its consists of cytoplasm enclosed by plasma membrane and the cellwall. The cell wall consists of nucleus. The hyphae are of two types, namely aseptate hypha and septae hypha.The aseptate hypha, cellwalls are absent and nuclei are scattered in the continous mass of cytoplasm.The septate hypha crosswalls are absent and it may be uninucleate or multinucleate.

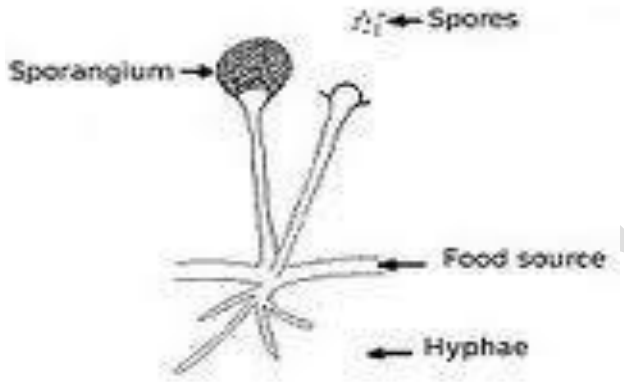
The mycelium without septa is called aseptate mycelium.The mycelium with septum is called a septate mycelium.

The fungi are sedentary and they are immobile. However, the motile cells appear in their life cycles.The motile cells have flagella. Each flagellum has a central axoneme and a cytoplasmic sheath. The axoneme has 9+2 fibrils.

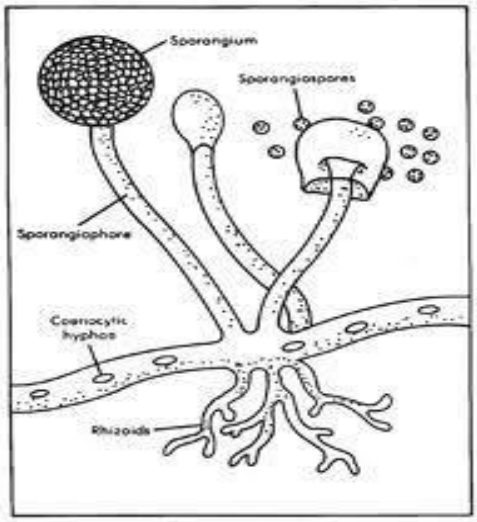
The nutrition in fungus is heterotrophic. They live as saprophytes or parasites or symbionts. Reproduction occurs by asexual and sexual methods.



Aspergillus species



Fungus



Asexual fruiting structure

Economic importance of fungi

Medicines: Antibiotics are obtained from fungi.

- Penicillum is obtained from *Penicillum notatum*.
- Streptomycin is obtained from Streptomyces griseus.

Fungal food: There are 200 species of edible fungus.Eg-Mushrooms

Alcoholic fermentation: Yeasts bring about alcoholic fermentation, baking industry:

Yeasts are extensively used in baking industry. They cause the dough to rise and make the bread light and spongy.

Enzymes: Various enzymes are produce by fungi Eg.*Aspergillus flavus* produce digestion.

Growth hormones: The fungus, *Gibberella fujikuroi* produce gibberelin.it is a plant hormone. It is used to accelerate the growth of several crops.

Soil fertility: Soil fungi maintain the fertility of soil

Plant disease: Fungi cause about 30.000 diseases in agricultural plants.Eg-Red root disease of sugar cane.

General studies: The fungus Neurospora is used for general studies.

Human fungal disease: Fungus cause the following diseases in man: *Aspergillosis*, athlets foot, ring worm, thrush etc.

Algae

Phycology or algology is the study of algae. The word phycology is derived from the Greek phykos, meaning seaweed. The term algae [s., alga] were originally used to define simple ―aquatic plants.‖ As noted above, it no longer has any formal significance in classification schemes. Instead the algae can be described as eucaryotic organisms that have chlorophyll a and carry out oxygen-producing photosynthesis. They differ from other photosynthetic eucaryotes in lacking a well-organized vascular conducting system and in having very simple reproductive structures. In sexual reproduction the whole organism may serve as a gamete; unicellular structures (gametangia) may produce gametes; or gametes can be formed by multicellular gametangia in which every cell is fertile. Unlike the case with plants, algal gametangia do not have nonfertile cells.

Distribution of Algae

Algae most commonly occur in water (fresh, marine, or brackish) in which they may be suspended (planktonic) or attached and living on the bottom (benthic). A few algae live at the water-atmosphere interface and are termed neustonic. Plankton [Greek plankos, wandering] consists of free-floating, mostly microscopic aquatic organisms. Phytoplankton is made up of algae and small plants, whereas zooplankton consists of animals and nonphotosynthetic protists.

Some algae grow on moist rocks, wood, trees, and on the surface of moist soil. Algae also live as endosymbionts in various protozoa, mollusks, worms, and corals. Several algae grow as endosymbionts within plants, some are attached to the surface of various structures, and a few lead a parasitic existence. Algae also associate with fungi to form lichens.

Classification of Algae

According to the five-kingdom system of Whittaker, the algae belong to seven divisions distributed between two different kingdoms. This classical classification is based on cellular, not organismal, properties. Some more important properties include: (1) cell wall (if present) chemistry and morphology; (2) form in which food or assimilatory products of photosynthesis are stored; (3) chlorophyll molecules and accessory pigments that contribute to photosynthesis; (4) flagella number and the location of their insertion in motile cells; (5) morphology of the cells and/or body (thallus); (6) habitat; (7) reproductive structures; and (8) life history patterns. Based on these properties the algae are arranged by divisions.

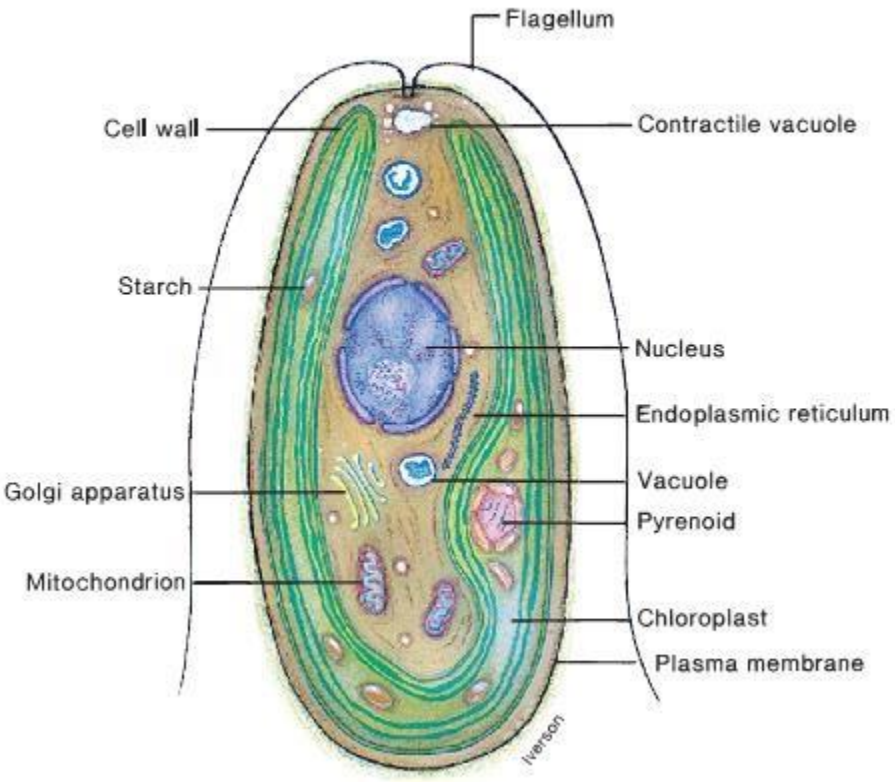
Classical Classification of Algae[a]

Division (Common Name)	Kingdom
Chrysophyta (yellow-green and golden-brown algae; diatoms)	*Protista* (single cell or colonial; eucaryotic)
Euglenophyta (photosynthetic euglenoid flagellates)	*Protista*
Pyrrhophyta (dinoflagellates)	*Protista*
Charophyta (stoneworts)	*Protista*
Chlorophyta (green algae)	*Protista*
Phaeophyta (brown algae)	*Plantae* (multicellular; eucaryotic)
Rhodophyta (red algae)	*Plantae*

Ultrastructure of the Algal Cell

The eucaryotic algal cell is surrounded by a thin, rigid cell wall. Some algae have an outer matrix lying outside the cell wall. This usually is flexible and gelatinous, similar to bacterial capsules. When present, the flagella are the locomotor organelles. The nucleus has a typical nuclear

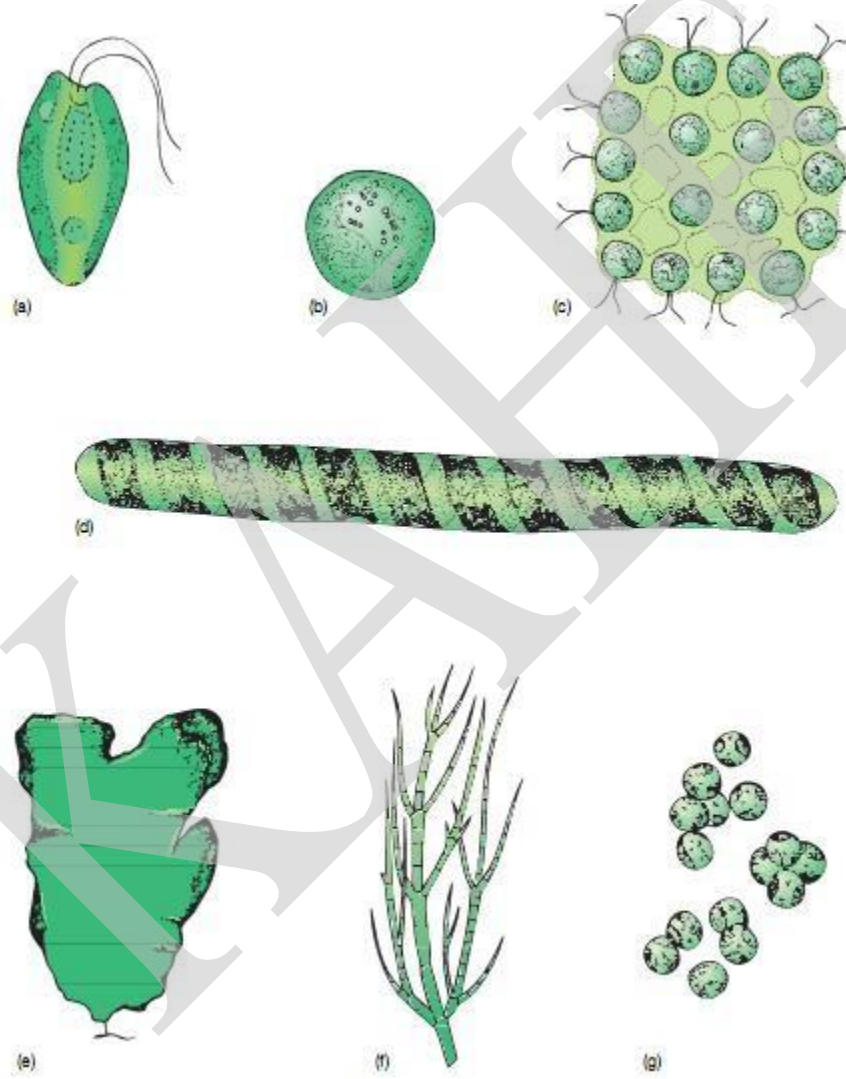
envelope with pores; within the nucleus are a nucleolus, chromatin, and karyolymph. The chloroplasts have membrane-bound sacs called thylakoids that carry out the light reactions of photosynthesis. These organelles are embedded in the stroma where the dark reactions of carbon dioxide fixation take place. A dense proteinaceous area, the pyrenoid that is associated with synthesis and storage of starch may be present in the chloroplasts. Mitochondrial structure varies greatly in the algae. Some algae (euglenoids) have discoid cristae; some, lamellar cristae (green and red algae); and the remaining, (golden-brown and yellow-green, brown, and diatoms) have tubular cristae.



Algal Morphology. Schematic drawing of a typical eucaryotic algal cell showing some of its organelles and other structures.

Structure of the Algal Thallus (Vegetative Form)

The vegetative body of algae is called the thallus [pl., thalli]. It varies from the relative simplicity of a single cell to the more striking complexity of multicellular forms, such as the giant kelps. Single-celled algae may be as small as bacteria, whereas kelp can attain a size over 75 m in length. Algae are unicellular, colonial, filamentous, membranous and bladelike or tubular.



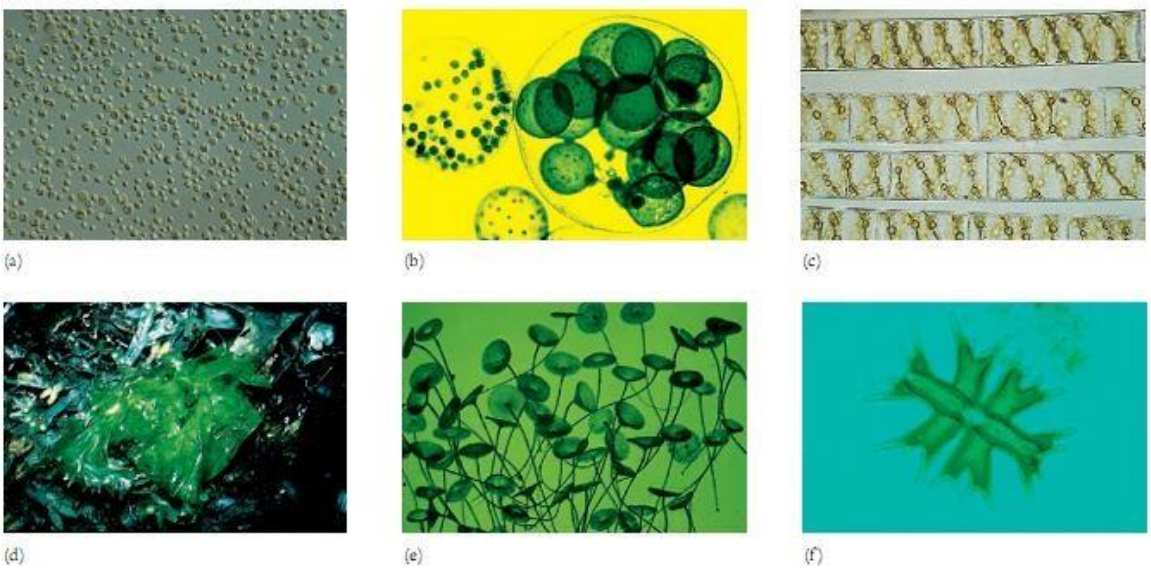
Diagrammatic Algal Bodies: (a) unicellular, motile, Cryptomonas; (b) unicellular, nonmotile, almellopsis; (c) colonial, Gonium; (d) filamentous, Spirotaenia; (e) bladelike

kelp, Monostroma; (f) leafy tubular axis, branched tufts or plumes, Stigeoclonium; (g) unicellular, nonmotile, Chrysocapsa.

Characteristics of the Algal Divisions

Chlorophyta (Green Algae)

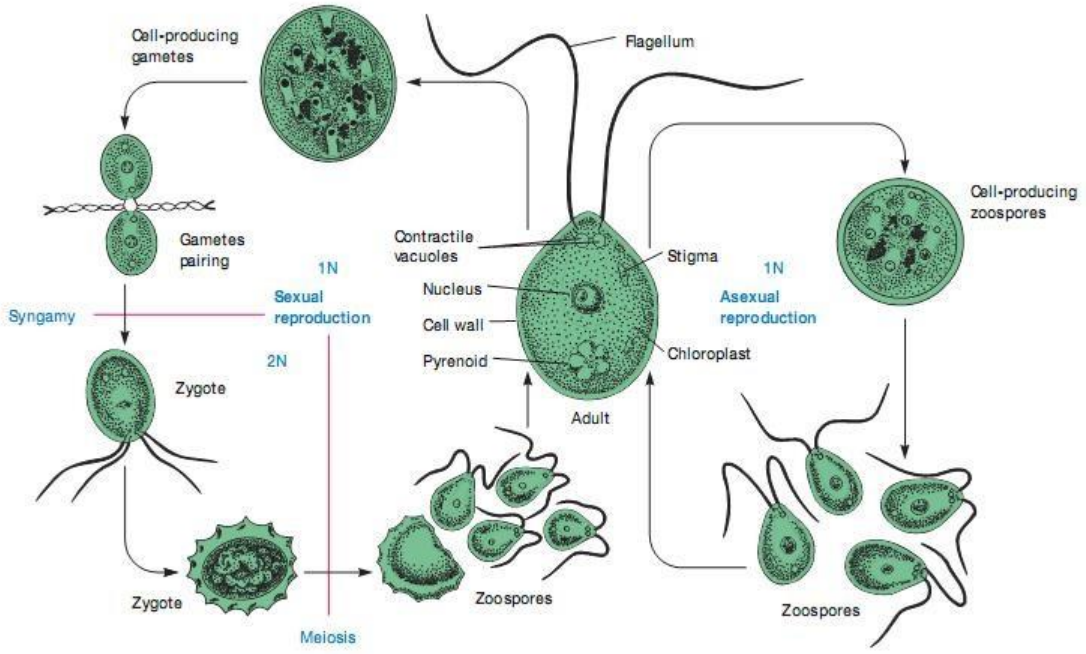
The Chlorophyta or green algae [Greek chloros, green] are an extremely varied division. They grow in fresh and salt water, in soil, on other organisms, and within other organisms. The Chlorophyta have chlorophylls a and b along with specific carotenoids, and they store carbohydrates as starch. Many have cell walls of cellulose. They exhibit a wide diversity of body forms, ranging from unicellular to colonial, filamentous, membranous or sheetlike, and tubular types.



Chlorophyta (Green Algae); Light Micrographs. (a) Chlorella, a unicellular nonmotile green alga (160). (b) Volvox, a typical green algal colony (450). (c) Spirogyra, a filamentous green alga (100). Four filaments are shown. Note the ribbonlike, spiral chloroplasts within each filament. (d) Ulva, commonly called sea lettuce, has a leafy appearance. (e) Acetabularia, the mermaid's wine goblet. (f) Micrasterias, a large desmid (150).

Chlamydomonas is a representative unicellular green alga (figure 26.4). Individuals have two flagella of equal length at the anterior end by which they move rapidly in water. Each cell has a

single haploid nucleus, a large chloroplast, a conspicuous pyrenoid, and a stigma (eyespot) that aids the cell in phototactic responses. Two small contractile vacuoles at the base of the flagella function as osmoregulatory organelles that continuously remove water. Chlamydomonas reproduces asexually by producing zoospores through cell division. The alga also reproduces sexually when some products of cell division act as gametes and fuse to form a four flagellated diploid zygote that ultimately loses its flagella and enters a resting phase. Meiosis occurs at the end of this resting phase and produces four haploid cells that give rise to adults.



Chlamydomonas: The Structure and Life Cycle of This Motile Green Alga. During asexual reproduction, all structures are haploid; during sexual reproduction, only the zygote is diploid.

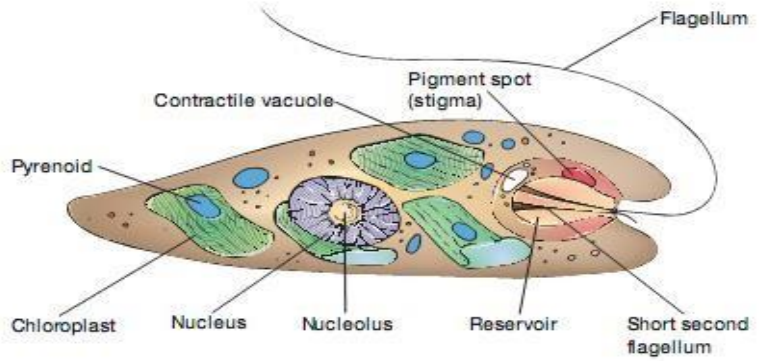
Charophyta (Stoneworts/Brittleworts)

The stoneworts are abundant in fresh to brackish waters and have a worldwide distribution. Often they appear as a dense covering on the bottom of shallow ponds. Some species precipitate calcium and magnesium carbonate from the water to form a limestone covering, thus giving the Charophyta their common names of stoneworts or brittleworts.

Euglenophyta (Euglenoids)

The euglenoids share with the Chlorophyta and Charophyta the presence of chlorophylls a and b in their chloroplasts. The primary storage product is paramylon (a polysaccharide composed of β-1,3 linked glucose molecules), which is unique to euglenoids. They occur in fresh, brackish, and marine waters and on moist soils; they often form water blooms in ponds and cattle water tanks. In molecular classification schemes, euglenoids are associated with the amoeboflagellates (flagellated protozoa) and kinetoplastids because all members have related rRNA sequences and mitochondria with discoid cristae at some stage in their life cycle.

The representative genus is Euglena. A typical Euglena cell is elongated and bounded by a plasma membrane. Inside the plasma membrane is a structure called the pellicle, which is composed of articulated proteinaceous strips lying side by side. The pellicle is elastic enough to enable turning and flexing of the cell, yet rigid enough to prevent excessive alterations in shape. The several chloroplasts contain chlorophylls a and b together with carotenoids. The large nucleus contains a prominent nucleolus. The stigma is located near an anterior reservoir. A large contractile vacuole near the reservoir continuously collects water from the cell and empties it into the reservoir, thus regulating the osmotic pressure within the organism. Two flagella arise from the base of the reservoir, although only one emerges from the canal and actively beats to move the cell. Reproduction in euglenoids is by longitudinal mitotic cell division.



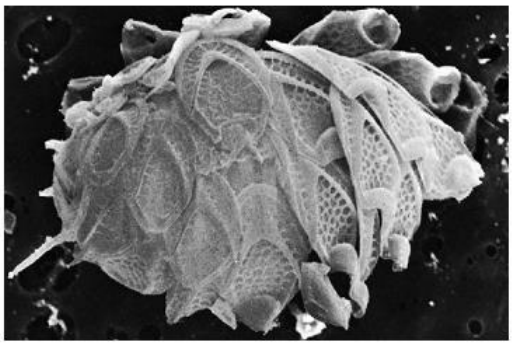
Euglena. A Diagram Illustrating the Principal Structures Found in This Euglenoid. Notice that a short second flagellum does not emerge from the anterior invagination. In some euglenoids both flagella are emergent.

Chrysophyta (Golden-Brown and Yellow-Green Algae; Diatoms)

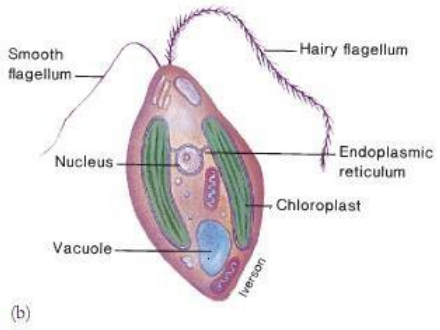
The division Chrysophyta is quite diversified with respect to pigment composition, cell wall, and type of flagellated cells. In molecular classification schemes, these algae are associated with the stramenopiles and have mitochondria with tubular cristae. The division is divided into three major classes: golden-brown algae [Greek chrysos, gold], yellow-green algae, and diatoms. The major photosynthetic pigments are usually chlorophylls a and c1/c2, and the carotenoid fucoxanthin. When fucoxanthin is the dominant pigment, the cells have a golden-brown color. The major carbohydrate reserve in the Chrysophyta is chrysolaminarin (a polysaccharide storage product composed principally of -1,3 linked glucose residues).

Some Chrysophyta lack cell walls; others have intricately patterned coverings external to the plasma membrane, such as scales (figure 26.6a), walls, and plates. Diatoms have a distinctive two-piece wall of silica, called a frustule. Two anteriorly attached flagella of unequal length are common among Chrysophyta (figure 26.6b), but some species have no flagella, and others have either one flagellum or two that are of equal length. Most Chrysophyta are unicellular or colonial. Reproduction usually is asexual but occasionally sexual. Although some marine forms are known, most of the yellow-green and golden-brown algae live in fresh water. Blooms of some species produce unpleasant odors and tastes in drinking water. The diatoms (figure 26.6c,d; see also figure 4.1b) are photosynthetic, circular or oblong chrysophyte cells with frustules composed of two halves or thecae that overlap like a petri dish [therefore their name is from the Greek diatomsos, cut in two]. The larger half is the epitheca, and the smaller half is the hypotheca. Diatoms grow in freshwater, salt water, and moist soil and comprise a large part of the phytoplankton (Box 26.1). The chloroplasts of these chrysophytes contain chlorophylls a and c as well as carotenoids. Some diatoms are facultative heterotrophs and can absorb carboncontaining molecules through the holes in their walls. The vegetative cells of diatoms are

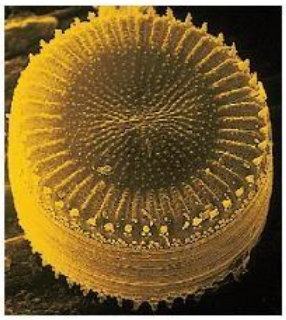
diploid; exist as unicellular, colonial, or filamentous shapes; lack flagella; and have a single large nucleus and smaller plastids. Reproduction consists of the organism dividing sexually, with each half then constructing a new theca within the old one. Because of this mode of reproduction, diatoms get smaller with each reproductive cycle. However, when they diminish to about 30% of their original size, sexual reproduction usually occurs. The diploid vegetative cells undergo meiosis to form gametes, which then fuse to produce a zygote. The zygote develops into an auxospore, which increases in size again and forms a new wall. The mature auxospore eventually divides mitotically to produce vegetative cells with normal frustules.



(a)



(b)



(c)

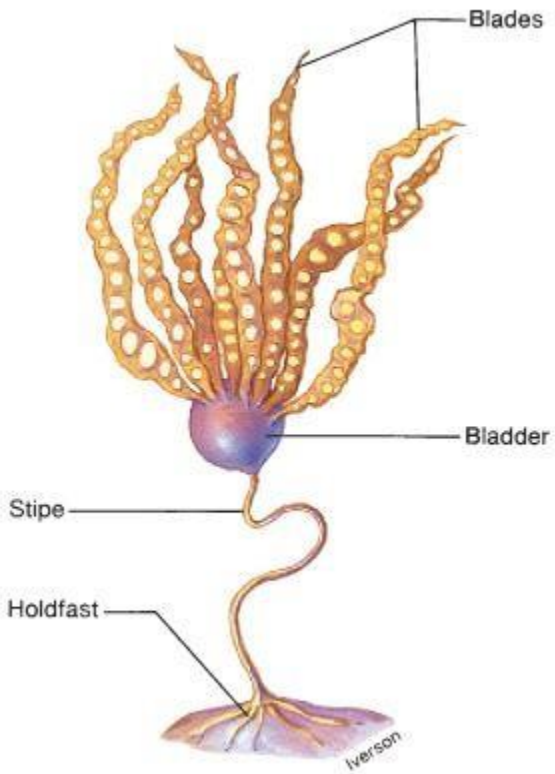
(d)

Chrysophyta (Yellow-Green and Golden-Brown Algae; Diatoms). (a) Scanning electron micrograph of Mallomonas, a chrysophyte, showing its silica scales. The scales are

embedded in the pectin wall but synthesized within the Golgi apparatus and transported to the cell surface in vesicles (9,000). (b) Ochromonas, a unicellular chrysophyte. Diagram showing typical cell structure. (c) Scanning electron micrograph of a diatom, Cyclotella meneghiniana (750). (d) Assorted diatoms as arranged by a light microscopist (900).

Phaeophyta (Brown Algae)

The Phaeophyta or brown algae [Greek phaeo, brown] consist of multicellular organisms that occur almost exclusively in the sea. Some species have the largest linear dimensions (length) known in the eucaryotic world (chapter opening figure). Since the brown algae have tubular cristae, they are associated with stramenopiles in molecular classification schemes. Most of the conspicuous seaweeds that are brown to olive green in color are assigned to this division. The simplest brown algae consist of small openly branched filaments; the larger, more advanced species have a complex arrangement. Some large kelps are conspicuously differentiated into flattened blades, stalks, and holdfast organs that anchor them to rocks. Some, such as Sargassum, form huge floating masses that dominate the Sargasso Sea. The color of these algae reflects the presence of the brown pigment fucoxanthin, in addition to chlorophylls a and c, β-carotene, and violaxanthin. The main storage product is laminarin, which is quite similar in structure to chrysolaminarin.



Phaeophyta (Brown Algae). Diagram of the parts of the brown alga, Nereocystis. Due to the holdfast organ, the heaviest tidal action and surf seldom dislodge brown algae from their substratum. The stipe is a stalk that varies in length; the bladder is a gas-filled float.

Rhodophyta (Red Algae)

The division Rhodophyta, the red algae [Greek rhodon, rose], includes most of the seaweeds (figure 26.8). A few reds are unicellular but most are filamentous and multicellular. Some red algae are up to 1 m long. The stored food is the carbohydrate called floridean starch (composed of α-1,4 and α -1,6 linked glucose residues).

The red algae contain the red pigment phycoerythrin, one of the two types of phycobilins that they possess. The other accessory pigment is the blue pigment phycocyanin. The presence of these pigments explains how the red algae can live at depths of 100 m or more. The wavelengths of light (green, violet, and blue) that penetrate these depths are not absorbed by chlorophyll a but

instead by these phycobilins. Not surprisingly the concentrations of these pigments often increases with depth as light intensity decreases. The phycobilins, after absorbing the light energy, pass it on to chlorophyll a. The algae appear decidedly red when phycoerythrin predominates over the other pigments. When phycoerythrin un- dergoes photodestruction in bright light, other pigments predominate and the algae take on shades of blue, brown, and dark green. The cell walls of most red algae include a rigid inner part composed of microfibrils and a mucilaginous matrix. The matrix is composed of sulfated polymers of galactose called agar, funori, porphysan, and carrageenan. These four polymers give the red algae their flexible, slippery texture. Agar is used extensively in the laboratory as a culture medium component. Many red algae also deposit calcium carbonate in their cell walls and play an important role in building coral reefs.

Rhodophyta (Red Algae). These algae (e.g., Corallina gracilis) are much smaller and more delicate than the brown algae. Most red algae have a filamentous, branched morphology.

It is important to know the different modes of nutrition in all living organisms in order to understand energy flow within the ecosystem. Plant produces high energy organic food from inorganic raw materials. They are called autotroph and the mode of nutrition is known as **autotrophic nutrition**. Animals feed on those high energy organic food, are called as

heterotrophs and their mode of nutrition is known as **heterotrophic nutrition** Heterotrophic nutrition further sub-categorise in **holozoic**, **parasitic**, and **saprophytic** mode of nutrition based on the pattern and class of food that is taken inside.

Autotrophs have been reported to survive weeks without an input of nutrients, while heterotrophs decrease in numbers very rapidly without food. During periods of stress – such as limited food sources and low dissolved oxygen – autotrophs can survive through inactivity while heterotrophs form durable, long-lasting spores. Both spores and inactive autotrophic cells are activated when the right environmental conditions are encountered. Autotrophic and heterotrophic bacteria exist in a complementary relationship. Heterotrophic bacteria produce carbon dioxide as an end product, which provides a carbon source for autotrophs, which in turn create biomass that will be eventually consumed by heterotrophs. Heterotrophs as Sources of Nutrition Nutrients from uneaten feed and excretion from culture animals in ponds can be efficiently recycled into valuable biomass by bacteria. Heterotrophic bacteria are important dietary components of detritivores such as shrimp, tilapia, and carp. Detritivores consume plant and animal remains or waste, sequentially reducing the particle sizes so that bacteria and fungi can break them down to their constituent chemical parts for recycling. About 10% of the available energy is passed on from one trophic level to the next, so organisms lower on the food chain, like detritivores, actually need less energy to grow.

Bright field microscopy

Bright field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Bright field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright field microscopy image is a dark sample on a bright background, hence the name

Light path

Bright field microscopy typically has low contrast with most biological samples as few absorb light to a great extent. Staining is often required to increase contrast, which prevents use on live cells in many situations. Bright field illumination is useful for samples which have an intrinsic colour, for example chloroplasts in plant cell.

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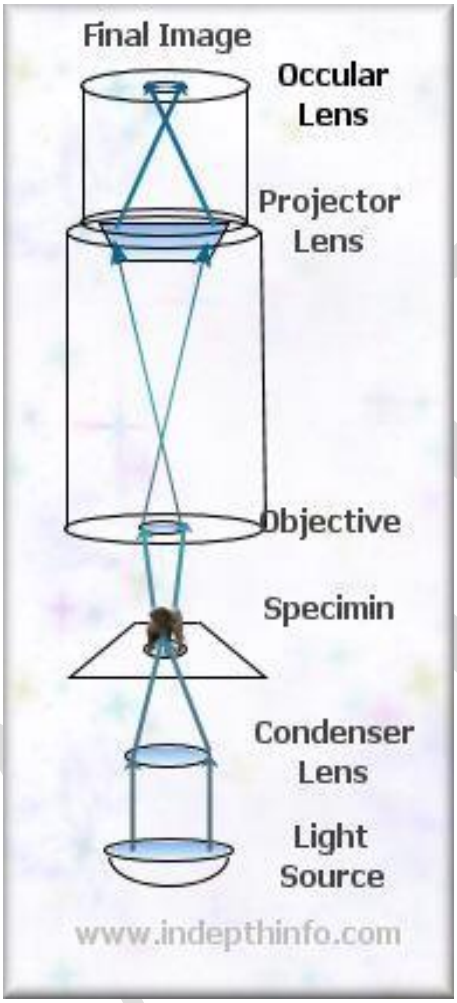


Advantages

- Simplicity of setup with only basic equipment required.

Limitations

- Very low contrast of most biological samples.
- Low apparent optical resolution due to the blur of out of focus material.
- The sample often has to be stained before viewing. Therefore, live cells cannot usually be viewed. There are some exceptions, however, including the observation of cytoplasmic streaming in Chara cell.

Enhancements

- Reducing or increasing the amount of the light source via the iris diaphragm.
- Use of an oil immersion objective lens and a special immersion oil placed on a glass cover over the specimen. Immersion oil has the same refraction as glass and improves the resolution of the observed specimen.

Phase contrast Microscopy

Unpigmented living cells are not clearly visible in the bright field microscope because there is little difference in contrast between the cells and water. Thus microorganisms often must be fixed and stained before observation to increase contrast and create variations in color between cell structures. A phase-contrast microscope converts slight differences in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells.

The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light. As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1/4 wavelength. The deviated light is focused to form an image of the object. Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate. If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1/2 wavelength out of phase and will cancel each other when they come together to form an image (figure 2.10). The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined. This type of microscopy is called dark-phase-contrast microscopy. Color filters often are used to improve the image.

Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain poly-hydroxybutyrate, poly-metaphosphate, sulfur, or other

substances. These are clearly visible because they have refractive indexes markedly different from that of water. Phase-contrast microscopes also are widely used in studying eucaryotic cells.

The Fluorescence Microscope

The microscopes thus far considered produce an image from light that passes through a specimen. An object also can be seen because it actually emits light, and this is the basis of fluorescence microscopy. When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light. Any light emitted by an excited molecule will have a longer wavelength (or be of lower energy) than the radiation originally absorbed. Fluorescent light is emitted very quickly by the excited molecule as it gives up its trapped energy and returns to a more stable state.

Fluorescence Microscopy. The principles of operation of a fluorescence microscope.

The above fluorescence microscope exposes a specimen to ultraviolet, violet, or blue light and forms an image of the object with the resulting fluorescent light. A mercury vapor arc lamp or other source produces an intense beam, and heat transfer is limited by a special infrared filter. The light passes through an exciter filter that transmits only the desired wavelength. A dark-field condenser provides a black background against which the fluorescent objects glow. Usually the specimens have been stained with dye molecules, called fluorochromes that fluoresce brightly upon exposure to light of a specific wavelength but some microorganisms are auto fluorescing. The microscope forms an image of the fluorochrome-labeled microorganisms from the light emitted when they fluoresce. A barrier filter positioned after the objective lenses removes any remaining ultraviolet light, which could damage the viewer‘s eyes, or blue and violet light, which would reduce the image‘s contrast.

Confocal Microscopy

A conventional light microscope, which uses a mixed wavelength light source and illuminates a large area of the specimen, will have a relatively great depth of field. Even if not in focus, images of bacteria from all levels within the field will be visible. These will include cells above, in, and below the plane of focus. As a result the image can be murky, fuzzy, and crowded.

Confocal Scanning Laser Microscopy: Light Collection Depth and Image Clarity. (a) Conventional light microscopic observation. (b) Confocal scanning laser microscopic observation.

The solution to this problem is the confocal scanning laser microscope (CSLM) or confocal microscope. Fluorescently stained specimens are usually examined. A focused laser beam strikes a point in the specimen.

Light from the illuminated spot is focused by an objective lens onto a plane above the objective. An aperture above the objective lens blocks out stray light from parts of the specimen that lie above and below the plane of focus. The laser is scanned over a plane in the specimen (beam scanning) or the stage is moved (stage scanning) and a detector measures the illumination from each point to produce an image of the optical section. When many optical sections are scanned, a computer can combine them to form a three-dimensional image from the digitized signals. This image can be measured and analyzed quantitatively.

The confocal microscope improves images in two ways. First, illumination of one spot at a time reduces interference from light scattering by the rest of the specimen. Second, the aperture above the objective lens blocks out stray light as previously mentioned. Consequently the image has excellent contrast and resolution. A depth of 1 m or less in a thick preparation can be directly observed. Special computer software is used to create high-resolution, three-dimensional images of cell structures and complex specimens such as biofilms.

Electron Microscopy

For centuries the light microscope has been the most important instrument for studying microorganisms. The electron microscope now has transformed microbiology and added immeasurably to our knowledge. The nature of the electron microscope and the ways in which specimens are prepared for observation are reviewed briefly in this section.

The Scanning Electron Microscope

The scanning electron microscope (SEM) has been used to examine the surfaces of microorganisms in great detail; many instruments have a resolution of 7 nm or less. The SEM

differs from other electron microscopes in producing an image from electrons emitted by an object‘s surface rather than from transmitted electrons.

Specimen preparation is easy, and in some cases air-dried material can be examined directly. Most often, however, microorganisms must first be fixed, dehydrated, and dried to preserve surface structure and prevent collapse of the cells when they are exposed to the SEM‘s high vacuum. Before viewing, dried samples are mounted and coated with a thin layer of metal to prevent the buildup of an electrical charge on the surface and to give a better image.

The SEM scans a narrow, tapered electron beam back and forth over the specimen. When the beam strikes a particular area, surface atoms discharge a tiny shower of electrons called secondary electrons, and these are trapped by a special detector. Secondary electrons entering the detector strike a scintillator causing it to emit light flashes that a photomultiplier converts to an electrical current and amplifies. The signal is sent to a cathode-ray tube and produces an image like a television picture, which can be viewed or photographed.

The number of secondary electrons reaching the detector depends on the nature of the specimen‘s surface. When the electron beam strikes a raised area, a large number of secondary electrons enter the detector; in contrast, fewer electrons escape a depression in the surface and reach the detector. Thus raised areas appear lighter on the screen and depressions are darker. The actual in situ location of microorganisms in ecological niches such as the human skin and the lining of the gut also can be examined.

The Transmission Electron Microscope

The very best light microscope has a resolution limit of about 0.2 m. Because bacteria usually are around 1 m in diameter, only their general shape and major morphological features are visible in the light microscope. The detailed internal structure of larger microorganisms also cannot be effectively studied by light microscopy. These limitations arise from the nature of visible light waves, not from any inadequacy of the light microscope itself.

Recall that the resolution of a light microscope increases with a decrease in the wavelength of the light it uses for illumination. Electron beams behave like radiation and can be focused much as light is in a light microscope. If electrons illuminate the specimen, the

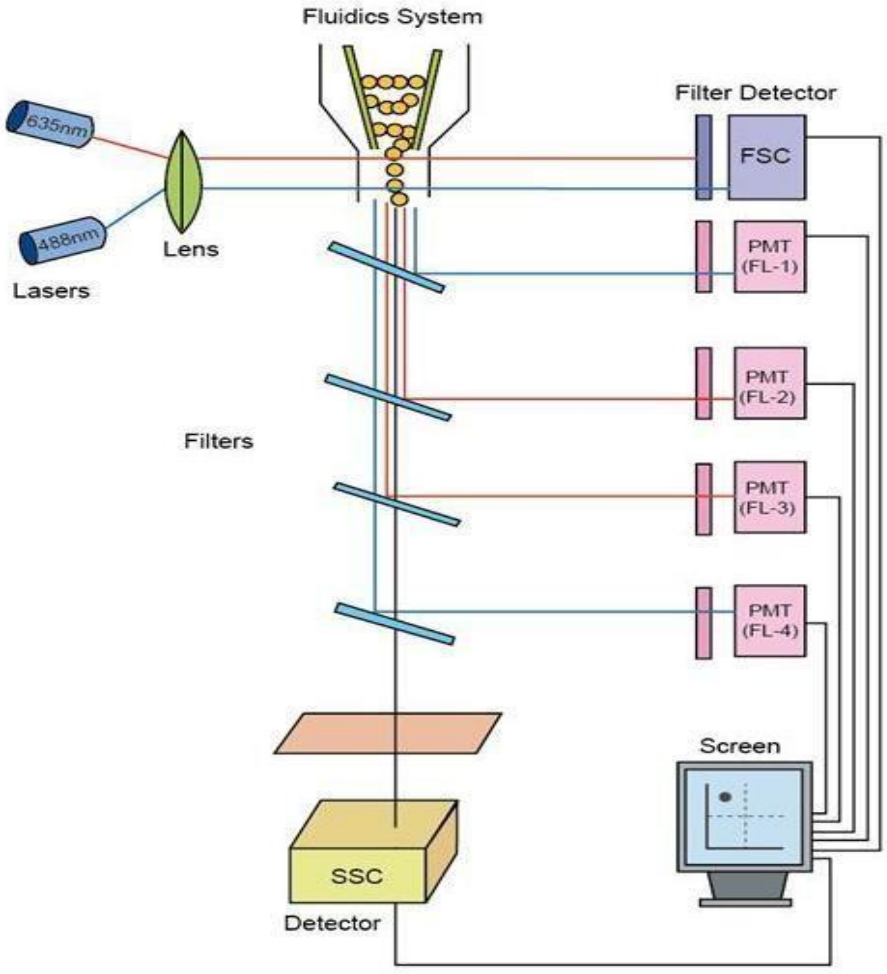
microscope's resolution is enormously increased because the wavelength of the radiation is around 0.005 nm, approximately 100,000 times shorter than that of visible light. The transmission electron microscope has a practical resolution roughly 1,000 times better than the light microscope; with many electron microscopes, points closer than 5 Å or 0.5 nm can be distinguished, and the useful magnification is well over 100,000X.

A modern transmission electron microscope (TEM) is complex and sophisticated, but the basic principles behind its operation can be understood readily. A heated tungsten filament in the electron gun generates a beam of electrons that is then focused on the specimen by the condenser. Since electrons cannot pass through a glass lens, doughnut-shaped electromagnets called magnetic lenses are used to focus the beam. The column containing the lenses and specimen must be under high vacuum to obtain a clear image because electrons are deflected by collisions with air molecules. The specimen scatters electrons passing through it, and the beam is focused by magnetic lenses to form an enlarged, visible image of the specimen on a fluorescent screen. A denser region in the specimen scatters more electrons and therefore appears darker in the image since fewer electrons strike that area of the screen. In contrast, electron-transparent regions are brighter. The screen can also be moved aside and the image captured on photographic film as a permanent record.

Cytophotometry and Flow Cytometry

Flow cytometry is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It has established itself as a useful, quick and novel method to determine efficiently and reproducibly the relative nuclear DNA content and ploidy level of a large number of plant species. Basically, a flow cytometer is a fluorescence microscope which analyses moving particles in a suspension. These particles are excited by a source of light usually a laser and in turn emit an epifluorescence, which is filtered through a series of dichroic mirrors. The inbuilt programme of the equipment converts these signals into a graph plotting the intensity of the epifluorescence emitted against the count of cells emitting it at a given time. Thus, a flow cytometer consists of fluidics, optics and electronics, as it measures cells in suspension that flow

in single-file through an illuminated volume where they scatter light and emit a fluorescence that is collected, filtered and converted to digital values for storage on a computer. It allows simultaneous multi - parametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. For the fluorescence to be detected by the photomultiplier, the cells have to be labelled with an appropriate fluorescent molecule whose properties will change on binding to nucleic acids.



Schematic overview of a typical flow cytometry principle

Signals are collected by an array of photo detectors. When light hits a photo detector, a small current is produced. Its associated voltage has amplitude that is proportional to the total number of light photons received by the detector. This voltage is then amplified by a series of amplifiers into electrical signals which can be plotted graphically.

Both fluorescence and scatter data are displayed in the form of univariate histogram or bivariate scatter plots by specialized software. In the case of histograms, the x-axis will correspond to either fluorescence or scatter intensity collected from a single photo-detector on either a linear or logarithmic scale or the y-axis corresponds to the number of particles with the corresponding light intensity. For example, if a cell is tagged with a fluorescent labelled antibody directed towards a surface protein, the fluorescence will be directly proportional to the expression level of this protein. By using multiple antibodies, one could assess the expression level of several membrane bound and / or intracellular proteins of a single cell.

Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

Simple stain techniques. Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the simple stain procedure.

Gram stain procedure. This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria. Crystal violet is first applied, followed by the mordant iodine, which fixes the stain.Then the slide is washed with alcohol, and the Gram-positive bacteria retain the crystal-violet iodine stain; however, the Gram-negative bacteria lose the stain. The Gram-negative bacteria subsequently stain with the safranin dye, the counterstain,

used next. These bacteria appear red under the oil-immersion lens, while Gram-positive bacteria appear blue or purple, reflecting the crystal violet retained during the washing step.

Spore stain procedure

A special stain technique is used to examine bacterial spores. Malachite green is used with heat to force the stain into the cells and give them color. A counterstain, safranin, is then used to give color to the nonspore forming bacteria. At the end of the procedure, spores stain green and other cells stain red.

Preparation and Staining of Specimens

Although living microorganisms can be directly examined withthe light microscope, they often must be fixed and stained to increasevisibility, accentuate specific morphological features, and preserve them for future study.

Fixation

The stained cells seen in a microscope should resemble living cells as closely as possible. **Fixation** is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and toughens cell structures so that theydo not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation.

There are two fundamentally different types of fixation.

(1) Bacteriologists heat-fix bacterial smears by gently flame heatingan air-dried film of bacteria. This adequately preserves overallmorphology but not structures within cells. (2) Chemical fixationmust be used to protect fine cellular substructure and the morphologyof larger, more delicate microorganisms. Chemical fixatives penetrate cells and react with cellular components, usually proteinsand lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain such components as ethanol, acetic

acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Dyes and Simple Staining

The many types of dyes used to stain microorganisms have twofeatures in common.

(1) They have **chromophore groups,**groups with conjugated double bonds that give the dye its color.

(2) They can bind with cells by ionic, covalent, or hydrophobicbonding. For example, a positively charged dye binds to negatively charged structures on the cell. Ionizable dyes may be divided into two general classes based
on the nature of their charged group.

Basic dyes—methylene blue, basic fuchsin, crystal violet,safranin, malachite green—have positively charged groups(usually some form of pentavalent nitrogen) and are generally sold as chloride salts. Basic dyes bind tonegatively charged molecules like nucleic acids and manyproteins. Because the surfaces of bacterial cells also are negatively charged, basic dyes are most often used in
bacteriology.

Acid dyes—eosin, rose bengal, and acid fuchsin—possess negatively charged groups such as carboxyls (—COOH)and phenolic hydroxyls (—OH). Acid dyes, because oftheir negative charge, bind to positively charged cell structures. The pH may alter staining effectiveness since the nature and degree of the charge on cell components change with pH. Thus anionicdyes stain best under acidic conditions when proteins and many other molecules carry a positive charge; basic dyes are most effective at higher pHs. Although ionic interactions are probably the most common means of attachment, dyes also bind through covalent bonds or because of their solubility characteristics. For instance, DNA can be stained by the Feulgen procedure in which Schiff‘s reagent iscovalently attached to its deoxyribose sugars after hydrochloricacid treatment. Sudan III (Sudan Black) selectively stains lipids because it is lipid soluble but will not dissolve in aqueous portions nof the cell.

Microorganisms often can be stained very satisfactorily by **Simple staining,** in which a single staining agent is used. Simple staining‘s value lies in its simplicity and ease of use. One covers the fixed smear with stain for the proper length of time,washes the excess stain off with water, and blots the slide dry.Basic dyes like crystal violet, methylene blue, and carbolfuchsinare frequently used to determine the size, shape, and arrangement of bacteria.

Differential Staining

Differential staining procedures divide bacteria into separategroups based on staining properties. The **Gram stain,** developedin 1884 by the Danish physician Christian Gram, is the mostwidely employed staining method in bacteriology. It is a differentialstaining procedure because it divides bacteria into two
classes—gram negative and gram positive.

Gram-positive and gram negative bacteria

In the first step of the Gram-staining procedure, the smear is stained with the basic dye crystal violet, the primary stain. It is followed by treatment with an iodine solutionfunctioning as a **mordant.** That is, the iodine increases the interactionbetween the cell and the dye so that the cell is stainedmore strongly. The smear is next decolorized by of the Gram stain; gram-positive bacteria retain the crystal violet,whereas gram-negative bacteria lose their crystal violetand become colorless. Finally, the smear is counterstained with a simple, basic dye different in color from crystal violet. Safranin, the most common counterstain, colors gram-negativebacteria pink to red and leaves gram-positive bacteria dark purple.

Acid-fast staining is another important differential staining procedure. A few species, particularly those in the genus *Mycobacterium* do not bind simple stains readilyand must be stained by a harsher treatment: heating with a mixtureof basic fuchsin and phenol (the Ziehl-Neelsen method).Once basic fuchsin has penetrated with the aid of heat and phenol,acid-fast cells are not easily decolorized by an acid-alcoholwash and hence remain red. This is due to the quite high lipid contentof acid-fast cell walls; in particular, mycolic acid—a groupof branched chain hydroxy lipids—appears responsible for acidfastness.Non-acid-fast bacteria are decolorized by acid-alcoholand thus are stained blue by methylene blue counterstain. Thismethod is used to identify *Mycobacterium tuberculosis* and *M.leprae* the pathogens responsible for tuberculosisand leprosy, respectively.

Staining Specific Structures

Many special staining procedures have been developed over theyears to study specific bacterial structures with the light microscope.One of the simplest is **negative staining,** a

technique thatreveals the presence of the diffuse capsules surrounding manybacteria. Bacteria are mixed with India ink or Nigrosin dye andspread out in a thin film on a slide. After air-drying, bacteria appearas lighter bodies in the midst of a blue-black background becauseink and dye particles cannot penetrate either the bacterialcell or its capsule. The extent of the light region is determined bythe size of the capsule and of the cell itself. There is little distortion of bacterial shape, and the cell can be counterstained for evengreater visibility.

The Common Nutritional Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen hydrogen, nitrogen, sulfur, phosphorous, potassium, Calcium, magnesium and iron. These are called macro elements or macronutrients because they are required by microorganism in relatively large amounts. The first six are components of carbohydrates, proteins ,lipids and nucleic acids. The remaining four macronutrients exist in the cell as cat ions and play a variety of roles. For example potassium (K+) is required for activity for a number of enzymes including some of those involved in protein synthesis. Calcium, among other functions, contributes to the heat résistance bacterial endospores. Magnesium serves as cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron is a part of cytochromes and cofactor for enzymes and electron carrying proteins.

All organisms , including microorganisms, require several micronutrients or trace elements besides macro elements. The micronutrients- manganese, cobalt, zinc, molybdenum, nickel, and copper- are needed by most cells. However, cells require regular media components are often adequate for growth. Therefore it is very difficult to demonstrate a micronutrient requirement. In nature ,micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors , and they aid in the catalysis of reactions and maintenance of protein structure. For example zinc is present at the active sites of some enzymes but is also involved in the regulatory and catalytic subunits of E.coli aspartate carbamoyltransferase.

Besides the common macroelements and trace elements, microorganisms may have particular requirements that affect the nature of their morphology or environment. Diatoms

require silicic acid to construct their beautiful cell walls of silica. Although most bacteria do not require large amounts of sodium, many bacteria growing I n saline lakes and oceans depend on the presence of high concentrations of sodium ions.

Finally it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of concentration of other nutrients.

Growth Factors

Microorganisms often grow and reproduce when minerals and sources of energy, carbon nitrogen, phosphorous and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components required for their well being. Many microorganisms on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic components required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called growth factors. There are three major classes of growth factors: (1) amino acids (2) purines and pyrimidines, and (3) vitamins.

Amino acids are required for protein synthesis, purines and pyrimidines for nucleic acid synthesis. Vitamins are small organic molecules that usually make up all or part of enzyme cofactors and only very small amounts sustain growth. Some microorganisms require many vitamins for growth. Other growth factors are also seen heme (from hemoglobin) is required by Haemophilus influenzae , and some mycoplasms need cholesterol.

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth-response assays for a variety of substances. For example species from the bacterial genera Lactobacillus and Streptococcus can be used in microbiological assays of mostly vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factors to be assayed. A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. Ideally the amount of growth resulting is directly proportional to

the quantity of growth factor present : if the growth factor concentration doubles the final extent of bacterial growth doubles.

Impact of environmental factors on growth

Nutrients

Nutrients such as carbohydrates, fats, proteins, vitamins, minerals and water, required by, man are also needed by microorganisms to grow. Microbes differ in their abilities to use substrates as nutrient sources. Their enzyme systems are made available according to their genetic code. They vary in ability to use nitrogen sources to produce amino acids and, therefore, proteins. Some require amino acids to be supplied by the substrate. When organisms need special materials provided by their environment, we refer to them as fastidious. Difference in the utilization of nutrients and the waste products they produce are important in differentiating between organisms.

Oxygen

Microbes also differ in their needs for free oxygen. Aerobic organisms must grow in the presence of free oxygen and anaerobic organisms must grow in the absence of free oxygen. Facultative organisms can grow with or without oxygen, while microaerophilic organisms grow in the presence of small quantities of oxygen.

Water

Water is necessary for microbes to grow, but microbes cannot grow in pure water. Some water is not available. A measurement of the availability of water is aw or water activity. The aw of pure water is 1.0 while that of a saturated salt solution is 0.75. Most spoilage bacteria require a minimum aw of 0.90. Some bacteria can tolerate an aw above 0.75 as can some yeasts and most molds. Most yeasts require 0.87 water activity. An aw of 0.85 or less suppresses the growth of organisms of public health significance.

Temperature

Microorganisms can grow in a wide range of temperatures. Since they depend on water as a solvent for nutrients, frozen water or boiling water inhibits their growth. General terms are applied to organisms based on their growth at different temperatures. Most organisms grow best

at or near room and body temperature. These are mesophiles. Those growing above 400C (1050F) are called thermophiles while those growing below 250 C (750F) are called psychrotrophs.

Acidity

The nature of a solution based on its acidity or alkalinity is described as pH. The pH scale ranges from 0, strongly acidic, to 14, strongly basic. Neutral solutions are pH 7, the pH of pure water. Most bacteria require near neutral conditions for optimal growth with minimums and maximums between 4 and 9. Many organisms change the pH of their substrate by producing by-products during growth. They can change conditions such that the environment can no longer support their growth. Yeasts and molds are more tolerant of lower pH than the bacteria and may outgrow them under those conditions.

Light & Chemicals

Ultraviolet light and the presence of chemical inhibitors may also affect the growth of organisms. Many treatments such as hydrogen peroxide and chlorine can kill or injure microbes. Under certain conditions those given a sublethal treatment are injured, but can recover.

Growth

Characteristic growth patterns can be illustrated on a graph. There is a selected portion of the normal growth curve which is referred to as the logarithmic growth phase or the log phase. When cells begin to grow, we usually observe a period of no apparent growth which we refer to as the lag phase. This occurs because cells are making necessary adjustments to adapt. Next we experience the rapid growth or the log phase previously described. As cell mass becomes large, nutrients are exhausted and metabolic byproducts collect. Growth tapers off and the population remains constant for a time. This is referred to as the stationary phase of growth. With no intervention in the system the population will enter a death phase and total numbers of organisms will decline.

Microbial Growth curve

Principle

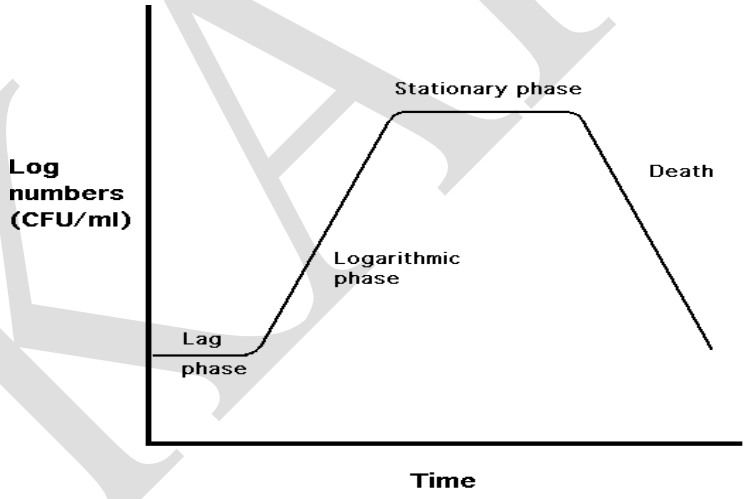
Bacterial population growth studies require inoculation of viable cells into a streile broth medium and incubation of the culture under optimum temperature, PH, and gases conditions. under these conditions , the cells while reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell number versus time of incubation the curve can be used to delineate stages of the growth cycle. It also facilitates measurement of cell numbers and the rate of growth of a particular organism under standardized conditions as expressed by its generation time the time required for a microbial population to double.

1. **Lag phase :** during this stage the cells are adjusting to their new environment cellular metabolism is accelerated , resulting in raid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of the cycle .Although the cells are increasing in size , there is no cell division and therefore no increase in number
2. **Logarithmic (log) phase :** under optimum nutrition and physical conditions the physiologically robust cells reproduce at a uniform and rapid rate by binary fission thus there is a rapid exponential increase in population , which doubles regularly until a maximum number of cells is reached .the time required for the population to doubles is the generation time. The length of the log phase varies, depending on the organism composition of the medium . the average may be estimated to last 6 to 12 hours .
3. **Decline, or death /phase :** because of the continuing depletion of nutrients and buildup of metabolic wastes, the micro organism die at a rapid and uniform rate . the decrease in population closely parallels its increase during the log phase . theoretically, the entire population should die during a time interval equal should die during a time interval equal to that of the log phase. This does not occur, however, since a small number of highly resistant organism persist for an indeterminate length of time.

   Construction of a complete Bacteria growth curve require that aliquots of a 24-4 hours shake –flask culture the inoculation period .such a procedure does not lend itself to a

regular laboratory session. Therefore this experiment follows a modified procedure designed to demonstrate only the lag and log phase. The curve will be plotted on semilog paper by using two values for the measurement of growth. the direct method requires enumeration of viable cells in serially diluted sample of the test culture taken at 30-minute intervals as described in Experiment 19. the indirect method uses spectrophotometric measurement developing turbidity at the same 30- minute intervals, as an index of increasing cells mass.

Determinination of generation time with indirect and direct methods by using data on the growth curve indirect determination is made by simple extrapolation from the log phase..Select two points On the optical density scale , such as 0.2 and 0.4 that represent doubling of turbidity. Using a ruler ; extrapolate by drawing line between each of the selected optical densities on the ordinate (x axis) and the plotted line of the growth curve to their respective time intervals on the abscissa (y axis). With this information, determine the generation time as follows.



Hypothetical bacterial growth curve.

Principle

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic

parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

Culturing

Since normal microbial culturing occurs in atmospheric air, which is an aerobic environment, the culturing of anaerobes poses a problem. Therefore, a number of techniques are employed by microbiologists when culturing anaerobic organisms, for example, handling the bacteria in a glovebox filled with nitrogen or the use of other specially sealed containers, or techniques such as injection of the bacteria into a dicot plant, which is an environment with limited oxygen. The GasPak System is an isolated container that achieves an anaerobic environment by the reaction

of water with sodium borohydride and sodium bicarbonate tablets to produce hydrogen gas and carbon dioxide. Hydrogen then reacts with oxygen gas on a palladium catalyst to produce more water, thereby removing oxygen gas. The issue with the Gaspak method is that an adverse reaction can take place where the bacteria may die, which is why a thioglycollate medium should be used. The Thioglycollate supplies a medium mimicking that of a Dicot, thus providing not only an anaerobic environment but all the nutrients needed for the bacteria to thrive.

Continuous Culture

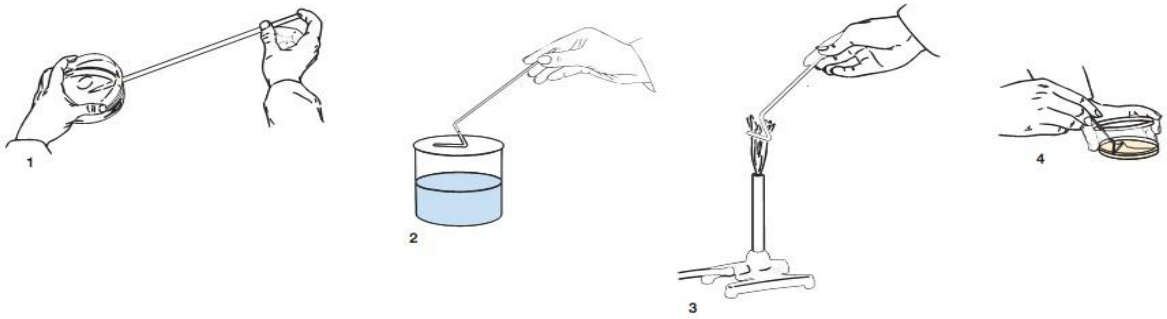
The continuous culture of micro-organisms is a technique of increasing importance in microbiology. The essential feature of this technique is that microbial growth in a continuous culture takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment. Such factors as pH value, concentrations of nutrients, metabolic products and oxygen, which inevitably change during the _ growth cycle ‘ of a batch culture, are all maintained constant in a continuous culture; moreover, they may be independently controlled by the experimenter. These features of the continuous culture technique make it a valuable research tool, while it offers many advantages, in the form of more economical production techniques, to the industrial microbiologist.

Isolation of Pure Cultures

In natural habitats microorganisms usually grow in complex, mixed populations containing several species. This presents a problem for the microbiologist because a single type of microorganism cannot be studied adequately in a mixed culture. One needs a pure culture, a population of cells arising from a single cell, to characterize an individual species. Pure cultures are so important that the development of pure culture techniques by the German bacteriologist Robert Koch transformed microbiology.

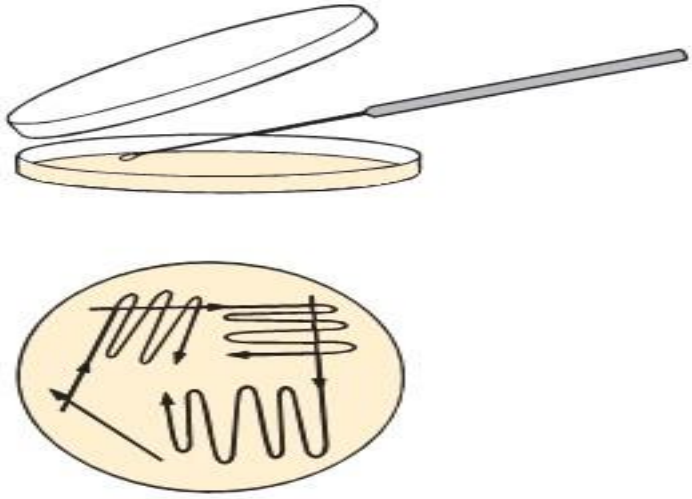
The Spread Plate and Streak Plate

If a mixture of cells is spread out on an agar surface so that every cell grows into a completely separate colony, a macroscopically visible growth or cluster of microorganisms on a solid medium, each colony represents a pure culture. The spread plate is an easy, direct way of achieving this result. A small volume of dilute microbial mixture containing around 30 to 300 cells is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies. Because the number of colonies should equal the number of viable organisms in the sample, spread plates can be used to count the microbial population.



Spread-Plate Technique. The preparation of a spread plate. (1) Pipette a small sample onto the center of an agar medium plate. (2) Dip a glass spreader into a beaker of ethanol. (3) Briefly flame the ethanol soaked spreader and allow it to cool. (4) Spread the sample evenly over the agar surface with the sterilized spreader.

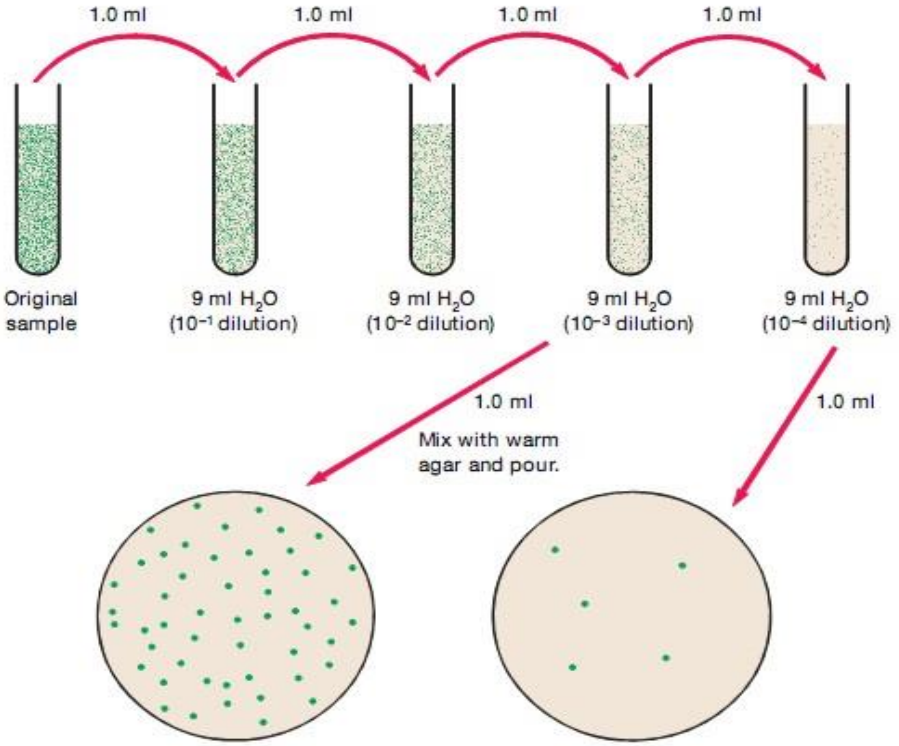
Pure colonies also can be obtained from streak plates. The microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. At some point in the process, single cells drop from the loop as it is rubbed along the agar surface and develop into separate colonies. In both spread-plate and streak-plate techniques, successful isolation depends on spatial separation of single cells.



Streak-Plate Technique. Preparation of streak plates. The upper illustration shows a petri dish of agar being streaked with an inoculating loop. A commonly used streaking pattern is pictured at the bottom.

The Pour Plate

Extensively used with bacteria and fungi, a pour plate also can yield isolated colonies. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes of several diluted samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes. Most bacteria and fungi are not killed by a brief exposure to the warm agar. After the agar has hardened, each cell is fixed in place and forms an individual colony. Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the diluted sample. Colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures.



The Pour-Plate Technique. The original sample is diluted several times to thin out the population sufficiently. The most diluted samples are then mixed with warm agar and poured into petri dishes. Isolated cells grow into colonies and can be used to establish pure cultures. The surface colonies are circular; subsurface colonies would be lenticular or lens shaped.

The preceding techniques require the use of special culture dishes named petri dishes or plates after their inventor Julius Richard Petri, a member of Robert Koch‘s laboratory; Petri developed these dishes around 1887 and they immediately replaced agar-coated glass plates. They consist of two round halves, the top half overlapping the bottom.. Petri dishes are very easy to use, may be stacked on each other to save space, and are one of the most common items in microbiology laboratories

Influence of Environmental factors on growth of microorganisms

The rate of growth or death of a particular microbial species is influenced by a variety of physical factors in its environment including temperature, osmotic pressure, pH, and oxygen concentration.

he major physical factors which affect microbial growth are solutes and water activity, pH, temperature, oxygen level, pressure and radiation.

Solutes and Water activity: Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings. Microorganisms need to keep the osmotic concentration of their cytoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. In a hypertonic environment, the prokaryotes increase their internal osmotic concentration through the synthesis or uptake of choline, proline, glutamic acid and other amino acids. A few prokaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions. The enzymes of these bacteria are altered for the requirement of high salt concentrations for normal activity. Halophiles grow optimally in the presence of NaCl or other salts at a concentration above about 0.2M. These have extensively modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes. They require higher potassium levels for stability and activity. The plasma membrane of halophiles is also stabilized by high concentration of sodium ions.

Water activity (a_w) is the amount of water available to microorganisms and this can be reduced by interaction with solute molecules (osmotic effect). Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's a w is low. Microorganisms differ greatly in their ability to adapt to habitats with low water activity. In a low a w habitat, the microorganisms must expend extra effort to grow as it should maintain a high solute concentration to retain water. Such microorganisms are osmotolerant or can grow over wide range of water activity or osmotic concentration. Most of the microorganisms grow at a w =0.98 or higher.

pH: It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$pH = -\log [H^+] = \log (1/H^+)$

The pH scale ranges from 1.0 to 14.0 and most microorganisms grow vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH values between 9.0 and 10. The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity.*Cyanidium caldarium* (algae) and archaeon *Sulfolobus acidocaldarium* are inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperature. Drastic changes/variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Prokaryotes die if the internal pH drops much below 5.0 to 5.5. External pH alterations also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. The microorganism needs to maintain a neutral cytoplasmic pH and for this the plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkalophiles maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. The antiport systems probably correct small variations in pH. In case of too much acidity (below 5.5 to 6.0) *S. typhimurium* and *E.coli* synthesize an array of new proteins as part of what has been called as their acidic tolerance response. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. Microorganisms can change the pH of their own habitat by producing acidic or basic metabolic waste products. In order to maintain the pH, buffers are often included in the media to prevent growth inhibition. Phosphate is commonly used buffer and a good example of buffering agent. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature:

Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. Beyond a certain point of higher temperature, slow growth takes place and damages the microorganisms by denaturing enzymes, transport carriers and other proteins. The plasma membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don't work rapidly. In summary, when organisms are above their optimum temperature, both the function and cell structure is affected at low temperature, function is affected. The cardinal temperatures vary greatly between microorganisms. Optimum usually range from 0°C to as high as 75°C, where as microbial growth occurs at temperature extending from -20°C to over 120°C. Archaen *Geogemma barossii* grows anaerobically at 121°C. The major microbial groups differ from one another regarding their maximum growth temperature. Upper limit for protozoans is around 50°C, some algae and fungi can grow at temperatures as high as 55°C to 60°C.

Pressure:

Most microorganisms always are subjected to pressure of 10 atmospheres (atm). The hydrostatic pressure can reach to 600 to 1100 atm in the deep sea with temperature about 2°C to 3°C. Organisms can survive and adapt at these extreme conditions and many are barotolerant, increased pressure does adversely affect them but not as much as it does to nontolerant bacteria. The barophillic organisms are those growing in the guts of deep sea invertebrates such as amphipods and holothurians and grow more rapidly at high pressures. These bacteria may play an important role in nutrient recycling in the deep sea. Bacterial genera of *Photobacteria, Shewanella, Colwellia* are barophiles. Some members of the Archaea are thermophiles for example *Pyrococcus spp., Methanococcus janaschii* .

Most organisms on land or on the surface of water is always subjected to a pressure of 1 atm. The hydrostatic pressure can reach 600 to 1100 atm in the deep sea. Despite these extremes, bacteria survive and adapt. Many are barotolerant. Some bacteria in the gut of deep sea

invertebrates such as amphipods and holothurians are truly barophilic and grow more rapidly at high pressures (Ex.*Photobacterium, shewanella, Colwellia*).

Radiation:

Electromagnetic radiation of various types bombards our world. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases – gamma rays and X rays are much more energetic than visible light or infrared waves (Fig. 7). Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation, infrared rays and radio waves. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun as visible light. Many forms of electromagnetic radiation are very harmful to microorganisms. Ionizing radiation, radiation of very short wavelength or high energy can cause atoms to lose electrons or ionize. The two major forms of ionizing radiation, X rays which are artificially produced and gamma rays which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations, higher levels are directly lethal. Some prokaryotes like *Deinococcus radiodurans* and bacterial endospores are resistant and can cause a variety of changes in cells like; it breaks hydrogen bonds, oxidises double bonds, destroys ring structures and polymerizes some molecules. Oxygen enhances these destructive e effects, probably through the generation of hydroxyl radicals (OH.). Destruction of DNA is the most important cause of death of microorganisms. Ultraviolet radiation kills all kinds of microorganisms due to its short wavelength (approximately 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. Formation of thymine dimmers in DNA is the primary mechanism of UV damage; these dimmers inhibit DNA replication and function. This damage is repaired by photo reactivation, where blue light is used by a photo reactivating enzyme (photolyase) to split the thymine dimmers. Dark reactivation, where a short sequence containing the thymine dimmers can also be excised and replaced in the absence of light. Damage can also be repaired by the recA protein in recombination repair and SOS repair.

Visible light:

Visible light when present in sufficieint intensity can damage or kill microbial cells. Pigments called photosensitizers and O_2 are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophull, cytochromes and flavins which absorb light energy, become exited or activated and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O_2 generating singlet oxygen ($_{=}O_2$).

$$P\ (light) \rightarrow P(activated)$$

$$P(activated) + O_2 \rightarrow P + {}^{\cdot}O_2$$

Singlet oxygen is very reactive, powerful oxidizing agent that will quickly destroy a cell. Many microorganisms that are airborne or live on exposed surface use carotenoid pigments for protection against photooxidation. Carotenoids effectively quench singlet oxygen that is absorbing energy from singlet oxygen and convert it back into the unexcited ground state.

Control of microorganisms by physical and chemical agents

Although microorganisms are beneficial and necessary or human well-being, microbial activities have undesirable consequences such as food spoilage and disease. To minimize their destructive effects, it is essential to kill a wide variety of microorganisms orinhibit their growth. The goal is twofold, to destroy pathogens and prevent their transmission and to reduce or eliminate microorganisms responsible for the contamination of water, food and other substances. Sometimes it is necessary to eliminate the microorganisms completely from an object, whereas sometimes only partial destruction may be required in other situations. The control of microbial populations on inanimate objects, like eating utensils, surgical instruments is of considerable importance. **Sterilization –** is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat. When sterilization is achieved by a chemical agent, the chemical is called a sterilant. **Disinfection –** is the killing, inhibition or removal of microorganisms that may cause disease. **Disinfectants** are agents, usually chemical used to carry out disinfection and does not necessarily sterilise an object because viable spores and few microorganisms may remain. **Sanitization** is closed related to disinfection. It is

sometimes necessary to control microorganisms on living tissue with chemical agents. **Antisepsis –** is the prevention of infection or sepsis and is accomplished with **antiseptics.** These chemical agents are applied to living tissue and they prevent infection by killing or inhibiting pathogen growth or they reduce the total microbial population. Substances that kill organisms often have the suffix – cide, **germicide** – kills pathogens but not necessary endospores. A disinfectant or antiseptic can be effective against a specific group and may be called a **bactericide, fungicide, algicide and viricide.** Other chemicals do not kill, but they do prevent growth, and if these are removed, growth will resume. Their names end in – static like, **bacteriostatic** and **fungistatic**

Heat

Heating is still one of the most popular ways to destroy microorganisms. Fire and boiling water have been used since the time of Greeks for sterilization and disinfection. Either moist heat or dry heat may be applied. Moist heat kills viruses, bacteria and fungi. Exposure to boiling water for 10 min is sufficient to kill or destroy vegetative cells and eukaryotic spores., but not enough to kill or destroy bacterial endospores, hence boiling does not sterilize but can be used for disinfection of drinking water and objects not harmed by water. In order to destroy bacterial endospores, moist heat sterilization must be carried out at temperatures above 100°C and this requires the use of saturated steam under pressure. This can be carried out with an **autoclave** (Chamberland, 1884).Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber. Hot, saturated steam enters the chamber and the desired temperature and pressure, usually 121°C and 15 pounds is reached (Fig. 9). At this temperature saturated steam destroys all vegetative cells and endospores. Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.

CLASS : I M. Sc., BT **COURSE NAME: Biochemistry and Microbiology**
BATCH : 2019 – 2021
COURSE CODE : 19BTP101
UNIT : IV (Microbial Diversity and Techniques)

Possible Questions

Part - A

Who is the father of microbiology?
A) Muller
B) Antony van leeuwenhoek
C) Louis pasteur
D) Robert koch

The process of heating liquid s to moderate temperature for a definite time which delays the bacterial growth are known as___?
A) pasteurization
B) sterilization
C) germination
D) fermentation

The efficiency of light microscope lies on its _____
A) magnification power
B) resolving power
C) virtual image
D lens system

In fluorescent microscope_____is used to illuminate the specimen
A) radiation
B) UV light
C) fluroscent
D) probes

_____microscope is used mainly to observe living unstained cells
A) fluorescence
B) light
C) phase contrast
D) electron

Exact composition of defined media is
A) glucose
B) latose
C) biotin
D) yeast extract

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Part – B

1. Comment on _Light microscope‘.
2. What are the advantages of light microscope?
3. What is Bright field microscope?
4. What is TEM?
5. Write short notes on Electron microscope?
6. List the different types of media.
7. What is sterilization?
8. Give a detailed note on isolation of pure culture.

Part – C

1. Describe the function and application of fluorescence microscopy.
2. Write in detail on isolation of pure culture.
3. Give a detailed account on microbial growth curve.
4. Describe the principle and applications of TEM/SEM in a concise manner.
5. Describe principles and applications of any two microscopic techniques.
6. Write a short note on Serial dilution method /Media preparation.
7. What are factors affecting growth of microorganisms?
8. What is staining? Explain in detail about the staining methods.

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CLASS : I M. Sc., BT **COURSE NAME: Biochemistry and Microbiology**
BATCH : 2019 – 2021
COURSE CODE : 19BTP101
UNIT : V (Applications, Diseases and Control measures)

UNIT - V

SYLLABUS

Applications, Diseases and Control measures: Causative agent, pathology, diagnosis, control and treatment of Bacterial - TB, Cholera and Typhoid. Protozoan – Amoebiasis and Malaria. Viral - AIDS. Control of microorganisms – drugs, chemotheraphy, antimicrobial agents.

Microbial Diseases

Tuberculosis (TB)

Tuberculosis came from the Latin word "Tuberculum" which means small swelling , principle. It is also known as Kochs disease, white plague, people plague. It is caused by a bacterium. TB can be present in someone for many years without individual knowing it. The entity of TB became evident only in the 17^{th} and 18^{th} century.

According to estimates of the world Health organisation, in 2000 there were approximately 8 million active cases. Death due to TB occur in 1.5-2million people worldwide each year. It is still a major cause of disease and death and its elimination will be externally difficult and HIV infection characterize large portion of earth.

Cause of disease

Pulmonary tuberculosis is caused by bacteria, ***Mycobacterium tuberculosis*.** The infection may stay asleep or non active ordormant for years.However in some people it can reactivate.

It usually attacks the lungs, but can also affect other part of body. Most people acquires the infection through air borne inhalation of bacilli expelled by another person with active pulmonary disease . Rarely there are cases where in there is transplacental infection. Invasion into blood stream is sufficient for such an infection.

People at higher risk

- Elderly
- Infants
- People with weakened immune system

Factors affecting rate of infection

- Poor nutrition
- Frequent contact with affected people
- Unsanitary living conditions
- Increase in HIV infection
- Appearance of drug resistant strains

Worldwide the most important risk factor is HIV co-infection present in 13% of cases. Tuberculosis is closely linked to both overcrowding and malnutrition making it one of the principle diseases of poverty. Chronic lung disease is a risk factor with smoking more than 20 cigarettes a day, increasing risk by two or four times.

Their types

It is classified in to two:

Pulmonary tuberculosis

- Primary tuberculosis pneumonia
- Tuberculosis pleurisy
- Cairtary tuberculosis
- Miliary tuberculosis
- Laryngeal tuberculosis

Symptoms

- The primary stage of the disease usually doesn‟t cause symptoms.
- Cough
- Excessive sweating, especially at night
- Fever

- Unintentional weight loss
- Breathing difficulty
- Chest pain
- Wheezing

Prevention

Bacilli calmette Guerin (BCG) is the current vaccine for tuberculosis. It was first used in 1921. BCG is the only vaccine available today for protection against tuberculosis . It is most effective in protecting children from the disease. It contains a live attenuated or weakened strain of Mycobacterium bovis. It was originally isolated from a cow with tuberculosis by calmette and Guerin who worked in paris at the Institute Pasteur. This strain was carefully structured every three weeks for many years. After thirteen years strain was seen to be less virulent for animals. In these years some genetic changes occurred and altered the strain. This was called BCG.

BCG is widely used. There is a concern that use of the vaccine in persons who are immune compromised may result in an infection caused by BCG itself.

Diagnosis

Diagnosis relies on radiology, a tuberculin skin test, blood test, as well as microscopic examination and microbiological culture of body fluids.

Adults commonly manifest distinctly symptomatic pulmonary disease that is associated with positive sputum smears and cultures. Examination may show

- Clubbing of fingers or toes
- Fluid around a lung
- Unusual breath sounds

Drugs used

Isoniazid

Ribampicin

Pyrazinamide

Streptomycin

Ethionamide

Cholera

Cholera is caused by the gram-negative ***vibrio cholarae*** bacterium of the family vibrionaceae.

Symptoms

Abdominal muscle cramps, Fever Watery diarrhea and Vomiting.

- Once the bacteria enter the body, the incubation period is from 24 to 72 hours. The bacteria adhere to the intestinal mucosa of the small intestine, where they are not invasive (tending to invade) but secrete choleragen, a cholera toxin.
- The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance and death in some cases,
- An untreated person with cholera may produce 10 to 20 liters of diarrhea for day with fatal results.
- The typical symptoms of dehydration include low blood pressure, poor skin turgor (wrinkled hands). Sunken eyes and a rapid pulse.
- Cholera effects children also, with two to four years old having the highest rates of infection.
- Cholera is also affected by their blood type, those with type „O" blood being the most susceptible.
- Persons with lower immunity, such as persons with AIDS of children who are malnourished, are more likely to experience a severe case if they became infected.

- Cholera has been found in only two other animal populations.
    a) Shell fish and
    b) Plankton.
- Cholera is rarely spread directly from person to person.
- Cholera has been nick named as "blue death" due to a patient"s skin turning a bluish-grey colour from extreme loss of fluids.
- Transmission is primarily by the fecal contamination of food and water caused by poor sanitation.

Treatment

There are several points along the cholera transmission path at which its spread may be halted.

I) Sterilization

- Proper disposal and treatment of infected faecal waste water produced by cholera victims and all contaminated materials (e.g: clothing bedding etc) are essential.
- All material that comes in contact with cholera patients should be sanitized by washing in hot water using chlorine, bleach is possible.

II) Sewage

Antibacterial treatment of general sewage by chlorine, ozone, ultraviolet light or other effective treatment before it enters the water ways or underground water supplies helps prevent undiagnosed patients form inadvertently spreading the disease.

III) Water purification

All water used for drinking water, washing or cooking should be sterilized by either boiling, chlorination, ozone water treatment, ultraviolet light sterilization (e.g: by solar water disinfection) or antimicrobial filtration in any area where cholera may be present.

Prevention

Prevention of the disease is normally straight forward if proper sanitation practices are followed.

Antibiotics

Antibiotic treatment for one to three days shorter the course for the disease and reduce the severity of the symptoms.

Few antibiotics are,- Doxycyline, Cotrimoxazole, Erythromycin, Tetracycline, Chloramphenicol, Furazolidone and Norfloxacin used.

Typhoid

Typhoid [Greek „typhodes" means smoke fever is caused by several virulent strains of *salmonella typhi.*

- It is acquired by ingestion of food or water contaminated by feces of infected humans or animals.
- In the small intestine the incubation period is about 10 to 14 days.
- This bacteria spread to the lymphoid tissue, blood, liver and gall bladder.

Symptoms

- Red colored spots may appear
- Fever, sweating
- Headache
- Abdominal pain
- Anorexia
- Malaise

Various names for typhoid,

Gastric fever, abdominal typhus, Slow fever, Nervous fever

- Typhoid fever is characterized by a slowly progressive fever as high as 40°C.
- The bacteria which causes typhoid fever may be spread through poor hygiene habits and public sanitation conditions at sometimes also by flying insects.

- A person may become an asymptomatic carrier of typhoid fever, suffering no symptoms, but capable of infecting others.

Diagnosis

Diagnosis is made by any blood, bone marrow or stool cultures and with the widal test.

Purification

- Chlorination of drinking water had led to dramatic decreases in the transmission of typhoid fever.
- Purification of drinking water, milk pasteurization and prevention of food handling by carriers are the most successful measures.
- Sanitation and hygiene are the critical measures that can be taken to prevent typhoid.
- Typhoid does not affect animals and therefore transmission is only from human to human.
- Antibiotics, such as Amphicillin, chloramphenicol, amoxicillin and ciprofloxacin have been commonly used to treat typhoid fever in developed countries.
- Typhoid fever in most cases is not fatal. When untreated, typhoid fever persists for 3 weeks to a month. Death occurs in between 10% and 30%..

Protozoan

Amoebiasis

Amoebiasis, or **amebiasis**, refers to infection caused by the amoeba *Entamoeba histolytica*. The term *entamoebiasis* is occasionally seen but is no longer in use; it refers to the same infection. A gastrointestinal infection that may or may not be symptomatic and can remain latent in an infected person for several years, amoebiasis is estimated to cause 70,000 deaths per year worldwide.

Symptoms can range from mild diarrhea to dysentery with blood and mucus in the stool. *E. histolytica* is usually a commensal organism. Severe amoebiasis infections (known as invasive

or *fulminant*amoebiasis) occur in two major forms. Invasion of the intestinal lining causes amoebic dysentery or amoebic colitis. If the parasite reaches the bloodstream it can spread through the body, most frequently ending up in the liver where it causes amoebic liver abscesses. Liver abscesses can occur without previous development of amoebic dysentery. When no symptoms are present, the infected individual is still a carrier, able to spread the parasite to others through poor hygienic practices. While symptoms at onset can be similar to bacillary dysentery, amoebiasis is not bacteriological in origin and treatments differ, although both infections can be prevented by good sanitary practices.

Signs and symptoms

Most infected people, about 90%, are asymptomatic, but this disease has the potential to make the sufferer dangerously ill. It is estimated that about 40,000 to 100,000 people worldwide die annually due to amoebiasis.

Infections can sometimes last for years. Symptoms take from a few days to a few weeks to develop and manifest themselves, but usually it is about two to four weeks. Symptoms can range from mild diarrheato severe dysentery with blood and mucus. The blood comes from lesions formed by the amoebae invading the lining of the large intestine. In about 10% of invasive cases the amoebae enter the bloodstream and may travel to other organs in the body. Most commonly this means the liver, as this is where blood from the intestine reaches first, but they can end up almost anywhere in the body.

Onset time is highly variable and the average asymptomatic infection persists for over a year. It is theorised that the absence of symptoms or their intensity may vary with such factors as strain of amoeba, immune response of the host, and perhaps associated bacteria and viruses.

In asymptomatic infections the amoeba lives by eating and digesting bacteria and food particles in the gut, a part of the gastrointestinal tract. It does not usually come in contact with the intestine itself due to the protective layer of mucus that lines the gut. Disease occurs when amoeba comes in contact with the cells lining the intestine. It then secretes the same substances it uses to digest bacteria, which include enzymes that destroy cell membranes and proteins. This process can lead to penetration and digestion of human tissues, resulting first in flask-

shaped ulcers in the intestine. *Entamoeba histolytica* ingests the destroyed cells by phagocytosis and is often seen with red blood cells (a process known as erythrophagocytosis) inside when viewed in stool samples. Especially in Latin America, a granulomatous mass (known as an amoeboma) may form in the wall of the ascending colon or rectum due to long-lasting immunological cellular response, and is sometimes confused with cancer.

Cause

Amoebiasis is an infection caused by the amoeba Entamoeba histolytica Likewise **amoebiasis** is sometimes incorrectly used to refer to infection with other amoebae, but strictly speaking it should be reserved for Entamoeba histolytica infection. Other amoebae infecting humans include:

- Parasites
  - *Dientamoeba fragilis,* which causes Dientamoebiasis
  - *Entamoeba dispar*
  - *Entamoeba hartmanni*
  - *Entamoeba coli*
  - *Entamoeba moshkovskii*
  - *Endolimax nana and*
  - *Iodamoeba butschlii.*

Except for Dientamoeba, the parasites above are not thought to cause disease.

- Free living amoebas. These species are often described as "opportunistic free-living amoebas" as human infection is not an obligate part of their life cycle.
  - *Naegleria fowleri,* which causes Primary amoebic *meningoencephalitis*
  - *Acanthamoeba,* which causes Cutaneous amoebiasis and *Acanthamoeba keratitis*
  - *Balamuthia mandrillaris*, which causes Granulomatous amoebic encephalitis and Primary amoebic meningoencephalitis
  - *Sappinia diploidea*

Transmission

Amoebiasis is usually transmitted by the fecal-oral route, but it can also be transmitted indirectly through contact with dirty hands or objects as well as by anal-oral contact. Infection is spread through ingestion of the cyst form of the parasite, a semi-dormant and hardy structure found in feces. Any non-encysted amoebae, or trophozoites, die quickly after leaving the body but may also be present in stool: these are rarely the source of new infections. Since amoebiasis is transmitted through contaminated food and water, it is often endemic in regions of the world with limited modern sanitation systems, including México, Central America, western South America, South Asia, and western and southern Africa.

Amoebic dysentery is often confused with "traveler's diarrhea" because of its prevalence in developing nations. In fact, most traveler's diarrhea is bacterial or viral in origin.

Diagnosis

Asymptomatic human infections are usually diagnosed by finding cysts shed in the stool. Various flotation or sedimentation procedures have been developed to recover the cysts from fecal matter and stains help to visualize the isolated cysts for microscopic examination. Since cysts are not shed constantly, a minimum of three stools should be examined. In symptomatic infections, the motile form (the trophozoite) can often be seen in fresh feces. Serological tests exist and most individuals (whether with symptoms or not) will test positive for the presence of antibodies. The levels of antibody are much higher in individuals with liver abscesses. Serology only becomes positive about two weeks after infection. More recent developments include a kit that detects the presence of amoeba proteins in the feces and another that detects ameba DNA in feces. These tests are not in widespread use due to their expense.

Microscopy is still by far the most widespread method of diagnosis around the world. However it is not as sensitive or accurate in diagnosis as the other tests available. It is important to distinguish the *E. histolytica* cyst from the cysts of nonpathogenic intestinal protozoa such as *Entamoeba coli* by its appearance. *E. histolytica* cysts have a maximum of four nuclei, while the commensal *Entamoeba coli* cyst has up to 8 nuclei. Additionally, in *E. histolytica*, the endosome is centrally located in the nucleus, while it is usually off-center

in *Entamoeba coli*. Finally, chromatoidal bodies in *E. histolytica* cysts are rounded, while they are jagged in *Entamoeba coli*. However, other species, *Entamoeba dispar* and *E. moshkovskii*, are also commensals and cannot be distinguished from *E. histolytica* under the microscope. As *E. dispar* is much more common than *E. histolytica* in most parts of the world this means that there is a lot of incorrect diagnosis of *E. histolytica* infection taking place. The WHO recommends that infections diagnosed by microscopy alone should not be treated if they are asymptomatic and there is no other reason to suspect that the infection is actually *E. histolytica*. Examination of stools under microscope. May need several samples over several days to determine if Amebiasis is present because it may not show up in every sample.

Typically, the organism can no longer be found in the feces once the disease goes extra-intestinal. Serological tests are useful in detecting infection by *E. histolytica* if the organism goes extra-intestinal and in excluding the organism from the diagnosis of other disorders. An Ova & Parasite (O&P) test or an *E. histolytica* fecal antigen assay is the proper assay for intestinal infections. Since antibodies may persist for years after clinical cure, a positive serological result may not necessarily indicate an active infection. A negative serological result however can be equally important in excluding suspected tissue invasion by *E. histolytica*.

Prevention

To help prevent the spread of amoebiasis around the home :

- Wash hands thoroughly with soap and hot running water for at least 10 seconds after using the toilet or changing a baby's diaper, and before handling food.
- Clean bathrooms and toilets often; pay particular attention to toilet seats and taps.
- Avoid sharing towels or face washers.

To help prevent infection:

- Avoid raw vegetables when in endemic areas, as they may have been fertilized using human feces.
- Boil water or treat with iodine tablets.
- Avoid eating street foods especially in public places where others are sharing sauces in one container

Good sanitary practice, as well as responsible sewage disposal or treatment, are necessary for the prevention of*E.histolytica* infection on an endemic level. *E.histolytica* cysts are usually resistant to chlorination, therefore sedimentation and filtration of water supplies are necessary to reduce the incidence of infection.

E. histolytica cysts may be recovered from contaminated food by methods similar to those used for recovering*Giardia lamblia* cysts from feces. Filtration is probably the most practical method for recovery from drinking water and liquid foods. *E. histolytica* cysts must be distinguished from cysts of other parasitic (but nonpathogenic) protozoa and from cysts of free-living protozoa as discussed above. Recovery procedures are not very accurate; cysts are easily lost or damaged beyond recognition, which leads to many falsely negative results in recovery tests.

Treatment

E. histolytica infections occur in both the intestine and (in people with symptoms) in tissue of the intestine and/or liver. As a result, two different classes of drugs are needed to treat the infection, one for each location. Such anti-amoebic drugs are known as amoebicides.

Malaria

Causative Agent

Malaria is a mosquito borne infection disease of humans and other animals caused by eukaryotic protests of the gene plasmodium. The disease results from the multiplication of plasmodium parasite with in RBC .in man, the infection takes place by the inoculation of the slender, sickle shaped nucleated sporozoid in the blood by the bite of an infected female mosquito belonging to the genus Anopheles. At least four species of plasmodium, P.vivax, P.falciparum, P.malariae and P.ovale are known to attack man causing different kinds of malaria.

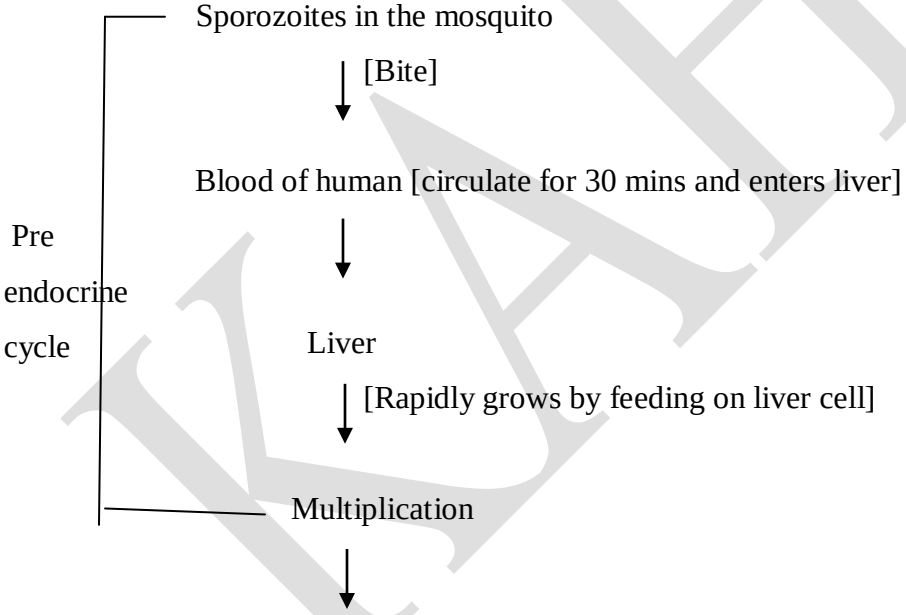
Pathology

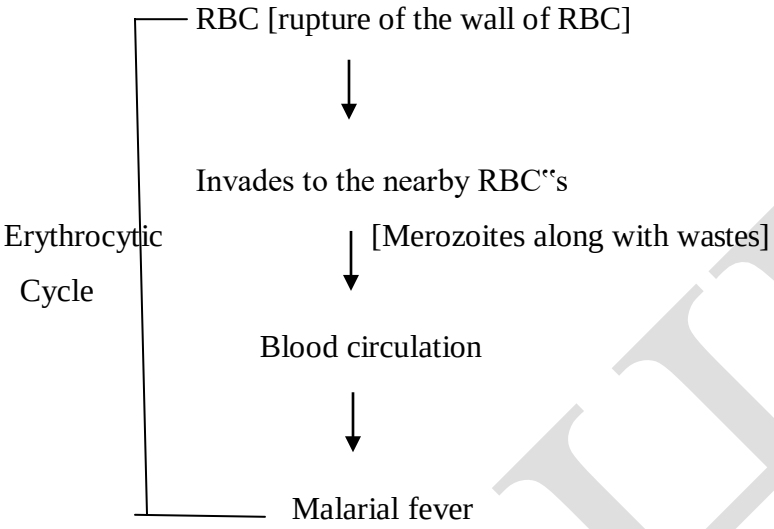
The life cycle of malaria parasite involves two hosts, the man and the mosquito. In man mode of reproduction is a sexual and in mosquito it is sexual.

There are two phases in the life cycle of malaria parasite in man. They are

- Pre erythrocytic cycle or exoerythrocytic cycle (in liver cells).
- Erythrocytic cycle or endo erythrocytic cycle (inside the red blood corpuscles)

Symptoms typically include fever and headache. Other symptoms include arthralgia (joint pain), jaundice, hemoglobinuria, renal damage and conclusions. The classic symptoms of malaria are cyclical coldness followed by rigor and the fever and sweating lasting from 4 to 6 hours. It also causes kidney failure, anemia, oedema, lactic acidosis, hypokalimia etc. Another name for malaria is black water fev





Diagnosis

The mainstay of malaria diagnosis has been the microscopic examination of blood, utilizing blood films. Although blood is the sample most frequently used to make a diagnosis, both saliva and urine have been investigated as alternative, less invasive specimens.

Modern techniques utilizing antigen tests or polymerase chain reaction have been discovered, though these are not widely implemented in malaria endemic region. Peripheral smear for malaria parasite is also one method of diagnosis.

Treatment

No vaccine for malaria is still not found. It can only be treated with drugs that may kill all stages of the parasite without affecting the patient. Chloroquine may be used where the parasite is still sensitive. Other drugs like quinine, Paludrine, Atabrin, Camoquin, Resochin, Pamaquin etc are used as suppressants of various stages of parasites.

Control

Malaria transmission can be reduced by preventing mosquito bites by distributions of mosquito nets, insect‟s repellents or by mosquito control measures such as spraying insecticides and draining standing water.

Acquired Immune Deficiency Syndrome (AIDS)

It is now recognized that AIDS (acquired immune deficiency syndrome) is the first great pandemic of the second half of the twentieth century. First described in 1981 AIDS is the result of an infection by the human immunodeficiency virus (HIV), a lentivirus within the family Retroviridae. The disease appears to have begun in central Africa as early as the 1950s; HIV may have developed in the human population in the 1930s, or even earlier. Simian immunodeficiency viruses (SIVs) related to HIV-1 and HIV-2, the strains primarily responsible for AIDS, have been isolated from African primates. The SIV from chimpanzees seems to have infected humans and developed into HIV-1; HIV-2 may have arisen from the SIV that infects sooty mangabeys. Once established, HIV-1 spread to the Caribbean and then to the United States and Europe. Epidemiologically AIDS occurs worldwide. The groups most at risk in acquiring AIDS are (in descending order of risk) homosexual/bisexual men; intravenous (IV) drug users; heterosexuals who have intercourse with drug users, prostitutes (sex trade workers), and bisexuals; transfusion patients or hemophiliacs who must receive clotting factor preparations made from donated blood; and children born of infected mothers. The mortality rate from AIDS is extremely high.

HIV-1 is an enveloped lentivirus and a member of the family Retroviridae with a cylindrical core inside its capsid. The core contains two copies of its plus single-stranded RNA genome and several enzymes. Thus far 10 virus-specific proteins have been discovered. One of them, the gp120 envelope protein, participates in HIV-1 attachment to CD4 cells.

The AIDS virus is acquired by direct exposure of a person‟s bloodstream to body fluids (blood, semen, vaginal secretions) containing the virus, through sexual contact, or perinatally from an infected mother to her fetus. It also is possible that a newborn can be infected through breast-feeding. Once inside the body, the virus gp120 envelope protein binds to the CD4 glycoprotein plasma membrane receptor on CD4 T cells, macrophages, dendritic cells, and

monocytes. (Dendritic cells are present throughout the body‟s mucosal surfaces and bear the CD4 protein. Thus it is possible that these are the first cells infected by HIV in sexual transmission.) Recent evidence shows that the virus requires a coreceptor in addition to the CD4 receptor. Macrophage-tropic strains, which seem to predominate early in the disease and infect both macrophages and T cells, require the CCR5 (CC-CKR-5) chemokine receptor protein as well as CD4. A second chemokine coreceptor, called CXCR-4 or fusin, is T cell–tropic and used by an HIV strain that is active at later stages of the infection. This strain induces the formation of syncytia, as described later. Individuals with two defective copies of the CCR5 gene do not seem to get AIDS; apparently the virus cannot infect their T cells. People with one good copy of the CCR5 gene do get AIDS but survive several years longer than those with no mutation. Host cellnreceptors and virion adsorption (pp. 399–403); Replication and transcription inn retroviruses.

Entry into the host cell begins when the envelope fuses with the plasma membrane, and the virus releases its core and two RNA strands into the cytoplasm. Inside the infected cell, the core protein remains associated with the RNA as it is copied into a single strand of DNA by the RNA/DNA-dependent DNA polymerase activity of the reverse transcriptase enzyme. The RNA is next degraded by another reverse transcriptase component, ribonuclease H, and the DNA strand is duplicated to form a double-stranded DNA copy of the original RNA genome. A complex of the double-stranded DNA (the provirus) and the integrase enzyme moves into the nucleus. Then the proviral DNA is integrated into the cell‟s DNA through a complex sequence of reactions catalyzed by the integrase. The integrated provirus can remain latent, giving no sign of its presence. Alternatively the provirus can force the cell to synthesize viral mRNA. Some of the RNA is translated to produce viral proteins by the cell‟s own ribosomes. Viral proteins and the complete HIV-1 RNA genome are then assembled into new virions that bud from the infected host cell. Eventually the host cell lyses.

CLASS : I M. Sc., BT **COURSE NAME: Biochemistry and Microbiology**
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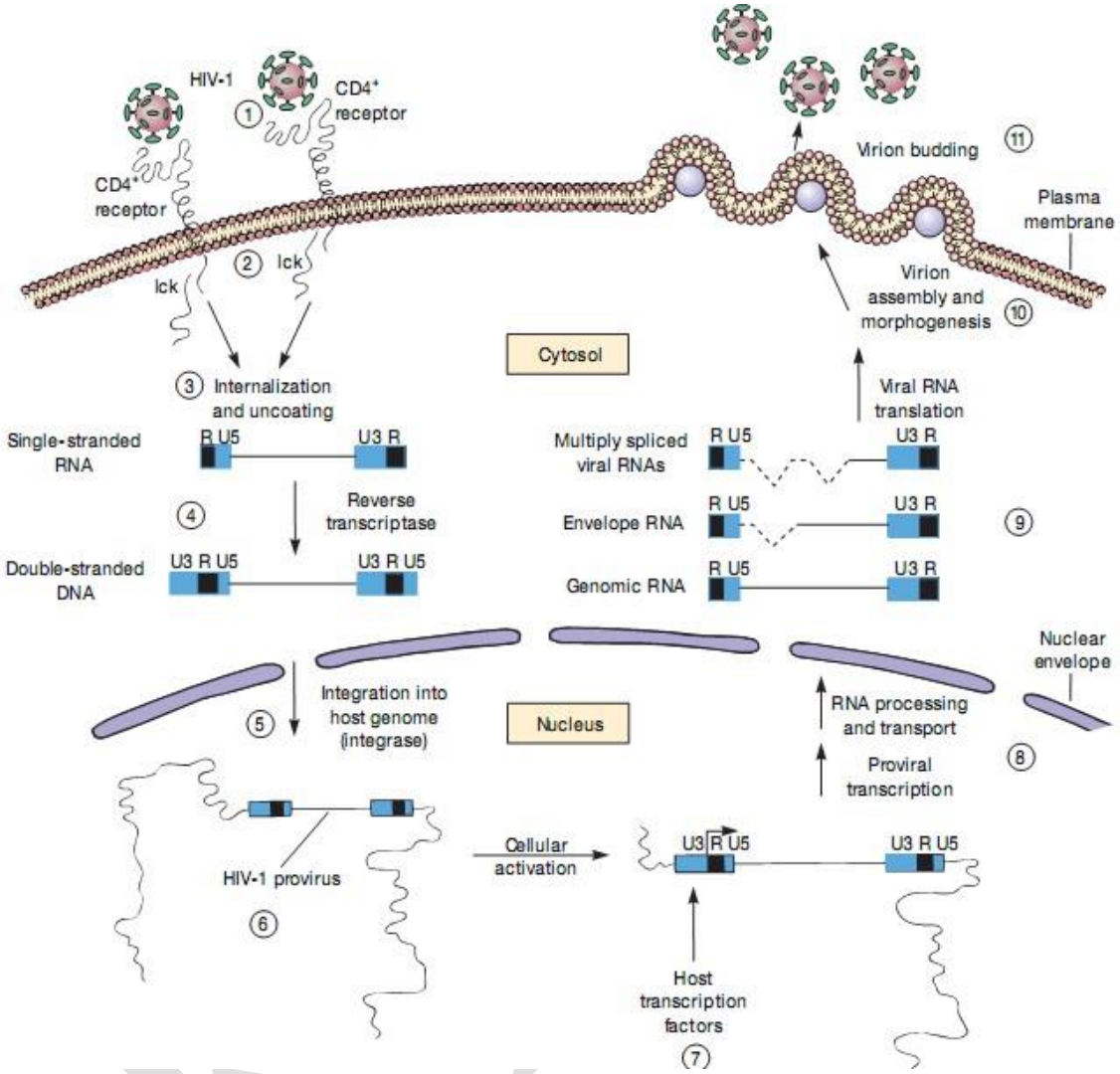
UNIT : V (Applications, Diseases and Control measures)



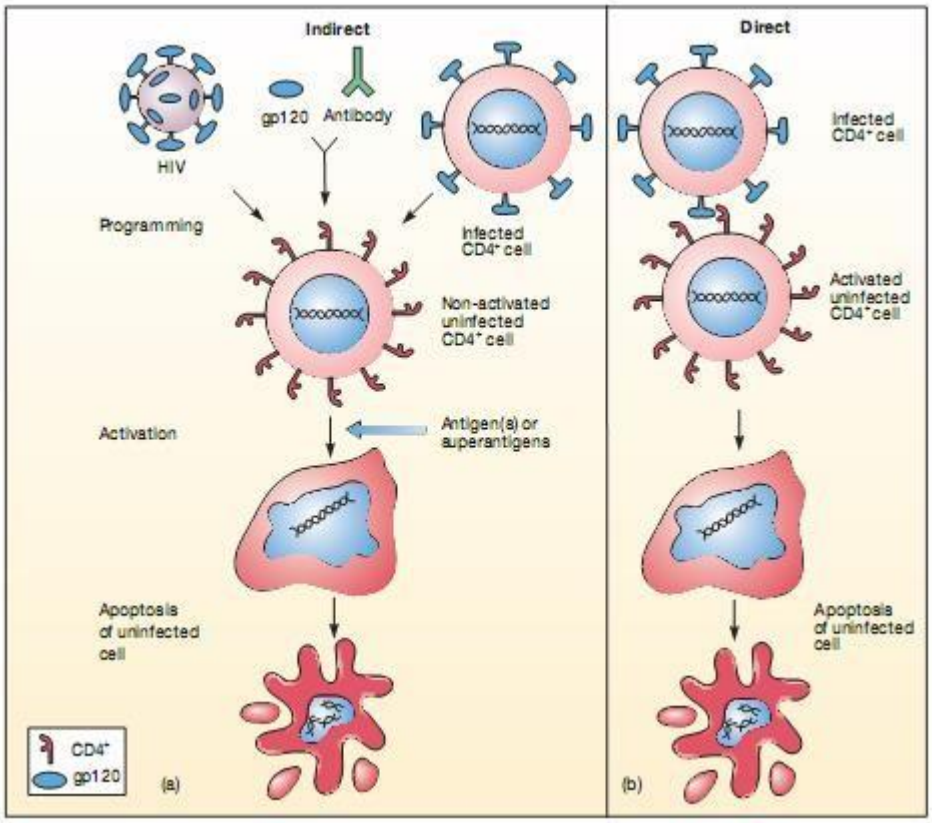
Life Cycle of HIV-1. (1) After interaction of gp120 with the CD4 cell plasma membrane receptor, gp41-mediated membrane fusion occurs. (2) This leads to the entry of HIV-1 into the cell. The lck denotes a lymphoid-specific tyrosine kinase that binds to CD4. (3) After internalization and uncoating, reverse transcription of viral RNA begins. (4) The double-stranded DNA form of the virus genome is produced in the presence of appropriate host factors. (5) The HIV-1 integrase promotes the insertion of this viral DNA duplex into the CD4 cell's genome after the DNA has entered the nucleus. (6) This gives rise to the HIV-1 provirus. (7) The expression of the HIV-1 gene is stimulated initially by the action of

specific inducible and constitutive host transcription factors with binding sites in the long terminal repeat. Their binding leads to the sequential production of various viral mRNAs. (8) The first mRNAs produced correspond to the multiply spliced species of approximately 2.0 kilobases encoding tat, rev, and nef regulatory proteins. (9) Subsequently the viral structural proteins are produced, allowing the (10) assembly and morphogenesis of the virions. (11) The new HIV-1 virions that are produced by viral budding from the host CD4 cell can then reinitiate the retroviral life cycle by infecting other CD4 target cells.

Second, a true case of AIDS can develop directly upon infection. The mean interval between HIV infection and the onset of AIDS appears to be about 8 to 10 years, although it varies considerably with each individual. At first, a person‟s immune system responds to the HIV-1 infection by manufacturing HIV-1 antibodies, but not in sufficient quantities to stop the viral attack. The virus becomes established within primarily CD4 T-helper cells, and HIV accumulates in lymphoid organs in large quantities even before symptoms appear. Initially, CD4 T-helper cells proliferate abnormally in the lymph nodes. Thereafter the lymph nodes‟ internal structure collapses due to viral replication. This leads to a decline in the number of lymphocytes within the lymph nodes and results in a selective depletion of the CD4 T-cell subset that is critical to the propagation of the entire T-cell pool. When this CD4 population declines, interleukin-2 (IL-2) production also decreases. Because IL-2 stimulates the production of T cells in general, the whole T-cell population may decline. This leaves the infected person open to opportunistic infections: invasion by pathogens that proliferate widely only because the immune system is defective.

It should be noted that factors other than direct T-cell destruction also may be involved in AIDS pathogenesis. HIV may reduce the immune response by destroying or disabling dendritic cells, which present foreign antigens to T cells. HIV also mutates exceptionally rapidly and thus could evade and eventually overwhelm the immune system. HIV may disrupt the balance between different types of T-helper cells and consequently decrease the
killer T-cell population. It is possible that several different mechanisms contribute to T-cell destruction.

New findings suggest still another potential mechanism for the depletion of CD4 cells . In HIV-infected individuals the loss of CD4 cells is associated with lymphocyte activation; however, this activation does not result in cell proliferation, as it does normally, but rather in cell death by a mechanism known as programmed cell death.



Apoptosis and AIDS. Apoptosis is a homeostatic physiological suicide mechanism in which cell death occurs naturally during normal tissue turnover. Usually apoptosis occurs after activation of a calciumdependent endogenous endonuclease. Cells undergoing apoptosis display profound structural changes such as a decrease in cell volume, blebbing of the plasma membrane, and nuclear fragmentation. The nuclear DNA is cleaved into short oligonucleosomal length DNA fragments. The dying cell sheds small membrane-bound apoptotic bodies, which are phagocytosed and digested. (a) There may be several ways in which an HIV infection can

indirectly trigger apoptosis. In all cases an initial event would program or prime the target cell so that apoptosis would be triggered by the binding of antigens or superantigens to the cell‟s T-cell receptors. Possibly the external gp120 envelope glycoprotein of the HIV virion binds to the CD4 protein on lymphocytes and programs the lymphocyte. A combination of free gp120 and antibodies to gp120 also could stimulate programmed cell death. First, the gp120 would bind to CD4 receptors. Then antibodies would attach to the gp120 and cause clustering of the receptors, thus priming the uninfected CD4 cell. It also is possible that binding of the infected cell‟s surface gp120 proteins to the CD4 receptors on an uninfected cell will program the uninfected cell for apoptosis in response to antigens. (b) Apoptosis may be directly triggered in an uninfected cell. The gp120 envelope proteins on the surface of an infected cell may combine with the CD4 proteins of an uninfected cell and directly stimulate programmed cell death without activation by antigens.

Disease Processes Associated with AIDS

Candidiasis of bronchi, trachea, or lungs
Candidiasis, esophageal
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cyclospora, diarrheal disease
Cytomegalovirus disease (other than liver, spleen, or lymph nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex: chronic ulcer(s) (>1 month's duration); or bronchitis, pneumonitis, or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *M. kansasii*
Mycobacterium tuberculosis, any site
Mycobacterium, other species or unidentified species
Pneumocystis carinii pneumonia
Pneumonia, recurrent
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to AIDS

Control of Microorganisms

Antimicrobial agents and Chemotherapy

Adult mosquito can be most effectively controlled by spraying DDT, malalhion or any other insecticide in the house; fumigating pyrethrum, cresol and other compounds of naptha, sterilization of male mosquitoes. The young stages of mosquito can be controlled by introducing larvivorous fishes like Gambusia and Lebistes in ponds, lakes, canals and tanks. An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms, replacing heavy metals and toxins and may someday be a viable alternative. Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting it is considered "community acquired".

Main classes

There are mainly two classes of antimicrobial drugs:

1. Those obtained from natural sources:
    1. Beta-lactam antibiotic (such as penicillins, cephalosporins)
    2. Protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides)
2. Synthetic agents:
    1. Sulphonamides, cotrimoxazole, quinolones
    2. Anti-virals
    3. Anti-fungals
    4. Anti-cancer drugs
    5. Anti-malarials
    6. Anti-tuberculosis drugs
    7. Anti-leprotics
    8. Anti-protozoals

Control of Microorganisms - Drugs

Antibiotics

Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. However, prolonged use of certain antibiotics can decrease the number of gut flora, which can have a negative impact on health. Some recommend that, during or after prolonged antibiotic use, one should consume probiotics and eat reasonably to replace destroyed gut flora.

The term antibiotic originally described only those formulations derived from living organisms, but is now applied also to synthetic antimicrobials, such as the sulfonamides. The discovery, development, and clinical use of antibiotics during the 20th century has decreased substantially the mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. Paralleled to this there has been an alarming increase in bacterial resistance to existing agents.

Antibiotics are among the most commonly used drugs. For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy.[*citation needed*] However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory tract infections. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Possible strategies towards this objective include the increased sampling from diverse environments and application of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms as well as the development of small-molecule libraries customized for bacterial targets.

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host, and therefore can be used to treat infections. They should be distinguished from viricides, which actively deactivate virus particles outside the body.

Many of the antiviral drugs available are designed to treat infections by retroviruses, mostly HIV. Important antiretroviral drugs include the class of protease inhibitors. Herpes viruses, best known for causing cold sores and genital herpes, are usually treated with the nucleoside analogue acyclovir. Viral hepatitis (A-E) are caused by five unrelated hepatotropic

viruses and are also commonly treated with antiviral drugs depending on the type of infection. influenza A and B viruses are important targets for the development new influenza treatments to overcome the resistance to existing neuraminidase inhibitors such as oseltamivir.

Antiviral drugs work by inhibiting the virus before it enters the cell, stopping it from reproducing, or, in some cases, preventing it from exiting the cell. However, like antibiotics, viruses may evolve to resist the antiviral drug.

Antifungals

An antifungal drug is medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly.

Antimicrobials and Home Mold remediation - Anti-fungal treatments are frequently sought-after to treat mold growth in damp or wet home materials that exhibit mold growth. Note that most home mold problems are moisture/water-caused and the solution for conquering the mold growth is most dependent upon the water/moisture control and removal/discarding of the mold-damaged materials. Cleaning mold-damaged materials may result in a visually acceptable appearance but most cleaning methods do not kill mold or prevent its return. For this reason, moisture management is the primary focus for mold prevention. Generally, Relative Humidity levels in the home above 54% will support mold growth on most cellulose containing materials (fabrics, carpeting and carpet backing, wood, paper, boxes, dust and lint). Mold also readily grows on most latex paints and leather.

Antimicrobials used in home remediation follow a variety of chemistry and functions. One common method of mold remediation utilizes Sodium Bicarbonate (Baking soda) as a blasting medium much in the way that sand is used to blast (clean) surfaces under the propulsion

of compressed air. This 'Soda Blasting' makes a large cloud of dust that is pH Alkalai and its residue that permeates the wood and painted surfaces is also naturally antimicrobial as a result of the high pH and the presence of Sodium Bicarbonate. If used alone, repeated wetting can wash away the Sodium Bicarbonate residue and mold can return to the materials if the water source is not managed. Dry ice (frozen Carbon Dioxide (CO2)) is also used as a blasting agent where clean-up is more restrictive (i.e. attics) and while the dry ice blasting leaves no antimicrobial residue, it does prep the blasted surface to receive one as a secondary step. One popular, professional anti-microbial (Serum) is often applied after or without blasting by soda or dry ice and in one of its variants, is a mix of Hydrogen Peroxide and thin surface coating that neutralizes the mold (making it non-viable) and encapsulating the surface to prevent spore release. Other anti-microbial surface treatments typically contain variants of metals known to suppress mold growth; i.e. pigments or solutions involving Copper, Silver, Zinc or other metals (some of which can be toxic to humans if improperly applied). Most antimicrobial solutions are professionally applied and are not sold to the public.

Many people use either white vinegar or laundry bleach as an inexpensive anti-microbial solution. These liquids are best used in combination and they can be safely combined. When combined in equal amounts (i.e. 2 cups bleach + 2 cups vinegar in 2 gallons of warm water + 2 drops of dish soap), these make a solution known as "Acidified Bleach" and it is considerably more effective as an antimicroboial and as a disinfectant. On porous foundation surfaces, a secondary step of scrubbing with Tri-Sodium Phosphate (TSP) or Spic-N-Span is capable of leaving behind a phosphate or carbonate reside that will be antimicrobial. The key to successful foundation cleaning with longer-lasting results hinges on leaving the cleaner residue on the wall and force-drying the area with a fan to drive off the water. Long-term success requires that the water source be corrected and that the area's relative humiidity be kept as far below 54% as possible.

Antimicrobials and paints - Kitchen and Bath paint formulations are often manufactured with the understanding that these areas are often cleaned and may experience elevated humidity levels from bathing/cooking. As a result, leading manufacturers often design Kitchen and Bath

formulations to be less porous, more scrubable, and often, the paint formulation is adjusted to be more antimicrobial than other interior paints. During color mixing, some stores also offer the option of buying and adding an antimicrobial packet to the paint as a booster. It is ill-advised to use a normal latex paint on a foundation since this naturally moist area will readily cause mold to grow on latex. Foundation painting should be done with products designed for that purpose and when applied over virgin masonry, these products perform very well.

Antiparasitics

Antiparasitics are a class of medications which are indicated for the treatment of infection by parasites, such as nematodes, cestodes, trematodes, infectious protozoa, and amoebae. Like antifungals, they must kill the infecting pest without serious damage to the host.

Non-pharmaceutical antimicrobials

Antimicrobials wide range of chemical and natural compounds are used as antimicrobials. Organic acids are used widely as antimicrobials in food products, e.g. lactic acid, citric acid, acetic acid, and their salts, either as ingredients, or as disinfectants. For example, beef carcasses often are sprayed with acids, and then rinsed or steamed, to reduce the prevalence of E. coli O157:H7.

Traditional healers long have used plants to prevent or cure infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity, and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. So, it is worthwhile to study plants and plant products for activity against resistant bacteria.

Copper-alloy surfaces have natural intrinsic properties to effectively and quickly destroy microbes, including *E. coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus, Clostridium difficile*, influenza A virus, adenovirus, and fungi. The United States Environmental Protection Agency has approved the registration of 355 different antibacterial copper alloys that kill *E. coli* O157:H7, *methicillin*-resistant *Staphylococcus aureus*

(*MRSA*), *Staphylococcus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* in less than 2 hours of contact. As a public hygienic measure in addition to regular cleaning, antimicrobial copper alloys are being installed in healthcare facilities and in a subway transit system.

Essential oils

The antimicrobial properties of 21 plant essential oils and two essences were investigated against five food-borne pathogens, *Campylobacter jejuni, Salmonella enteriditis, Escherichia coli, Staphylococus aureus and Listeria monocytogenes*. The oils of bay, cinnamon, clove and thyme were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all five pathogens. (A. Smith-Palmer, J. Stewart and L. Fyfe. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters in Applied Microbiology 1998. 26. 118-122)

Many essential oils are included in pharmacopoeias as having antimicrobial activity, including:

- Cinnamon oil
- Clove oil - stomatology etc.
- Eucalyptus oil
- Garlic
- Oregano oil
- Lavender oil
- Leleshwa oil
- Lemon oil
- Lemon myrtle oil
- Mint oil - in medicine, cosmetics (tooth paste etc.)
- Neem oil
- Nigella sativa (Black cumin) oil Onion oil (*Allium cepe*) - phytoncides, in phytotherapy
- Peppermint oil
- Sandalwood oil - in cosmetics
- Sideritis or Greek Mountain Tea
- Tea tree oil - in cosmetics, medicine and Thyme oil

Metal ions

Many heavy metal cations such as Hg^{2+}, Cu^{2+}, and Pb^{2+} have antimicrobial activities, but are also very toxic to other living organisms, thus making them unsuitable for treating infectious diseases. Colloidal silver is commonly used as an antimicrobial in alternative medicine without clear scientific proof of effectiveness. To keep surfaces clean, in addition to regular cleaning, antimicrobial copper-alloys are used in a wide range of products to kill *E. coli O157:H7*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* in less than 2 hours.

Possible Questions

Part - A

Doubling time for Mycobacterium tuberculosis is _____
A) 14-16 seconds
B) 14-16 days
C) 14 -16 minutes
D) 14 -16 hours

Protozoan is a _____
A) single cell nucluei
B) single cell
C) micro organism
D) nucleus plasmid

The classic symptom malaria is _____
A) Paroxysm
B) Pentoxysm
C) Parentral
D) Internal

Severe malaria is usually caused by _____
A) P.ovale
B) P.vivax
C) P.falciform
D) P.malariae

The enzyme that helps to convert RNA into DNA in HIV virus is_____
A) Anylase

B) Invertase
C) Pepsin
D) Reverse transcriptase

The first modern Chemotherapeutic agent was
A) Arsenic
B) Arsphenamine
C) Lethenc
D) Proteonise

AIDS is diagnosed by:
A) Slide aggluitination
B) Latex agglutination
C) ELISA
D) CFT

Part – B

1. Mention any four symptoms of TB.
2. Write a short note on Cholera.
3. Give a brief account on symptoms of Cholera.
4. List the treatments for typhoid.
5. Discuss the important symptoms of AIDS.
6. Comment on antibiotics.
7. Write in detail about Microbial diseases.

Part – C

1. Describe the causative agents, pathology, treatment and diagnosis of TB.
2. Write about treatments and diagnosis of AIDS.
3. Explain the merits and limitations of chemotherapy.
4. Explain about causative agents, pathology and diagnosis of Malaria.
5. Comment on antibiotics with an example.
6. Discuss in detail about control of microorganisms by drug.
7. Explain in detail about the protozoan diseases.

Questions	A	B	C	D	Answer
UNIT I					
What are amphiphilic molecules?	Highly polar	Highly non-polar	Neutral	Having both polar and non-polar groups	**Having both polar and non-polar groups**
The shape of a water molecule is	Linear	Trigonal	Tetrahedron	Distorted Tetrahedron	**Distorted Tetrahedron**
In taxonomy, classifications of organisms are on the basis of	Overall morphology	Proteomics	Genomics	Evolution	**Overall morphology**
In phylogeny, classifications of organisms are on the basis of	Overall morphology	Proteomics	Genomics	Evolution	**Evolution**
How many H-bond a water molecule can make with neighboring water molecules?	1	2	3	4	**4**
What is osmosis?	Movement of solvent molecules across a semipermeable membrane.	Movement of solute molecules across a semipermeable membrane.	Movement of both solvent and solute molecules across a semipermeable membrane.	None of the above.	**Movement of solvent molecules across a semipermeable membrane.**
What is dialysis?	Movement of solvent molecules across a semipermeable membrane.	Movement of solute molecules across a semipermeable membrane.	Movement of both solvent and solute molecules across a semipermeable membrane.	None of the above.	**Movement of solute molecules across a semipermeable membrane.**
Buffers are	Mixture of weak acid and its conjugate base	Mixture of weak acid and weak base	Mixture of strong acid and its conjugate base.	Mixture of strong acid and strong base.	**Mixture of weak acid and its conjugate base**

At Acidosis condition, the blood pH is	7.3	< 7.2	7.4	> 7.4	**7.3**
At Alkalosis condition, the blood pH is	< 7.4	7.4	7.5	> 7.6	**> 7.6**
Physiological pH of the blood is	< 7.0	7	> 7.0	7.4	**7.4**
The blood pH is mainly maintained by the following components.	O_2 and CO_2	CO_2 and HCO_3^-	O_2 and CO	CO and CO_2	**CO_2 and HCO_3^-**
Hyperventilation may cause	Acidosis	Alkalosis	Both the acidosis and alkalosis	None of the above.	**Alkalosis**
Closed ventilation may cause	Acidosis	Alkalosis	Both the acidosis and alkalosis	None of the above.	**Acidosis**
All living things contain the element in some form	Iodine	Phosphorous	Carbon	All the above.	**Carbon**
The primary element of all biological macromolecules is	Iodine	Phosphorous	Carbon	All the above.	**Carbon**
Molecular self-assembly is directed through	Covalent interaction	Non-covalent interaction	Both covalent and non-covalent interaction	All the above.	**Non-covalent interaction**
Amyloid fibers are due to	Molecular self-assembly of correctly folded proteins	Molecular self-assembly of incorrectly folded proteins	Molecular self-assembly of lipids	Molecualr self-assembly of lipids and proteins.	**Molecular self-assembly of incorrectly folded proteins**
Biomolecules are	Endogenous	Exogenous	Either endogenous or exogenous	Neither endogenous nor exogenous	**Either endogenous or exogenous**
An atom is made up of central _____ containing positively charged protons	nucleus	molecule	nucleolus	shells	**nucleus**
A chemical bond that involves the sharing of electron pairs between atoms is called_____.	covalent	coordinate	electrovalent	non covalent	**covalent**

Chemical bond formed between two atoms due to transfer of electron(s) from one atom to the other. atom is called_____.	coordinate	electrovalent	noncovalent	covalent	**electrovalent**
Ionization of water can be described by an _____constant.	molecular	ionization	equilibrium	ionizable	**ionization**
The number of H+ ions present in a solution is a measure of_____of the solution.	Alkalinity	basicity	acidity	avidity	**acidity**
The_____of a solution is dependent upon the number for hydroxyl ions present.	basicity	acidity	alkalinity	neutrality	**alkalinity**
_____is defined as the negative logarithm of hydrogen ion concentration.	pH	[–H]	[-OH]	H+	**pH**
The pH of pure water at 25°C is_____.	6	8	7	10	**7**
A condition called_____occurs when pH of the blood is higher than normal.	acidosis	alkalosis	basidosis	avidosis	**alkalosis**
A_____is defined as a substance that has a greater tendency to lose its proton and completely dissociates.	strong acid	weak acid	strong base	weak base	**strong acid**
_____resists changes in pH on the addition of acid or base.	buffer	pH paper	acidohile	electrophile	**buffer**
The pK_a of the weak acid is given by a simple expression called_____equation.	Lowry-Bronsted	Lowry-Hasselbach	Henderson-Hasselbach	Hasselbach	**Henderson-Hasselbach**
The principal buffer for erythrocytes is _____.	bicarbonate	phosphate	protein	hemoglobin	**hemoglobin**
The pH of blood is maintained at_____.	7.8	7.4	6.4	7.1	**7.4**
_____is the number of isomers of glucose.	4	8	12	16	**16**
Epimers of Glucose_____.	Fructose	Galactose	Ribose	Deoxyribose	**Galactose**
Cellulose is made up of the molecules of_____.	α- Glucose	β-Glucose	Mannose	non of the above	**β-Glucose**
Glucose absorption may be decreased in _____.	Oedema	Nephritis	Rickets	Osteomyelitis	**Oedema**
The number of high energy phosphates contained by ATP is_____.	1	3	5	2	**2**

Keratin the protein of hair is synthesized from the aminoacid	glycine	Serine	Proline	Methionine	**Methionine**
Cytochrome Oxidase is poisoned by_____.	cyanide	sulphide	sulphite	sulphate	**cyanide**
An example for hydrophobic molecule is	water	heat	rosewater	oils	**oils**
The bond formed between atoms or groups carrying opposite charges is known as_____	covalent	electrostatic	hydrogen	metallic	**electrostatic**
pH of hydrochloric acid secreted by stomach lining is _____	6	7	3	1	**1**
_____is a substance which produces hydrogen ions(H^+) by dissociation.	Base	water	liquid	acid	**acid**
The pH scale ranges between _____	0 & 14	-114	-115	0 & 15	**0 & 14**
The bicarbonate ion is the conjugate base of	2 carbon atom	Carbonic acid	Carbamides	Carbondioxide	**Carbonic acid**
The pI of 'Lysine' can be calculated by using the formula	$pI = (pK_2 + pK_R)/2$	$pI = (pK_1 + pK_R)/2$	$pI = (pK_1 + pK_2)/2$	$pI = (pK_1 + pK_2 + pK_R)/3$	**$pI = (pK_2 + pK_R)/2$**
The pI of 'glutamic acid' can be calculated by using the formula	$pI = (pK_2 + pK_R)/2$	$pI = (pK_1 + pK_R)/2$	$pI = (pK_1 + pK_2)/2$	$pI = (pK_1 + pK_2 + pK_R)/3$	**$pI = (pK_1 + pK_R)/2$**
The pI of 'alanine' can be calculated by using the formula	$pI = (pK_2 + pK_R)/2$	$pI = (pK_1 + pK_R)/2$	$pI = (pK_1 + pK_2)/2$	$pI = (pK_1 + pK_2 + pK_R)/3$	**$pI = (pK_1 + pK_2)/2$**
The axial ratio of globular proteins is	> 10	< 10	> 20	< 20	**< 10**
The axial ratio of fibrous proteins is	> 10	< 10	> 20	< 20	**> 10**
Supercoiled DNA molecules have	W > 0	W < 0	W = 0	None of the above.	**W > 0**
Negatively supercoiled DNA molecules have	W > 0	W < 0	W = 0	None of the above.	**W < 0**
The sugar puckering effect in A-DNA is	C3' endo	C3' exo	C2' endo	C2' exo	**C3' endo**
The sugar puckering effect in B-DNA is	C3' endo	C3' exo	C2' endo	C2' exo	**C2' endo**
Reducing sugars have following structural and chemical features	Hemiacetal configuration	Exhibit mutarotation	Reduce Tollen's reagent	All the above.	**All the above.**

Non-reducing sugars have following structural and chemical features	Acetal configuration	Exhibit mutarotation	Reduce Tollen's reagent	All the above.	**Acetal configuration**
The monomeric unit of 'starch' is	β-D-Glucose	α-D-Glucose	α-L-Glucose	β-L-Glucose	**α-D-Glucose**
The monomeric unit of 'cellulose' is	β-D-Glucose	α-D-Glucose	α-L-Glucose	β-L-Glucose	**β-D-Glucose**
Triglycerides are composed of	Monohydric alcohols and fatty acids	Dihydric alcohols and fatty acids	Trihydric alcohols and fatty acids	None of the above.	**Trihydric alcohols and fatty acids**
Waxes are composed of	Monohydric alcohols and fatty acids	Dihydric alcohols and fatty acids	Trihydric alcohols and fatty acids	None of the above.	**Monohydric alcohols and fatty acids**
Phospholipids are	Simple lipids	Compound lipids	Derived lipids	All the above.	**Compound lipids**
Phosphosphingosides are	Simple lipids	Compound lipids	Derived lipids	All the above	**Compound lipids**
'Drying oils' exhibit	Low Iodine value	High Iodine value	Low acid number	High acid number	**High Iodine value**
Iodine solution produces no colour with _____.	cellulose	glycogen	starch	dextrin	**cellulose**
The distinguishing test between monosaccharides and dissacharides is _____.	barfoed's test	seliwanoff's test	fehling's test	benedict's test	**barfoed's test**
The non-protein part of rhodopsin is	Retinal	Retinol	Carotene	Repsin	**Retinal**
Heparin has a molecula weight of_____.	14000	14500	17000	17500	**17000**
Cellulose is made up of molecules of _____.	a glucose	b-glucose	d- glucose	g- glucose	**b-glucose**
Each branch of amylopectin is at an interval of_____glucose units.	14-20	24-30	34-40	44-50	**24-30**
UNIT II					
Cyclic AMP is formed from ATP by the enzyme adenylate cyclase which is activated by the hormone.	insulin	epinephrine	glucagons	progesterone	**epinephrine**

The absorption of glucose is interfered by the deficiency of_____.	vitamin A	thiamine	riboflavin	pyridoxine	**thiamine**
The branching enzyme acts on the glycogen chain between glucose units of_____.	1 and 6	2 and 7	3 and 9	6 and 11	**6 and 11**
The synthesis of adenylate cyclase is increased by_____.	thyroid hormones	growth hormones	ACTH	FTH	**thyroid hormones**
Inulin is a_____.	glucosan	fucosan	fructosan	pyranosan	**fructosan**
Glucose on treatment with strong mineral acids produces_____.	levulinic acid	levunyl acid	gluconic acid	glucuronic acid	**levulinic acid**
Heparin is used as an _____	anticoagulant	coagulant	depressent	antidepressent	**anticoagulant**
Each turn of the helix amylose consists of _____glucose units.	2	4	6	7	**6**
The method of formation of glucose and glycogen from non-carbohydrate sources is called as_____.	glycogenesis	glucogenesis	Gluconeogenesis	Glycolysis	**glucogenesis**
Pyruvate dehydrogenase is inhibited by _____.	fluoride	sulphide	arsenite	sulphate	**arsenite**
The carrier of citric acid cycle is_____.	succinate	fumarate	malate	oxaloacetate	**oxaloacetate**
When equal amounts of dextro and levo rotatory isomers are present in a mixture it is said to be_____.	isomeric	epimeic	racemic	enantiomeric	**racemic**
Fructokinase is present in_____.	intestine	adipose tissue	heart	brain	**intestine**
Pyruvate is accumulated by the dietary deficiency of_____.	folic acid	B6	b12	thiamine	**thiamine**
The glycogen content of_____is more than in muscle.	liver	brain	kidney	intestine	**liver**
In galactosemic individual UDP galactose is formed by epimerization from_____.	glucose	UDP glucose	CDP glucose	ITP glucose	**UDP glucose**
In the liver glyceraldehyde –3 phosphate is converted to_____.	glycol	formaldehyde	formic acid	glycerol	**glycerol**
The reaction involving the conversion of succinyl CoA to succinate requires_____.	CDP	GDP	ADP	ATP	**ADP**

The heptose ketose sugar formed as a result of chemical reactionin HMP shunt is _____.	glucoheptose	galactoheptose	sedoheptulose	manooheptose	**sedoheptulose**
The general formula for polysaccharide is _____.	$(C_6H_{10}O_5)n$	$(C_6H_{12}O_6)n$	$(C_6H_{12}O_5)n$	$1(C_6H_{10}O_6)n$	**$(C_6H_{10}O_5)n$**
Human heart muscle contains_____	D-Arabinose	D-Ribose	D-Lyxose	D-Xylose	**D-Lyxose**
Honey contains the hydrolytic product of	Lactose	Maltose	Inulin	Starch	**Inulin**
Iodine solution produces no colour with	Cellulose	Starch	Dextrin	Glycogen	**Cellulose**
Amylose contains glucose units _____	100-200	200-300	300-400	500-600	**300-400**
Glucose absorption may be decreased in	Oedema	Nephritis	Rickets	Osteomyelitis	**Oedema**
Sucrose is refered as _____	Sugar	Simple sugar	Fructosan	Invert sugar	**Invert sugar**
Starch is formed by_____chain	α-glucosidic	β- glucosidic	γ-glucosidic	All	**α-glucosidic**
Gluconeogenesis is a reversal of _____	Krebs cycle	HMP shunt	PMP	Glycolysis	**Glycolysis**
_____cannot be synthesized in man.	Lactic acid	HCl	Protein	Ascorbic acid	**Ascorbic acid**
Insulin is destroyed by _____	Lyases	Ligases	Aldolases	Peptidase	**Peptidase**
_____are antagonists to insulin.	Pyruvic acid	Glucokinase	Glycogenin	Glucagon	**Glucagon**
Gangliosides are the glycolipids occurring in	liver	brain	kidney	muscle	**brain**
The prostaglandins are synthesized from	arachidonic acid	oleic acid	linoleic acid	linolenic acid	**arachidonic acid**
The essential fatty acids retard	atherosclerosis	diabetes mellitus	nephritis	edema	**atherosclerosis**
Eicasonoids are formed from	arachidonate	palmitate	stearate	butyrate	**arachidonate**
The principal organ for cholesterol synthesis is	brain	thyroid	liver	lungs	**liver**
LDL contains the apoprotein	C-I	C-II	C-III	B	**B**
Fats are esters of_____with glycerol	fatty acids	waxes	Phospholipids	Cholesterol	**fatty acids**
HDL is synthesized and secreted from	pancreas	liver	kidney	muscle	**liver**

Liebermann- Burchard reaction is performed to detect	cholesterol	glycerol	fatty acid	vitamin D	**cholesterol**
Sulpholipids have been isolated from	heart	liver	brain	intestine	**brain**
Prostaglandins are liberated in the circulation by the stimulation of	posterior pituitary	anterior pituitary	adrenal gland	thyroid	**adrenal gland**
The great majority of absorbed fat appears in the form of	HDL	chylomicrons	VLDL	LDL	**chylomicrons**
The fatty acids containing even and odd numbers of carbon atoms and aiso unsaturated fatty acids are oxidized by_____	a- Oxidation	b - Oxidation	w-Oxidation	g- Oxidation	**b - Oxidation**
Long chain fatty acids are first activated to acyl- CoA in the	cytosol	mitochondria	microsomes	lysosomes	**cytosol**
Phospholipids help the oxidation of	glycerol	fatty acids	glycerophosphates	glycophosphates	**fatty acids**
The synthesis of prostaglandins is inhibited by	aspirin	arsenite	fluoride	cyanide	**aspirin**
Faty acids synthesis takes place in the presence of the coenzyme	NAD+	reduced F+	reduced NAD	reduced NADP	**reduced NADP**
The protein moiety of lipoproteins is known as	apoprotein	preprotein	post protein	pseudoprotein	**apoprotein**
In adipose tissue prostaglandins decrease	lipogenesis	ketogenesis	lipolysis	ketolysis	**lipogenesis**
The beta lipoprotein fraction increases in severe	diabetes mellitus	uremia	.nephritis	muscular dystrophy	**diabetes mellitus**
Acyl-CoA dehydrogenase converts acyl-CoA to a, b-unsaturated acyl-CoA in the presence of the coenzyme	NAD+	NADP	ATP	FAD+	**FAD+**
Before the action of lipase the fat is emulsified by	lipoproteins	phospholipids	ergosterols	digitoxin	**phospholipids**
Phosphadityl inositol is found in	cabbages	soyabeans	cauliflower	apples	**soyabeans**
Ketone bodis are utilized in	mitochondria	extrahepatic tissues	nuclei	chromosomes	**extrahepatic tissues**
Carboxylation of acetyl coA to malonyl CoA takes place bin the presence of	FAD+	biotin	NAD+	NADP+	**biotin**
Arachidonic acid contain_____number of double bond.	2	3	4	5	**4**

The synthesis of prostaglandins is inhibited by _____	Aspirin	Arsenite	Fluoride	Cyanide	**Aspirin**
Sunflower oil contains a high proportion of _____fattyacids.	Monounsaturated	Polyunsaturated	Monosaturated	Polysaturated	**Polyunsaturated**
All aminoacids are optically active except _____.	glycine	serine	threonine	tryptophan	**glycine**
The aminoacid which synthesizes many hormones is_____.	valine	phenylalanine	alanine	Histidine	**phenylalanine**
The sulphur containing aminoacid is _____.	glycine	methionine	valine	homoserine	**methionine**
The milk protein in the stomach of infants is digested by_____.	pepsin	trypsin	chymotrypsin	rennin	**rennin**
The half life of antibody proteins is _____	4 weeks	2 weeks	3 weks	1 week	**2 weeks**
Protein anabolism is stimulated by_____.	ACTH	Testosterone	Glucagons	Epinephrine	**Testosterone**
The metabolism of protein is integrated with that of carbohydrate and fat through _____.	Oxaloacetate	Citrate	Isocitrate	Malate	**Oxaloacetate**
Chymotrypsin in the small intestine hydrolyzes peptide linkages containing _____.	Phenylalanine	Alanine	Methionine	Valine	**Phenylalanine**
The building up and breakdown of protoplasm are concerned with the metabolism of_____.	Carbohydrates	Fats	Protein	Minerals	**Protein**
Aminoacids provide the nitrogen for the synthesis of_____.	the bases of the Phospholipids	uric acid	glycolipids	chondroitin sulphates	**the bases of the Phospholipids**
The end product of aminoacid nitrogen metabolism in uricotelic organisms is _____.	bilirubin	urea	uric acid	biliverdin	**uric acid**
Oxidative conversion of many aminoacids to their corresponding a-keto acids occurs in mammalian_____.	liver and kidney	adipose tissue	pancreas	intestine	**liver and kidney**
The biosynthesis of urea occurs mainly in the liver_____.	cytosol	mitochondria	microsomes	nuclei	**mitochondria**

One molecule of urea is synthesized at the expense of_____number of ATP.	1	2	4	3	**3**
The symptom of ammonia intoxication includes_____.	blurring of vision	constipation	mental confusion	diarrhea	**blurring of vision**
The only ketogenic amino acid is_____.	leucine	isoleucine	alanine	glycine	**leucine**
Both valine and isoleucine on catabolism produce_____	Alanine	Succinyl-CoA	Methionine	Valine	**Succinyl-CoA**
Transamination is a_____process.	Irreversible	Reversible	Inhibition	a & c	**Reversible**
In brain, the mechanism for the removal of ammonia is the formation of _____	Glutamate	Aspartate	Asparagine	Glutamine	**Glutamine**
UNIT III	**A**	**B**	**C**	**D**	**Answer**
The net numbers of ATP production of glycolysis under aerobic condition is	2	4	8	24	**24**
. The net numbers of ATP production of glycolysis under anaerobic condition is	2	4	8	24	**8**
The net numbers of ATP production in a TCA cycle is	2	4	8	24	**24**
Polyuria is	excessive thirst	excessive appetite	excessive excretion of urine	glucose in urine	**excessive excretion of urine**
Polydipsia is	excessive thirst	excessive appetite	excessive excretion of urine	glucose in urine	**excessive thirst**
Polyphagia is	excessive thirst	excessive appetite	excessive excretion of urine	glucose in urine	**excessive appetite**
Glucosuria is	excessive thirst	excessive appetite	excessive excretion of urine	glucose in urine	**glucose in urine**
How may irreversible steps occur in glycolysis	1	2	3	4	**3**
The important reducing power produced in HMP shunt pathway is	NADH	FADH	NADPH	FADH2	**NADPH**
Lactate is converted into glucose in	Liver	Muscle	Kidney	Lung	**Liver**

Which of the following enzyme links glycolysis and TCA cycle	Pyruvate carboxylase	Pyruvate kinase	Phosphoenol pyruvate kinase	Pyruvate dehydrogenase	**Pyruvate dehydrogenase**
Pyruvate is converted to oxaloacetate by	Pyruvate carboxylase	Pyruvate kinase	Phosphoenol pyruvate kinase	Pyruvate dehydrogenase	**Pyruvate dehydrogenase**
Oxaloacetate is converted to phosphoenolpyruvate by	Pyruvate carboxylase	Pyruvate kinase	Phosphoenol pyruvate kinase	Pyruvate dehydrogenase	**Phosphoenol pyruvate kinase**
Glucagon stimulates synthesizing of	Glucose from glycogen	Glucose from pyruvate	Glycogen from glucose	Glycogen from pyruvate	**Glucose from glycogen**
Epinephrine stimulates synthesizing of	Glucose from glycogen	Glucose from pyruvate	Glycogen from glucose	Glycogen from pyruvate	**Glucose from glycogen**
_____test is positive for the aminoacid Cysteine.	Millon	Ninhydrin	Nitroprussid e	Catalase	**Nitroprusside**
The first incoming NTP binds _____ at the start point of initiation	DNApolymerase I	polymerases	DNA polymerase	RNA polymerase	**RNA polymerase**
Non-protein chemical compound that is required for the protein's biological activity_____	Coenzyme	Cofactor	Isomerases	Synthetases	**Cofactor**
The reactions in which two molecules are joined at the expense of an energy source are catalyzed by _____	Ligases	Isomerases	Transferases	Hydrolases	**Ligases**
Retinal is reduced to retinol by retinene reductase in presence of the coenzyme_____	NAD+	NADP+	NADH+H+	NADPH+H+	**NADH+H+**
The unit of genetic information is the_____or cistron.	Genom	Codon	Gene	Anticodon	**Gene**
Chromatin consists of a long double stranded _____molecules	RNA	DNA	Subunit	tDNA	**DNA**
The sequences recognized by RNA polymerase are called	Terminator	Promoter	Both of the above	Non of the above	**Promoter**

DNA is refered as _____	Transforming factor	Range constants	Transplantation factor	Heterogenous factor	**Transforming factor**
Messenger RNA has a molecuar weight of_____	15000 to 30000	20000 to 35000	25000 to 40000	30000 to 50000	**30000 to 50000**
The 60S subunit contains 5s rRNA a 5.8S;rRNA and a_____	18S	30S	28S	40S	**28S**
DNA is denatured by _____	Acid	Alkali	Heat	All the above	**All the above**
A chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H2O)	Hydrophobic interaction	Hydrophilic interaction	Disulphide bonds	Peptide bonds	**Peptide bonds**
cGMP is antagonistic to _____	cAMP	CTP	Non of the above	All the above	**cAMP**
Michaelis-Menten model describes	Enzyme stability	Enzyme specificity	Enzyme kinetics	None of the above	**Enzyme kinetics**
cGMP is formed from_____by the enzyme adenyl cyclase	ATP	GDP	CTP	CDP	**ATP**
An example for a semipermeable membrane is _____	Plasma membrane	Cell membrane	Dialysis membrane	All the above	**All the above**
The lactam form is the predominant tautomer of	Uracil	Cytosine	Adenine	Xanthine	**Uracil**
The chemical name 2-amino-6-oxypurine is said to be_____	Adenine	Xanthine	Guanine	Hypoxinthine	**Guanine**
_____ is an important molecule in metabolism, used in many biochemical reactions	Pyruvate	Carboxide	Acetyl Co A	Acetamide	**Acetyl Co A**
A dicarboxylic acid with structure $CH_2(COOH)_2$_____	Succinic acid	Malonic acid	Pyruvic acid	Formic acid	**Malonic acid**
In Vmax, [Et] denotes _____	Enzyme at time t	Total Enzyme	Substrate	Product	**Total Enzyme**
Lyases belongs to_____class in the major classes of Enzymes.	3^{rd}	2^{nd}	5^{th}	4^{th}	**4^{th}**
The_____is a molecule upon which an enzyme acts	Substrate utilized	Substrate involved	Substrate oxidized	Substrate recovered	**Substrate oxidized**
The initiation of DNA synthesis requires priming by a short length of	RNA	DNA	Hydroxyl group	Alkyl group	**RNA**

International system of units is	SI	Anson	Katal	Newton	**SI**
A biochemically active compound formed by the combination of an enzyme with a coenzyme_____	Apoenzyme	Isoenzyme	Holoenzyme	Heyteroenzyme	**Holoenzyme**
Enzymes can be precipitated by	Ammonium Sulphate	Ammonium Oxalate	Ammonium Chloride	Ammonium oxide	**Ammonium Sulphate**
_____is the inorganic chemical component that is required for enzyme activity	Coenzyme	Protein	Aminoacids	Cofactor	**Cofactor**
In_____the international union of Biochemistrty gave the classification and naming system of enzymes on the basis of overall reaction catalysed.	1923	1961	1941	1963	**1961**
An active group of cysteine is _____	Alcoholic	Imidazole	Sulfhydryl	Phenolic	**Sulfhydryl**
Induced Fit Mechanism was proposed by	Fisher	Michael	Kunhe	Koshland	**Koshland**
The Substrate is specific towards of the enzyme.	Active site	Allosteric group	Hydroxyl group	Inactive group	**Active site**
For entrapping enzymes instead of cellulose acetate fibres is used	Calcium Chloride	Calcium oxalate	Calcium alginate	Cellulose oxide	**Calcium alginate**
DNA gyrases act to relieve the stress generated by_____	Gyrases	Helicases	polymerases	All the above	**Helicases**
The enzyme involved in hydrolysis is	Reductases	Lyases	Ligases	Hydrolases	**Hydrolases**
IUPAC is _____	International Unit of Pure and Applied Chemistry	International Union of Pure and Applied Chemistry	Indian Unit of Pure and Applied Chemistry	Indian Union of Pure and Applied Chemistry	**International Union of Pure and Applied Chemistry**
Fifth class enzyme is _____	Oxidoreductase	Lyases	Hydrolases	Isomerases	**Lyases**
Last digit number of E.C.Number represents the_____of enzyme within the subsub class	Register number	Code number	Serial number	Account number	**Serial number**
Systematic code number is otherwise known as _____	Enzyme cofactor number	Enzyme coenzyme number	Enzyme coordinate number	Enzyme Commission number	**Enzyme Commission number**

An example for yeast enzymes having E.C.number 3.2.1.23 is _____	Invertase	Raffinase	Lactase	Lipase	**Lactase**
E.C.number of α-amylase is _____	3.2.1.1	3.2.1.3	1.1.3.4	3.2.1.2	**3.2.1.1**
β-amylase is an_____enzyme.	intracellular	toxic	heterogenous	extracellular	**extracellular**
An example for intracellular enzyme is	Pectinase	Aminoacylase	Lipase	Papain	**Aminoacylase**
E.C number of Raffinase is _____	3.2.1.23	3.2.1.22	3.2.1.15	3.2.1.1	**3.2.1.22**
An example for extracellular enzyme is	Aminoacylase	Lipase	Raffinase	Catalase	**Lipase**
E.C.number of α-amylase is _____	3.2.1.1	3.2.1.3	1.1.3.4	3.2.1.2	**3.2.1.1**
An example for a animal enzyme is	Rennet	α-amylase	Pullulanase	Raffinase	**Rennet**
An example for a plant enzyme is	Rennet	Lipoxygenase	Lipase	Pullulanase	**Lipoxygenase**
An example for a yeast enzyme is	Rennet	Lipase	Lactase	Raffinase	**Lipase**
UNIT IV					
Microbiology is a branch of science that deals with_____?	virus	plants	microbes	bacteria	**microbes**
who is the father of microbiology ?	Muller	Antony van leeuwenhoek	Louis pasteur	Robert koch	**Antony van leeuwenhoek**
Microbes are_____?	prokaryotes	eukaryotes	both a and b	unicelluar	**both a and b**
The study of bacteria is known as_____?	bacteriology	microbiology	virology	phycology	**bacteriology**
The study of algae is called as_____?	bacteriology	microbiology	phycology	virology	**physology**
Bright field microscope is also known as	Phase contrast microscope	Electron microscope	Light microscope	Fluroscent	**Light microscope**
The magnification of objective lens in bright field microscope is _____	45x	100x	10x	all the above	**all the above**
The magnification of ocular lens in bright field microscope is _____	5x	10x	15x	all the above	**all the above**
The efficieny of light microscope lies on its	magnification power	resolving power	virtual image	lens system	**resolving power**

The resolving power of a microscope is a function of the wavelengthof light used and ____of the lens system	magnification power	virtual image	numerical aperture	fine adjustment	**numerical aperture**
Dark field microscope is most useful for observing bacteria less than_____µm	0.2	0.5	o.4	0.1	**0.2**
In SEM the image is created as the specimen is scanned with a beam of _____	UV	fluroscent	electron	illumination	**electron**
The TEM employs an electron beam generated by _____	a tungsten filament	lamp	UV	Fluroscent	**a tungsten filament**
The electron microscope uses electron beams and_____to produce image	light waves	magnetic fields	glass lenses	illuminator	**magnetic fields**
In SEM the scattered electrons are collected by electronic devices and displayed on _____	a cathode ray tube	objetive lens	magnetic field	image plan	**a cathode ray tube**
In SEM the specimens are coated with a thin layer of _____	gold	palladium	silver	gold or palladium	**gold or palladium**
In Phase contrast microscope , in living unstained condition of most microorganism are_____in liquid medium which they are suspended	transparent	similar to medium	visible	not visible	**transparent**
The exmination of stained smear preparation is often called as_____mount techinque	dry	wet	liquid	solid	**dry**
An acid dye is one which as_____charge in staining basic cell components	negative	positive	netural	negative and positive	**negative**
An basic dye is one which as_____charge in staining acidic cell components	negative	positive	netural	negative and positive	**positive**
_____are the dyes used for simple staining	methylene blue	crysrtal violet	carbol fuschin	all the above	**all the above**
Who develeped staining procedure?	Christain Gram	Muller	Robert koch	Leeu van hoeck	**Christain Gram**
In_____christain Gram developed staining procedure	1882	1883	1884	1886	**1884**
_____ is used in negative staining	methylene blue	crysrtal violet	nigrosin	eosin	**nigrosin**

Exact composition of complex media is	unknown	known	partially known	none of these	**unknown**
Exact composition of defined media is	unknown	known	partially known	none of these	**known**
Which of the following is a macronutrient	carbon	manganese	zinc	cobalt	**carbon**
Which of the following is a micronutrient	carbon	manganese	magnessium	nitrogen	**manganese**
The technique used to avoid all microorganisms is accomplished by	sterilization	disinfection	incineration	boiling	**Sterilization**
Seperation of single bacterial colony is called as	isolation	pure culturing	seperation	All of the above	**pure culturing**
Media which distinguish one type of bacteria from other is known as	complex media	defined media	differential media	enriched media	**differential media**
A_____permit growth of one group of bacteria	selective media	enriched media	complex media	differntial media	**selective media**
Which one is a selective media	potato dextrose	LB agar	Mannitol salt agar	eosin methylene blue	**mannitol salt agar**
Example of differential media is	potato dextrose	LB agar	Mac conkey's agar	mannito salt agar	**Mac conkey's agar**
Enrichment media is used to isolate bacteria from	sputum	soil	water	air	**sputum**
Urea media is used to check the presence of enteric bacteria producing	amylase	cellulase	urease	lactase	**urease**
Autoclaving is an example of _____ Sterilization	moist heat	dry heat	red heat	all of these	**moist heat**
Temperature in autoclaving must be	118	121	100	115	**121**
Sterilization of heat sensitive material is achieved by	dry heat	moist heat	red heat	radiation	**radiation**
Bacterians can be stored successfully for weeks by	refrigration	lyophilization	pasteurization	filteration	**refrigeration**
Technique used for long storage of bacterial culture is	cryopreservation	lyophilization	refrigeration	pasteurization	**cryopreservation**

Stabilizing agent used for cryopreservaton is	glycerol	glucose	acetone	ethanol	**glycerol**
Glycerol/DMSO is used in cryopreservation to prevent formation of	ice crystals	toxins	spores	none of these	**ice crystals**
Cultures can be stored for _____ by cryopreservation	10-20 months	1-2 years	infinitive	20-30 years	**20-30 years**
In lyophilization culture is dehydrated by	vaccum	heating	thawing	all of these	**vaccum**
Low temperature in lyophilization is achieved by	slow freezing	instant freezing	liquid nitrogen	dry ice	**slow freezing**
Which technique is used to visualize microbes in bacteria	smearing	spreading	staining	dyeing	**staining**
Mordant used in gram staining is	crystal violet	saffranine	iodine	ethanol	**iodine**
Grams staining is a_____staining Technique	simple	differential	complex	none of these	**differential**
Saffranine used in gram's staining is a	primary stain	counter stain	mordant	solublizer	**counter stain**
Primary stain used in grams stainig is	crystal violet	saffranine	iodine	ethanol	**crystal violet**
Stain used for staining spores is	crystal violet	malachite green	iodine	saffranine	**malachite green**
Methylene blue is an example of	acidic dye	basic dye	neutral dye	all of these	**basic dye**
An example of acidic dye is	methylene blue	saffranine	crystal violet	eosin	**eosin**
Grams staining was developed in the year	1884	1984	1887	1987	**1884**
Gram negative bacterial are visualized as _____colured after grams stainig	violet	blue	yellow	pink	**pink**
In gram's staining violet colured bacteria are	gram's positive	gram's negative	gram's neutral	norma	**gram's postive**
Acid fast staining is performed to differentiate _____	viruses	fungi	acetobacter	mycobacteria	**mycobacteria**
Maximum growth of bacteria occurs in	lag phase	log phase	stationary phase	death phase	**log phase**

Bacteria reaches to death phase due to	depletion of nutrients	production of metabolic wastes	both a and b	none of these	**both a and b**
Phase in which cells adjust to new enviornment is	log phase	declining phase	lag phase	stationary phase	**lag phase**
Normal doubling time of the bacteria is _____?	30mins-1hr	1-2hr	3hr	15minutes	**30minutes-1hr**
Microorganisms is dependent on the _____?	substrate	antibiotics	medium	none of the above	**substrate**
The miocroorganisms can be grown in _____?	Batch bioreactor	fed-batch bioreactor	continuous bioreactor	allof the above	**all of the above**
Theromophiles can be isolated by using_____?	High temperature	low temperature	Acidic pH	Aerosol dilution	**High temperature**
Acidophiles can be isolated from_____?	High temperature	low temperature	Acidic pH	Aerosol dilution	**Acidic pH**
UNIT V					
Sickness or ailment caused in animal and humans are called_____	viral diseases	Bacterial diseases	Fungal diseases	Microbial diseases	**Microbial diseases**
How many types of microbes?	2 types	3 types	4 types	5 types	**4 types**
protozoa also known as _____	Bacteria	Protoctistia	Fungus	Virus	**Protoctistia**
One of the following is a bacterial diseases	Chickungunya	Bird flu	Hepatitus	typhoid	**Chickungunya**
The organ infected by Hepatitus 'B' virus is	Heart	Kidney	Stomach	Liver	**Liver**
The enzyme that helps to convert RNA into DNA in HIV virus is____	Anylase	Invertase	Pepsin	Reverse transcriptase	**Invertase**
"Reduced platelet count in blood "is one of the symptoms of the diseases is ____	Dengue	Chickungunya	Bird flu	Syphilis	**Dengue**
TB caused by the____	Viral infection	Bacterial infection	Both A & B	None of the above	**Bacterialinfection**
Tuberculosis usually affected in the____	Heart	Kidney	Lungs	Liver	**Lungs**
s.typhi growing in the _____	Heart	Kidney	Intestine	Lungs	**Intestine**
How many types of Tuberculosis infection?	Only one	Two	Three	Four	**Two**
Live vaccine are used for specific prophylaxis of _____	Diphtheria	Hepatitis D	Tuberculosis	Botulism	**Tuberculosis**
Live vaccine for specific pro[hylaxis against tuberculosis is called____	DTP	HLA	MMR	BCG	**BCG**

Salmonella typhi	Gram positive cocci arranged single	Gram positive rod arranged in chains	Gram negative rod arranged singly	Gram negative cocci arranged in pairs	**Gram negative rod arranged singly**
Typhoid is also known as_____	Pneumoinic fever	Hepatic fever	vesical fever	Enteric fever	**Enteric fever**
Skin test with specific allergens is used for diagnosis of_____	Tuberculosis	Enteric fever	Staphylococcal infection	Cholera	**Tuberculosis**
what is the pathogen that causes typhoid fever?	S.pneumoniae	S.typhi	C.perfrigens	P.aeruginosa	**S.typhi**
which phage typing is widely used for epidemiological analysis?	Dysenetry	Typhoid fever	Botulidsm	Camphylobacteriosis	**Typhoid fever**
AIDS is diagnosed by:	Slide aggluitination	Latex agglutination	ELISA	CFT	**ELISA**
Doubling time for Mycobacterium tuberculosis is _____	14-16 seconds	14-16 days	14 -16 minutes	14 -16 hours	**14 - 16 hours**
which is used to treatment of drug____	Azidothymidine	Ribavirin	Pencillin G	Acyclovir	**Azidothymidine**
choose the microorganism which possesses cord-factor:	Mycoplasm pneumoniae	Mycobeacterium tuberculosis	Streptococcus pneumoniae	Neisseria meningitidis	**Mycobacterium tuberculosis**
AIDS is diagnosed by:	Bacteriology method	Biological method	Seriological method	Microscope	**Seriological method**
Chemotherapy is treatment of_____	Lungs	Heart	Cancer	Fever	**Cancer**
Main property of cancer has_____	killing the cells	replication the cell	growing the cell	doubling the cell	**killing the cell**
Chemotherapy using drugs that convert cytotoxic activity only upon light exposure is called_____	Photochemo therapy	Chemoradiotherapy	Targeted therapy	Radiotherapy	**Photochemo therapy**
Hiv is a_____	Immuno virus	Retrovirus	Pathogenic bacteria	fungi	**Retro virus**
HIV affected immunosystem as _____	CD4+ T Cell	Macrophage	Dentric cell	All the above	**All the above**
The bacteria then perforate through the wall and are phagocytosed by _____	Macrophage	dentric cell	white cell	RBC cell	**Macrophage**

Chemotherapy term was coined by____	Mendal	Edward genour	Paul Ehrlinch	John	**Paul Ehrlinch**
The first modern Chemotherapeutic agent was	Arsenic	Arsphenamine	Lethenc	Proteonise	**Arsphenamine**
Any treatment of diseases with drug called as	Pharmacodynamics	Pharmacotherapy	Pharmacokinetics	Pharmacology	**Pharmacotherapy**
protozoan is a ____	single cell nucluei	single cell micro organism	nucleus	plasmid	**single cell micro organism**
The classic symptom malaria is _____	Paroxysm	Pentoxysm	Parentral	Internal	**Paroxysm**
Severe malaria is usually caused by _____	P.ovale	P.vivax	P.falciform	P.malariae	**P.Falciform**
sympotms of Falciform symotms arise ____ after infection	9-25 days	7-14 days	1-10 days	9-30 days	**9-30 days**
How many types of malaria caused by humans?	3	2	4	5	**5**
Antibacterial are used against	Bacteria	Fungi	Protozoa	seaweed	**Bacteria**
Microbial agents that kill microbes are called	Microbicidal	Antimicrobial	Antibacterial	Antifungal	**Microbicidal**
Antifungal are used against	Bacteria	Fungi	Protozoa	Seaweed	**Fungi**
use of antimicrobial medicines to treat infection is known as	Photochemotherapy	Pharmacotherapy	antimicrobial chemotherapy	Chemoradiotherapy	**Antimicrobial chemotherapy**
The main classes of antimicrobial agens are	Microbes	Disinfactant	Contamination	Media	**Disinfactant**
HIV as affected to ____	Aquired immune deficiency syndrome	Innate immune deficiency sndrome	Iron deficiency	Calacium deficiency	**Acquired immune deficiency syndrome**
which immunity level as lost from the affecting of HIV	aquired immunity	cell mediate immunite	innate immunity	None of the above	**Cell mediate immunity**
AIDS syndrome caused by a virus is called	Immuno virus	Retrovirus	HIV virus	Bacteriophage	**HIV virus**
Typhoid can caused affected by ____	heart attack	Kidney failure	lens failure	Brain tumour	**Kidney failure**
Which of the following types of interferon is used in the treatment of hepatitis C virus:	Interferon-alpha	Interferon-beta	Interferon-delta	None of the above	**Interferon-alpha**

Which one of the following drugs produces hemorrhagic cystitis:	Nitrosurea	Cisplatinum	Cyclophosphamide	Bleomycin	**Cyclophosphamide**
All of the following are cell cycle non specific anticancer agents except ___	Nitrosurea	Cisplatinum	Cyclophosphamide	Bleomycin	**Bleomycin**
Which one of the following antibiotics is effective in treatment of typhoid fever____	Pencillin G	Kefaclor	Demicocycline	Ceprofloxacin	**Ceprofloxacin**
Which one of the following antibiotics is bacteriostatic	Erythromycin	Gentamycin	Cefradin	Ampicillin	**Cefradin**
Bone marrow depression is a sideeffect of all of the following drug except ____	Chloramphicol	Cyclosporin	Interferon A	Pencillin G	**Pencillin G**
Cotromizole is a combination of _____	Sulfadiaxine+trimethoprim	Sulfamethoxyzole+trimethoprim	Sulfamdioxine+dapose	Sulfadioxine+pyrimetheamine	**Sulfamethoxyzole+trimethoprim**
Fanconi syndrome is a side effect of which one of the following therapeutic agent____	Refampicin	Neomycin	Demicocycline	Clindamycin	**Demicocycline**
which drugs passes through blood brain barrier?	Cephalexin	Cefactor	Cefatriaxone	Micillinam	**Micillinam**
Which is the longest duration of action____	Oxytetracyclin	Demicocycline	Minocycline	Chlortetracyclin	**Minocycline**
Second generation of cephalosporin is ____	Cepharadine	Cefoperzone	Cefotaxine	Cefoxline	**Cefoxline**
More active against gram-ve bacilli is	Dicloxacilline	Erythromycine	Cephalexine	Micillinam	**Micillinam**
All of the following chemotherapeutic agents are effective in treatment of anerobic infection except:	Metronidazole	Imipenium	vancomycin	Cefoxitin	**Imipenium**