Crash

Biochemistry

and

Molecular Biology

Lecture Text

For Doctors

Of All Specialties

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Second Edition

Dear colleagues:

I hope you find this text useful for your exams and your practice.

Your feedback is most welcome.

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Best wishes

A. Hammouda

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Overview

This is an intensive course of medical biochemistry and molecular biology for doctors of all specialties. It is intended to refresh their information and update their awareness of the application of the science of biochemistry to their clinical practice. It is formulated as lectures that stress general viewpoints rather than minor details.

Why should we study biochemistry?

Like professionals dealing with specific machines, medical personnel should know well about the structure and function of the human body. Biochemistry is the branch of science that deals with the structure and function of living tissues at the molecular level.

What are we made of?

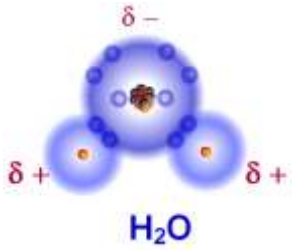
Living tissues can be chemically described in terms of molecules, ions, and atoms. The bodily functions result from the interaction of these components. An alteration in the structure or the interaction of these components can result in alteration of the function of the body, a disease state.

The major component, on weight basis, is water. The human body can be described as essentially water with which other components: organic and inorganic chemicals are mixed. The intracellular and extracellular fluids are nothing but aqueous solutions (aqua = water).

Organic components of living tissues can be studied as groups according to shared characteristics. Major organic compounds include proteins, lipids, carbohydrate, nucleotides, nucleic acids, porphyrins and various metabolites.

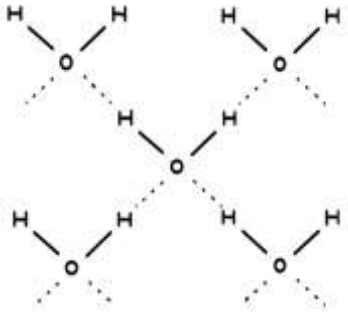
The major inorganic compound is the insoluble calcium phosphate of the bones and teeth. Soluble ions, though constituting only a minor fraction of body weight, are essential for the proper organization and function of any living organism.

Water



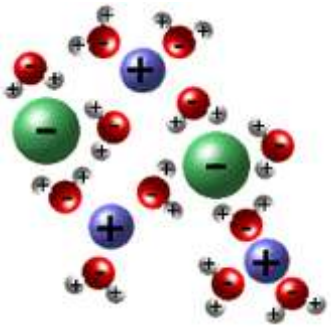
The unique properties of water make it an excellent solvent for many of the tissue components. A water molecule is formed of one oxygen atom connected to two hydrogen atoms by two covalent bonds. A covalent bond is a pair of electrons spinning around the two atoms. In water molecule, this electron pair of the oxygen-hydrogen bond is more associated with the big oxygen atom compared to the small hydrogen nucleus (one proton). This results in a partial negative charge ($\delta -$) on the oxygen atom and a partial positive charge ($\delta +$) on the hydrogen atoms. The water molecule is therefore described as a polar molecule.

Hydrogen bond



Water molecules associate with each other by the weak electrostatic attraction between their oppositely charged poles. These weak attraction forces are called hydrogen bonds, since the hydrogen atom is shared by two oxygen atoms: one in the same water molecule and another one from a neighboring molecule. Every water molecule can associate via hydrogen bonds with four other molecules.

How can water dissolve different substances?



Water molecules surround the ions of ionic compounds, e.g., sodium chloride with their oppositely charged poles. Thus, water molecules form shields around the ions preventing their association and ensuring their dissolution. The same applies to non-ionic polar compounds dissolved by water, e.g., glucose.

Solubility

Not all ionic and polar compounds are soluble in water. Ethanol (ethyl alcohol) for example is freely miscible with water at any ratio, while phenol (an aromatic alcohol) has a limited solubility in water. Glucose and sodium chloride are highly soluble, while calcium phosphate is very poorly soluble in water.

The low solubility of calcium phosphate makes the plasma virtually saturated at all times, making calcium phosphate concentration constant. Thus the product of soluble calcium and phosphate concentrations (solubility product) is constant:

$$\text{Calcium phosphate} \rightleftharpoons \text{Calcium} + \text{Phosphate}$$

$$\text{Solubility product} = [\text{Calcium}] \times [\text{Phosphate}] = \text{Constant}$$

Solubility of gases

Solubility of gases increases by increasing the pressure. Breathing pure oxygen delivers oxygen to the blood at a higher oxygen partial pressure, five times that from air, thus increasing the oxygen dissolved in the plasma. High pressure chambers (hyperbaric oxygen therapy) ensure more dissolution of oxygen in the patient's plasma.

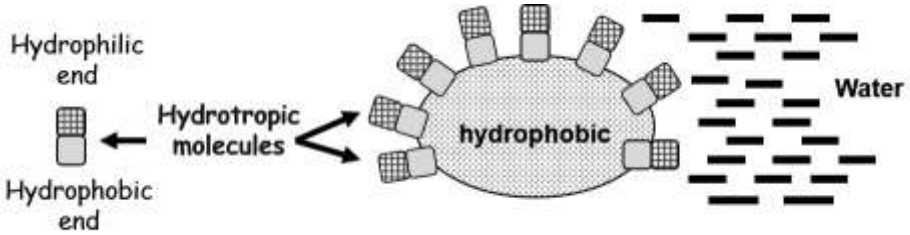
Scuba divers have more nitrogen dissolved in their plasma and tissue fluids. This extra nitrogen is released in the tissues upon rapid surfacing, causing decompression injury. Placing the patient in a hyperbaric chamber redissolves the released nitrogen, and breathing pure oxygen helps getting rid of nitrogen. The pressure should then be decreased gradually.

Hydrophilic and hydrophobic substances

Substances which are water miscible are called hydrophilic (water loving). Hydrophobic (water hating) substances carry no electric charges and are not water soluble. These include organic compounds with gross non-polar structure, such as hydrocarbons, chloroform, oils, cholesterol, etc.

Hydrotropic substances

Hydrotropic substances can render the hydrophobic molecules water miscible, without changing their chemical nature. These are amphipathic molecules like soaps and detergents. The amphipathic molecules surround the hydrophobic particles by their hydrophobic sides. The hydrophilic sides of these amphipathic molecules face water molecules and thus render the hydrophobic substance water-miscible.



Phospholipids, bile salts, and proteins act as hydrotropic substances in-vivo. Plasma lipids are kept in a miscible form due to the protein and phospholipid content of the plasma lipoproteins. Bile salts emulsify the dietary lipids in the intestine, facilitating their digestion and absorption. Cholesterol is kept in solution in the bile by bile salts and phospholipids. If the ratio of bile salts to cholesterol in the bile decreases, cholesterol precipitates forming biliary stones.

Concentration

Water is the universal solvent in tissues, and also the major solvent of medications and several preparations used with patients. We need to have a means of expressing how much substance is dissolved. A rough method is to say dilute or concentrated solution. The accurate way is to express the concentration in a numerical value.

Concentration is the amount of solute (dissolved substance) in a specified amount of the solution. A 70% ethyl alcohol solution used for disinfection contains 70 volumes of ethyl alcohol in 100 volumes of the solution. Plasma glucose concentration of 90 mg/dL means that every deciliter (100 milliliters) of plasma contains 90 milligrams of glucose. We should not use the wrong expression “90 milligrams per cent” to describe plasma glucose concentration or the concentration of any blood analyte. We should always specify the amount of solute and the amount of solution, or use a ratio that denotes a volume per volume (v/v) like 70% alcohol solution, or weight per weight (w/w). By convention, 5% glucose solution used for intravenous infusion contains 5 grams of glucose in 100 milliliters of the solution (about 100 grams).

Units of mass

For practical purposes, the terms mass and weight are used interchangeably. The basic international unit of mass is the kilogram (kg). Fractions of this unit are commonly used (please always observe the upper case or lower case letters for the symbols):

1 gram	(g)	$= 10^{-3}$	kg
1 milligram	(mg)	$= 10^{-3}$	g
1 microgram	(μg)	$= 10^{-6}$	g
1 nanogram	(ng)	$= 10^{-9}$	g
1 picogram	(pg)	$= 10^{-12}$	g

Units of volume

The derived international unit of volume is the liter (L). Fractions of this unit are commonly used (in some writings, the lower case “l” is used for the liter symbol):

1 liter	(L)	$= 1\ dm^3$ (cubic decimeter)		
1 deciliter	(dL)	$= 10^{-1}$	L	= 100 mL
1 milliliter	(mL)	$= 10^{-3}$	L	$= 1\ cm^3$ (cubic centimeter, cc)
1 microliter	(μL)	$= 10^{-6}$	L	$= 1\ mm^3$ (cubic millimeter)
1 femtoliter	(fL)	$= 10^{-15}$	L	$= 1\ \mu m^3$ (cubic micrometer)

The units of international system are now replacing the old units. The milliliter and deciliter are now replacing the cubic centimeter (cc) and the 100 cc, respectively. The microliter is replacing the cubic millimeter in blood cell count. The femtoliter is used for red blood cell volume.

Units for the amount of matter

When studying chemical reactions, it is important to look at the relative number of molecules or other reacting entities, rather than their mass or volume. Different substances, of the same mass, contain different number of molecules because they have different molecular weights. A unit is needed that contains the same number of molecules or other forming entities (atoms, ions or electrons) for any matter. This is the basic international unit of matter, the mole (mol). One mole of any substance equals its atomic weight or molecular weight in grams. Fractions of this unit are commonly used:

1 millimole	(mmol)	$= 10^{-3}$	mol
1 micromole	(μmol)	$= 10^{-6}$	mol, etc.

Unit conversion

Plasma analyte concentrations have been expressed in mg/dL units. These are being replaced by mmol/L (mM, millimolar) units. To convert to the new units, the following equation is used:

Concentration (mmol/L) = Concentration (mg/dL) x 10 ÷ molecular weight.

Knowing that the molecular weight of glucose is 180, a blood glucose concentration can be converted like in this example: 90 mg/dL = 90 x 10 ÷ 180 = 5 mmol/L or 5 mM

Knowing that the molecular weight of urea is 60, a blood urea concentration can be converted back like in this example: 20 mM or 20 mmol/L = 20 x 60 ÷ 10 = 120 mg/dL

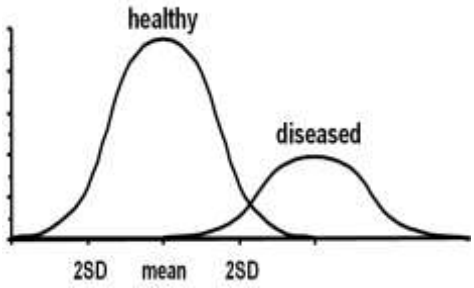
Knowing that the atomic weight of calcium is 40, a plasma calcium concentration can be converted like in this example: 10 mg/dL = 10 x 10 ÷ 40 = 2.5 mmol/L or 2.5 mM

Conversion factors can be found in conversion tables and applied directly. Glucose for example has a conversion factor of 18, while the factor for urea is 6 and for calcium is 4.

Monovalent ions: sodium, potassium, and chloride are measured in mEq/L (milli-equivalent per liter) or mmol/L. These need no conversion since one equivalent of a mono-valent ion equals one mole. For calcium (a divalent ion), 1 mmol/L = 2 mEq/L.

Reference values

Reference range or reference values are always given for different plasma analytes. These are wrongly described as normal values by many doctors, which may lead to problems in diagnosis.

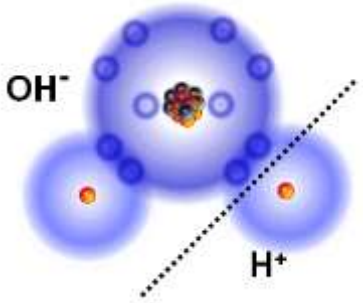


The reference range is usually two standard deviations (2 SD) below to 2 SD above the mean for a healthy population. This range is expected to include about 95% of healthy individuals. A value outside this range is not necessarily belonging to a diseased person (positive predictive value < 100%). In addition, there is usually an overlap of the values of healthy and diseased individuals. Therefore, a value inside the range is not necessarily belonging to a healthy individual (negative predictive value < 100%). Furthermore, the reference population may not even be the best for judging one's patient. An auto-analyzer, for example, may give a "High" flag for alkaline phosphatase in a normal child sample when comparing the result to an adult reference range.

If the reference range is widened, more patients will be missed (lower sensitivity). If the reference range is narrowed, sensitivity increases but more healthy people will be misdiagnosed as diseased (lower specificity). Sensitivity equals the ratio of test-positive patients to all patients. Specificity equals the ratio of test-negative in all healthy subjects.

Another important point to remember is that there is a physiological variation in an individual from time to time and from one state to another. In addition, sample collection and processing is always subject to variation, technically termed accepted error. The higher the precision of a lab, the lower this variation would be. Lab to lab variation is usually higher than within the same lab. Both high precision (almost the same result with repetition) and high accuracy (almost the true value) are required for diagnosis and monitoring patients' prognosis.

Water dissociation and pH



Water is a covalent compound, yet water molecules can dissociate giving positively and negatively charged ions. Water molecules poorly dissociate. One liter of pure water is about 55.5 moles of water, since the molecular weight of water is 18. The concentration of hydrogen ions, and of the hydroxyl ions, in pure water is only 10^{-7} mol/L. The dissociation of water molecules is shown as follows:

$$H_2O \rightleftharpoons H^+ + OH^-$$

This very low concentration of hydrogen ions is expressed in terms of pH, the negative logarithm of hydrogen ion concentration:

$$pH = -\log [H^+] = -\log 10^{-7} = 7$$

Thus, we can say that the pH of water is 7, which is the neutral pH. If an acid like hydrochloric acid (HCl) is added, more hydrogen ions will be present due to dissociation of the acid. In acidic solution hydrogen ion concentration increases and pH is lower than 7.

$$HCl \longrightarrow H^+ + Cl^-$$

If a base, e.g., sodium hydroxide (NaOH) is added, more hydroxyl ions will be present due to dissociation of sodium hydroxide:

$$NaOH \longrightarrow Na^+ + OH^-$$

This pushes the water dissociation to the left, with decrease of hydrogen ion concentration. Therefore, the pH of a basic solution is higher than 7.

Buffers

Chemical reactions depend largely on the pH of the medium. The plasma pH is about 7.4, which is kept constant by the blood buffers. A buffer is a solution that resists the change in pH when an acid or a base is added. It is a mixture of a weak acid and its conjugate base. A weak acid is an acid that does not dissociate completely in dilute solutions, e.g., carboxylic acids like acetic acid:

$$\text{weak acid} \rightleftharpoons \text{conjugate base}^- + H^+$$

$$\text{e.g., acetic acid} \rightleftharpoons \text{acetate}^- + H^+$$

If an acid is added to the buffer, $[H^+]$ increases. The conjugate base takes H^+ and resists the decrease in pH. If a base is added, $[H^+]$ decreases. The buffer acid donates H^+ and resists the increase in pH.

Biological buffers – carbonic anhydrase

Biological buffer systems include the monobasic/dibasic phosphate ($H_2PO_4^-/HPO_4^{2-}$), carbonic acid/bicarbonate (H_2CO_3/HCO_3^-), and the proteins like plasma proteins and blood hemoglobin.

Hemoglobin is quantitatively the major blood buffer. It owes its buffering capacity to the imidazole groups of histidine residues. It buffers the acidifying effect of metabolic products at tissue capillaries, in addition to its binding of a portion of carbon dioxide as carbamate. At the lungs, oxyhemoglobin is more acidic than deoxygenated hemoglobin, which compensates for carbon dioxide excretion.

The importance of carbonic acid/bicarbonate buffer arises from the rapid equilibrium between bicarbonate and carbon dioxide, achieved by the catalytically efficient carbonic anhydrase enzyme:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Carbonic anhydrase has an essential role in converting carbon dioxide released by the cells to bicarbonate, which is converted back to carbon dioxide in the lungs. The pH of the plasma is determined by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log ([\text{bicarbonate}] / [\text{carbonic acid}])$$

where pK_a is a fixed value for carbonic acid (=pH when $[HCO_3^-]=[H_2CO_3]$)

Since carbonic acid is in a rapid equilibrium with carbon dioxide, thanks to carbonic anhydrase, the plasma pH is a function of the ratio between bicarbonate concentration and carbon dioxide pressure (pCO_2). The bicarbonate concentration is physiologically regulated at the kidneys, while carbon dioxide is washed out by the lungs. The control of this buffer system at two different organs contributes to the fixation of plasma pH. Metabolic acidosis (a lower pH) is characterized by compensatory reduction of pCO_2. Respiratory acidosis is characterized by retention of carbon dioxide and high pCO_2. Respiratory alkalosis is characterized by low pCO_2, while in metabolic alkalosis there is compensatory elevation of pCO_2.

Blood gas analyzers use the Henderson-Hasselbalch equation to calculate the bicarbonate concentration, which is used for calculation of the anion gap. The latter increases with metabolic acidosis due to increased unmeasured acids resulting from metabolism, e.g., lactic acid and ketone bodies.

pH measurement

pH can be measured with the help of hydrogen ion-sensitive electrodes (in pH meters and blood gas analyzers). Rough estimation of pH is achieved by indicators. An indicator is a weak acid that has the protonated and the dissociated forms different in color. Since the ratio between the two forms is dependent on the pH of the medium, according to Henderson-Hasselbalch equation, the indicator color depends on the pH of the medium. Examples of pH indicators are phenolphthalein, methyl orange, litmus paper, etc. Urine dipsticks contain a pad with indicators for measuring the urine pH. A pad for protein measurement may use the principle of “protein error of indicators”; an indicator color changes in presence of protein. A pad for specific gravity may depend on release of hydrogen ions due to cations in the urine.

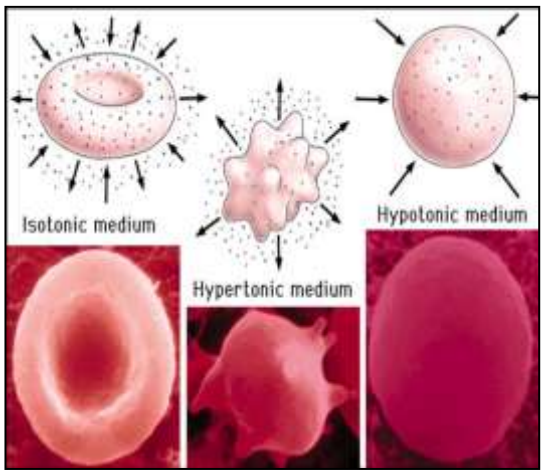
Diffusion

If a drop of ink is added to a glass of water, we find that the ink color spreads gradually to fill the whole glass. If a piece of sugar is left to dissolve in a glass of water, all the water in the glass becomes sweet. This is because freely mobile molecules or ions spread to fill all the available space. This is called diffusion. Diffusion is important for interchange of nutrients, gases, and waste products across body compartments, e.g., between intracellular and extracellular fluids.

Osmosis

If there is a barrier that allows the passage of water but prevents the passage of the solute, water moves towards the trapped solute. Water is forced from dilute to concentrated solutions across semi-permeable membranes that prevent the passage of solute particles. This is called osmosis. The pressure that results from difference in concentration of two solutions is thus osmotic pressure. Osmolarity is the concentration of particles (molecules or ions) that cannot pass through the semi-permeable membrane, thus they pull water.

The cell membrane acts as a semi-permeable membrane that allows only water and some other molecules to pass. If red blood cells are placed in water, they take water by osmosis, swell, and rupture leading to hemolysis. Therefore, we cannot give water or hypo-osmolar (hypotonic) fluids to a patient by intravenous infusion. On the other hand, hyper-osmolar (hypertonic) solutions take water from the cells, causing the cells to shrink and crenate. Intravenous infusion fluids should be isotonic, having the same osmolarity like that of the intracellular fluid.



Plasma proteins do not escape from the capillary endothelium, and thus exert an osmotic pressure (oncotic pressure) that normally pulls water from the interstitial space, preventing the development of edema. In cases of liver cirrhosis, with liver cell failure, there is hypo-albuminemia due to failure of the liver cells to synthesize adequate plasma albumin (major plasma protein). This leads to edema and ascites. Osmosis plays other several important roles in the human physiology as well as some pathological conditions, e.g., the concentration of urine by normal kidney function and the osmotic effect of non-absorbed sugars in the intestine.

In cases of brain edema, we may give the patient intravenously a substance that raises the plasma osmotic pressure, e.g., mannitol to pull water from the brain tissue. The purgative (anti-constipation) action of some used medications depends on the fact that these medications are not absorbed from the intestine, so they exert an osmotic effect that retains more fluid in the intestinal lumen. Diuretics too act by employing the osmosis phenomenon.

Plasma osmolarity

An empirical formula may be used to calculate plasma osmolarity, in mosmol/L (mOsm/L) units, using the concentrations of sodium, glucose and urea:

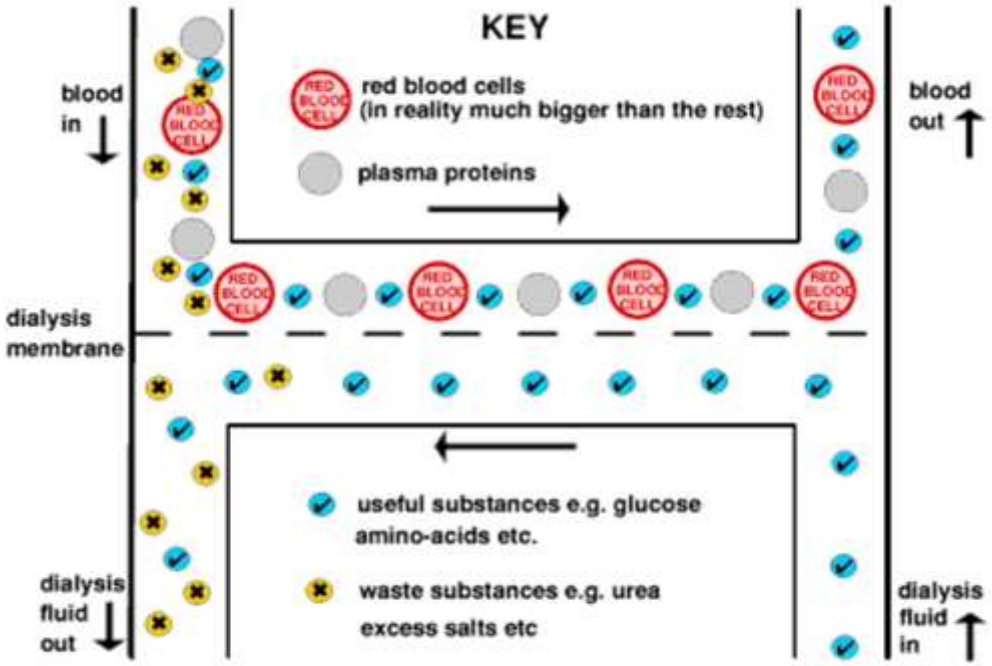
Plasma osmolarity (mOsm/L) = sodium x 1.86 + glucose (mmol/L) + urea (mmol/L) + 9

Osmolality

Osmolality is the concentration of osmotically active particles per kilogram of water. It is measured by an osmometer, which determines the depression of freezing point as a function of the concentration. It can be applied to the urine or any liquid. Normal plasma osmolality equals 275-295 mOsm/kg. An osmolal gap, a difference between measured osmolality of the plasma and the calculated osmolarity >10, is caused by alcohol, hyperproteinemia, hyperlipidemia, etc.

Dialysis

Dialysis is the separation of small molecules and ions from large molecules and particles in a solution using a semi-permeable membrane. In hemodialysis procedure, the patient's blood is cleared of the harmful substances through the semi-permeable membrane (filter) of the dialysis unit.



Adsorption

Adsorption is the attachment of gas molecules or solute particles to the surface of a solid. Adsorption forces increase with increase of the surface area of the adsorbent. Strong adsorbents include charcoal, alumina and silica gel. It may be used clinically for getting rid of gases or poisons.

Study Questions

Choose one best answer for every question of the following:

1- A hydrogen bond is
(A) Attraction between positively and negatively charged ions.
(B) Attraction between hydrogen atoms.
(C) A covalent binding of oxygen and hydrogen.
(D) A weak electrostatic attraction.

2- Which of the following is freely soluble in water?
(A) Cholesterol.
(B) Calcium phosphate.
(C) Ethyl alcohol.
(D) Phenol.

3- Which of the following may be considered a hydrophobic substance?
(A) Glucose.
(B) Ethanol.
(C) Cholesterol.
(D) Bile salts.

4- A hydrotropic substance is amphipathic meaning that it …. …. ….
(A) has hydrophilic and hydrophobic parts of its molecules.
(B) reacts with acids and bases.
(C) has no electric charges.
(D) is not miscible with water.

5- Molecular weight of glucose = 180. What is the concentration of blood glucose that equals 180 mg/dL?
(A) 1 mmol/L.
(B) 1.8 mmol/L.
(C) 10 mmol/L.
(D) 18 mmol/L.

6- Specificity of a blood test is which of the following?
(A) Ability of the test to detect all diseased people.
(B) Percentage of test-negative subjects in a healthy population.
(C) Low variation in the lab results.
(D) Ratio of diseased people in test-positive subjects.

7- Positive predictive value of a test is which of the following?
(A) Ratio of positive results in diseased subjects.
(B) Ratio of diseased subjects in test-positive subjects.
(C) Ability to detect all diseased subjects.
(D) The numerical accuracy of the test.

8- Sensitivity of a blood test is which of the following?
(A) Ratio of test-positive to test-negative samples.
(B) Ratio of test-positive samples in a healthy population.
(C) Ratio of test-positive samples in a diseased population.
(D) Ratio of test-negative samples in a healthy population.

9- Accuracy of a test is defined as which of the following?
(A) Ratio of true positive plus true negative to the whole assayed population.
(B) Ratio of true positive plus false negative to the diseased population.
(C) The difference between positive and negative predictive values.
(D) Positive predictive value plus negative predictive value.

10- pH indicators may be used for which of the following?
(A) Controlling plasma pH.
(B) Measuring the urine pH.
(C) Raising the pH of the stomach.
(D) Lowering the pH of the intestine.

11- A patient with liver cell failure may be given albumin injection in order to
(A) give nutrition to the liver cells.
(B) make glucose in liver cells by gluconeogenesis.
(C) help the liver cells to synthesize more albumin.
(D) increase the osmotic pressure of plasma.

12- The phrase “5% glucose” is commonly used to describe
(A) 5 g glucose in 100 L of water.
(B) 5 mg glucose in 100 mL of solution.
(C) 5 g glucose per dL of solution.
(D) 5 mg/L glucose solution.

13- One gram of a toothpaste contains 8 μg of sodium fluoride. Fluoride constitutes 45% of sodium fluoride. Fluoride content in the toothpaste is
(A) 3.6 ppm.
(B) 4.5 ppm.
(C) 8.0 ppm.
(D) 845 ppm.

14- In osmotic fragility test,
(A) red blood cells are subjected to hypertonic solutions of various strengths.
(B) weak erythrocytes hemolyze at salt concentrations that do not cause hemolysis of normal red blood cells.
(C) normal red blood cells hemolyze at salt concentrations that do not cause hemolysis of weak red blood cells.
(D) weak red blood cells can stand dilute solutions more than normal red blood cells can do.

15- The phenomenon of adsorption may be used clinically for treatment of
(A) flatulence by adsorbing gases in the intestine on charcoal.
(B) diarrhea by adsorbing toxins in the intestine on kaolin.
(C) accidental poisoning by adsorbing the poison on charcoal .
(D) all the above.

Match each item to the proper type of unit symbol:

(A) mass (C) concentration
(B) matter (D) none

16- kg
17- mg/dL
18- mmol
19- μmol
20- mEq/L
21- mol/L
22- g/L
23- M
24- mmol/L
25- mM
26- %
27- ppm
28- mg/L
29- mM/L
30- Mmol/L
31- Kg
32- gm/L
33- Mol/L
34- Mg/L
35- G/L
36- mG
37- MG/DL
38- mmole/L
39- m mol/L
40- m/L
41- MMOL/L
42- mMol/L
43- gm
44- mg /dL
45- Gm/L
46- mg%
47- m.g/dL

Proteins

Structure

Proteins are the major organic compounds of the tissues. Besides, proteins are an essential component of the diet. They are formed of the major elements of organic compounds. In addition to carbon, hydrogen, and oxygen, they are essentially nitrogenous compounds. Sulfur is also an important component of proteins.

Proteins are aminoacid polymers, where the aminoacids form a long-chain molecule. Thus, proteins are macromolecules. Their molecular weight depends on the number and types of their aminoacids. An average protein, for example, may be about five hundred times larger than glucose.

Proteins are synthesized by the cells from aminoacids according to genetic code. Therefore, proteins differ from one species to another, and from one individual to another.

A short list of important functions of proteins:

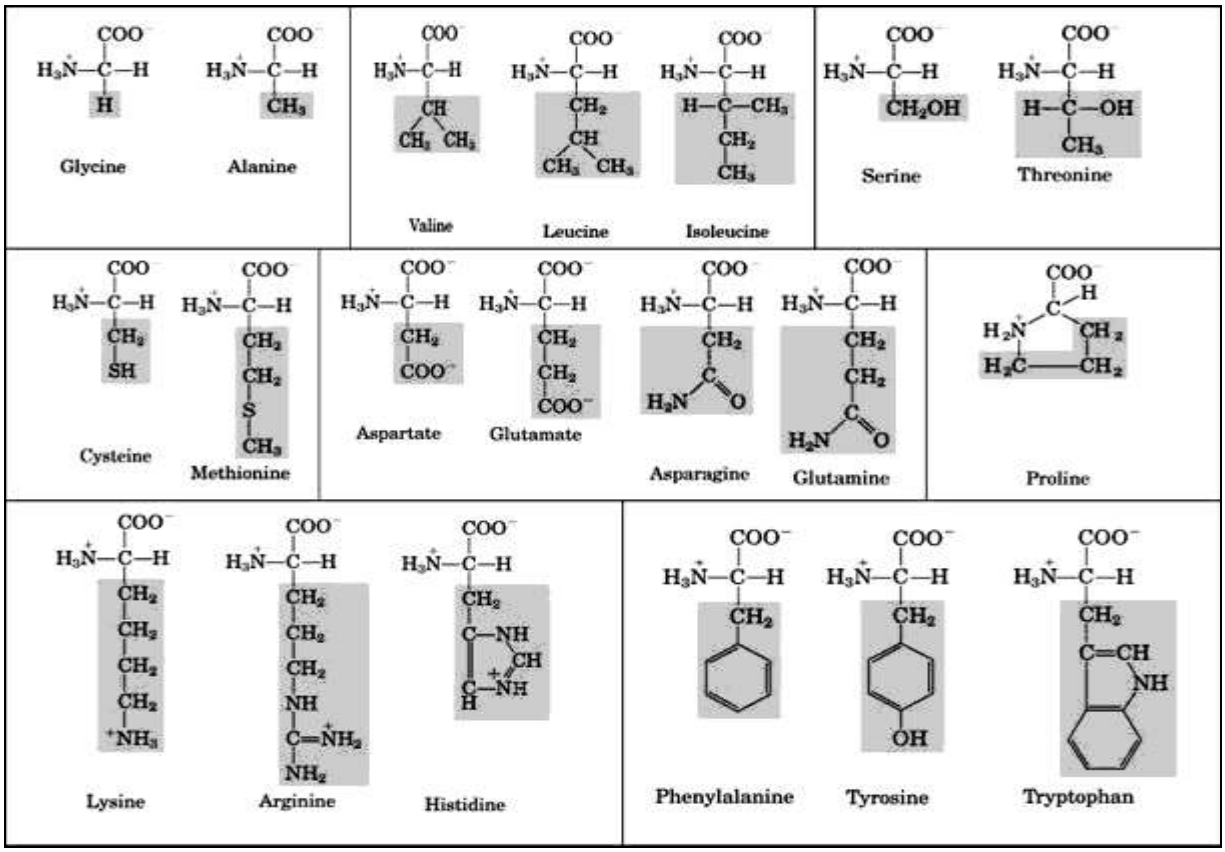
- Structural function: biological membranes, the cytoskeleton and the intercellular matrix.
- Contractile function: by actin and myosin.
- Catalytic function: by enzymes.
- Transport function:
  - Respiratory gases: oxygen and carbon dioxide by hemoglobin.
  - Lipids by the plasma lipoproteins.
  - Metals like iron and copper by plasma transferrin and ceruloplasmin respectively.
  - Steroid and thyroid hormones by their specific hormone-binding proteins.
- Regulatory function: by many hormones and the hormone receptors.
- Protective function:
  - Antibodies against invading pathogens.
  - Coagulation proteins for preventing excessive bleeding.
  - Antiproteases for protection of tissue proteins against the harmful effect of protease enzymes.
- Vision: by rhodopsin and other proteins in the eye retina.
- Chromosome packing: by histones and other proteins in the cell nucleus.
- Buffering action of hemoglobin and plasma proteins.
- Osmotic pressure (oncotic pressure) of plasma proteins, mainly albumin.
- Cell fuel: aminoacids by proteolysis on starvation.
- Diet proteins are the source of essential aminoacids and the main source of nitrogen and sulfur.

Simple and conjugated proteins

Proteins may be simple proteins, formed of only aminoacid chains, e.g., plasma albumin. Usually, proteins are conjugated with other chemical groups, forming complex proteins. Glycoproteins, like anterior pituitary hormones and immunoglobulins, contain conjugated carbohydrate. Lipoproteins contain lipids, e.g., plasma lipoproteins and membrane lipoproteins. Nucleoproteins have nucleic acids, e.g., ribosomes. Phosphoproteins have attached phosphate groups, e.g., casein of milk. Chromoproteins contain conjugated pigments, e.g., heme-proteins like hemoglobin. Metalloproteins contain metals like copper in ceruloplasmin, zinc in carbonic anhydrase enzyme and iron in ferritin and transferrin.

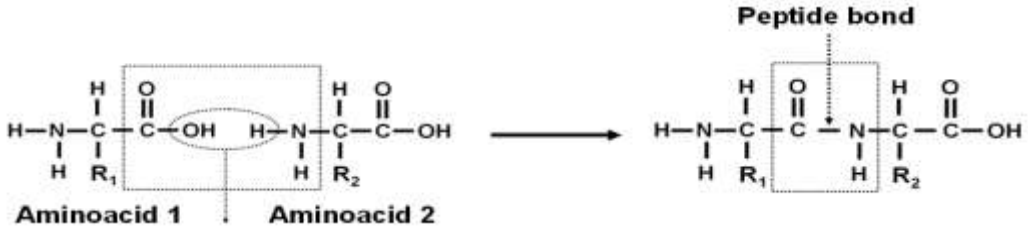
Aminoacids

Twenty different aminoacids share in protein biosynthesis. All are alpha-amino carboxylic acids. The amino group is attached to the α-carbon (carbon # 2), which is the first carbon next to the carboxyl group. The rest of the molecule, attached also to the α-carbon, is different from one aminoacid to the other. These different parts of the aminoacids, referred to as "R" become side groups of the polypeptide chain, while the amino group, the α-carbon and the carboxyl group constitute the backbone of the chain. The twenty aminoacids are shown below.

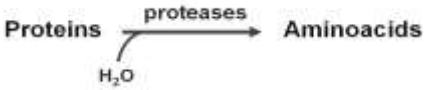


Peptide bond and peptides

Aminoacids are joined together by covalent bonds that result from condensation of the carboxyl group of one aminoacid and the amino group of the next aminoacid. These bonds are called peptide bonds. Two aminoacids form a dipeptide molecule, while three aminoacids form a tripeptide, and so on. These are called oligopeptides. Many hormones are oligopeptides in nature, e.g., thyrotropin releasing hormone (tripeptide), oxytocin (nonapeptide), and angiotensin II (octapeptide). Polypeptide chains contain a higher number of aminoacids (more than 20 aminoacids), e.g., insulin. Proteins contain hundreds of aminoacids linked by peptide bonds.



Proteolysis

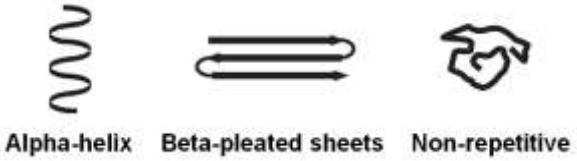


Proteins are hydrolyzed by proteolytic enzymes: proteases. These enzymes catalyze the hydrolysis of peptide bonds, yielding smaller peptides and ultimately free aminoacids. This process takes place in the gastrointestinal tract where the proteolytic enzymes, e.g., pepsin and trypsin digest the diet proteins. Tissue proteins are also subject for proteolysis, where old molecules are broken down and new molecules are synthesized. This is called turnover of tissue proteins.

Protein organization

The biological function of a protein is dependent on its shape and active groups. A protein molecule is highly organized, described in four levels of organization. The specific sequence of aminoacids, their number and types is called the primary structure of a protein.

Secondary structure is the description of the folding of the polypeptide chain. The aminoacid chain may be folded in the form of alpha-helix, beta-pleated sheets, non-repetitive pattern or a combination of these.

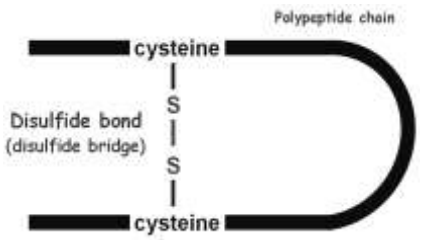


Tertiary structure describes the super-folding to give the final 3D shape of the protein molecule. For instance, a protein molecule can be a fibrous protein, or a globular one. The molecule can still have specifically shaped regions (domains) to suit the function of the protein.

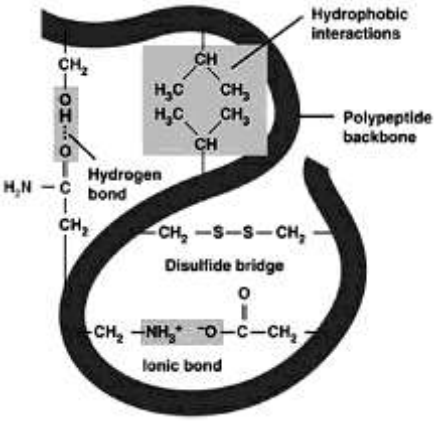
Quaternary structure describes only the proteins that are formed of a combination of more than one polypeptide chain, giving a dimer, trimer, tetramer, etc. These proteins contain more than one fully organized polypeptide chain combined by non-covalent bonds. An example of these proteins is creatine kinase enzyme, a dimer that has two types of chains: M and B. Thus, we have creatine kinase: MM, MB, and BB. Another example of proteins with quaternary structure is hemoglobin, a tetramer with two types of chains.

Bonds in proteins

The primary structure of a protein is dependent on peptide bonds between the aminoacids in series. Higher levels of organization are maintained by other forces. The disulfide bond, or the disulfide bridge, between to cysteine residues of the protein is another covalent bond that adds to the stability of protein structure.



Non-covalent bonds involved in protein conformation include the hydrogen bond and the ionic bond (salt bridge) between oppositely charged groups of constituent aminoacids. Hydrophobic interaction is important for proper protein conformation, whereby hydrophobic groups are directed away from the aqueous phase and brought in close proximity to each other. Hydrophobic groups characterize the aminoacids: alanine, the three branched chain aminoacids (valine, leucine, isoleucine), methionine, proline, phenylalanine, and tryptophan. Van der Waals forces (nonspecific attraction forces) also play a role in protein conformation.



Shape of proteins

Fibrous proteins have an axial ratio (length/width) > 10. They are usually insoluble, and perform structural functions. They are often called scleroproteins. Fibrous proteins include collagen, elastin, and keratin. These are animal proteins, not found in plants. Globular proteins, on the other hand, are soluble and perform catalytic and other functions. They include plasma albumin and globulins and soluble cytosolic proteins.

Protein denaturation

The physical, chemical & biological properties of a protein are dependent on its proper conformation. Many physical and chemical agents can cause a disruption of protein organization, leading to a change of the physical, chemical and biological properties of the native protein. This is called denaturing of the protein, or protein denaturation.

Protein denaturation can also be defined as disruption of protein organization, short of its primary structure. Only non-covalent bonds are disrupted. Peptide bonds are not affected. Denaturation of protein is usually irreversible.

Denaturing agents include heat, irradiation, vigorous shaking, repeated freezing and thawing, organic solvents, strong acids and alkalis, and salts of heavy metals.

Denatured protein loses its physiological function. It becomes more susceptible to digestive enzymes, less soluble and less antigenic.

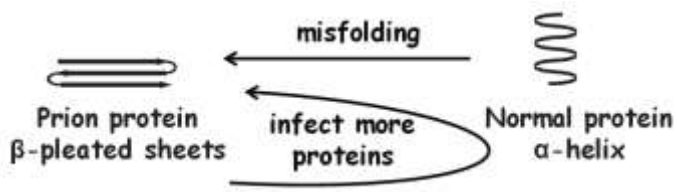
Knowing the effect of denaturing agents on proteins encourages us to deal carefully with blood samples intended for analysis. Clinically used disinfectants depend largely on their denaturing of the proteins of microorganisms. Cooking of food makes it easier to digest by proteolytic enzymes and less apt to produce a hypersensitivity reaction.

Folding and misfolding of proteins

Proteins are folded during synthesis (folding for one time). Chaperones are specific proteins that help in proper protein folding. They bind reversibly to unfolded segments to prevent misfolding. They may help in destruction of misfolded proteins. Heat shock proteins is a name given to a class of chaperones first discovered in bacteria exposed to high temperature. They may help keeping proper folding of proteins. Protein misfolding (spontaneous or due to gene defect) leads to an abnormal protein, which is degraded by the cell proteasomes.

Amyloidosis is a pathological condition characterized by accumulation of a misfolded protein called amyloid. This is formed of beta pleated sheets that form long fibrillar aggregates. Degenerative disorders such as Alzheimer disease are characterized by the deposition of similar proteins.

Transmissible spongiform encephalopathy (TSE), also known as Creutzfeldt-Jakob disease (mad cow disease in animals), is another disease of protein misfolding. It is caused by infectious protein particles (prions) thought to be transmitted from cadavers. The prion is folded as β-pleated sheets and it has the capacity to transform other normally folded proteins (α-helix), propagating the pathological process.



Study Questions

Choose one best answer for every question of the following:

1- The types and number of aminoacids in a protein molecule is determined by
   (A) the type of diet.
   (B) source of the aminoacids.
   (C) nucleotide sequence on DNA.
   (D) enzymes that share in protein synthesis.

2- Diet proteins are important because they are the main source of which of the following?
   (A) Carbon.
   (B) Carbon and hydrogen.
   (C) Hydrogen and oxygen.
   (D) Nitrogen and sulfur.

3- Aminoacids of proteins differ from each other in which of the following?
   (A) Carbon number 1.
   (B) Site of the amino group.
   (C) Presence or absence of carboxyl group.
   (D) The group attached to alpha-carbon.

4- The primary structure of proteins is a chain of aminoacids connected by
   (A) hydrophobic bonds.
   (B) covalent bonds.
   (C) ionic bonds.
   (D) hydrogen bonds.

5- The aminoacid chain of a protein may bend due to attraction between
   (A) lysine and glutamic acid.
   (B) lysine and arginine.
   (C) serine and proline.
   (D) glutamic acid and aspartic acid.

6- Amino acid number 1 in a protein is the
   (A) aminoacid at the N terminus.
   (B) aminoacid at the C terminus.
   (C) smallest aminoacid.
   (D) aminoacid with the biggest side chain.

7- Essential aminoacids are the aminoacids that
   (A) are not produced by protein hydrolysis.
   (B) are present in all proteins.
   (C) should be present in the diet proteins.
   (D) can be synthesized in the body.

8- High biological value proteins are those proteins that
   (A) have high caloric value.
   (B) are not hydrolyzed by digestive enzymes.
   (C) are obtained usually from plants.
   (D) contain all the essential aminoacids.

9- A dipeptide is formed of
   (A) two aminoacids and one peptide bond.
   (B) two aminoacids and two peptide bonds.
   (C) two aminoacids and three peptide bonds.
   (D) three aminoacids and two peptide bonds.

10- Regarding the structure-function relationship of proteins,
   (A) a biological function depends only on the primary and secondary structures.
   (B) non-repetitive regions have no biological significance.
   (C) both (A) and (B).
   (D) neither (A) nor (B).

11- The biological function of a protein is dependent on its
(A) shape.
(B) active groups.
(C) both (A) and (B).
(D) neither (A) nor (B).

12- What is meant by protein denaturation?
(A) Breaking of the peptide and ionic bonds.
(B) Breaking of covalent bonds only.
(C) Disturbed primary, secondary and tertiary structure.
(D) Disturbed organization with preservation of primary structure.

13- Which of the following is not expected to cause protein denaturation?
(A) Shaking.
(B) Heating.
(C) Ethyl alcohol.
(D) Physiological saline.

14- Denatured proteins become
(A) non-functional.
(B) more antigenic.
(C) difficult to digest.
(D) of no nutritional value .

15- Why may we get decreasing values of hormone immunoassay when repeating the analysis of a frozen serum sample?
(A) Denaturing of proteins, probably by interfering with normal hydrogen bonding.
(B) Altered immunological properties of the protein hormone.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

16- Why is protein denaturation usually irreversible?
(A) The primary structure is changed by denaturation.
(B) Covalent bonds are broken.
(C) Proper folding occurs once during synthesis.
(D) A high temperature is needed for regaining proper folding.

17- Chaperones are responsible for which of the following?
(A) Preventing misfolding of proteins being synthesized.
(B) Helping proper protein folding.
(C) Tagging of wrongly folded proteins to be hydrolyzed by proteasomes.
(D) A + B.

18- Which of the following is associated with protein misfolding?
(A) Amyloidosis and Alzheimer.
(B) Prion diseases such as transmittable spongiform encephalopathy.
(C) Alpha 1-antitrypsin deficiency.
(D) All the above.

19- Which of the following best describes prions?
(A) DNA core covered by protein.
(B) Viral envelop protein.
(C) Infective protein.
(D) Post-mortem decayed protein.

20- Which of the following is true about prions?
(A) They are proteins having alpha-helix structure in their normal form.
(B) They are folded as beta-sheets in their infective form.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

Carbohydrates

Composition

As their name indicates, carbohydrates are formed of carbon, hydrogen and oxygen. Some carbohydrates may also contain nitrogen. Carbohydrate molecules vary in size from simple sugars (monosaccharides) to disaccharides, oligosaccharides, and polysaccharides.

Carbohydrate Function

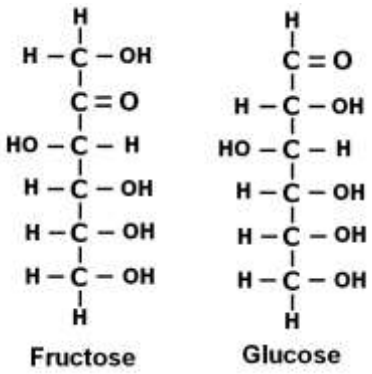
Carbohydrates constitute only a minor part of our tissues, but they form the major part of food we take. They give the food its bulk, the sweet taste, and the main calorie source. In the tissues, carbohydrates are a major component of the extracellular matrix. As a part of glycoproteins and glycolipids, they are important as structural components of biological membranes and a component of several biologically active molecules. Carbohydrates also constitute the major cell fuel, oxidized by all cells for energy production.

Monosaccharides

Monosaccharides are simple sugars that cannot be hydrolyzed to simpler form. Major monosaccharides include hexoses, having six carbon atoms, and pentoses, which have five carbon atoms per molecule. Monosaccharides may be aldo-sugars, containing an aldehyde group, or keto-sugars, which contain a keto group. Major keto-hexose is fructose, while the major aldo-hexoses are glucose, galactose, and mannose. Major aldo-pentoses include ribose and 2-deoxyribose.

Isomerism

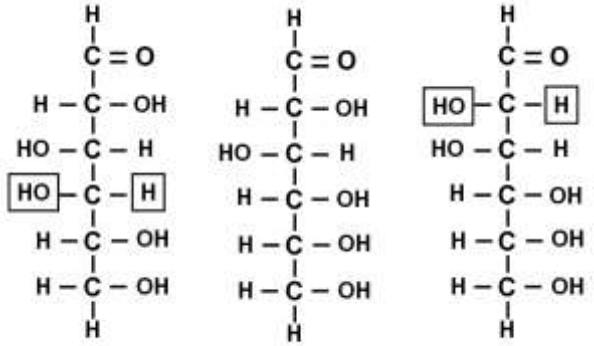
Isomerism is the presence of two or more compounds having the same molecular formula. It is a common property of organic compounds that results from the possibility of the arrangement of the same atoms in different chemical groups. Thus, glucose and fructose are isomers, the first is an aldo-hexose while the latter is a keto-hexose. Both have the same molecular formula, $C_6H_{12}O_6$.



Stereoisomerism

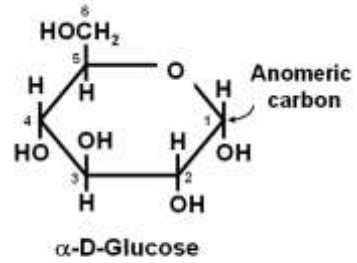
Stereoisomerism is a special form of isomerism, where two compounds share not only the molecular formula, but also the structural groups. They differ in the orientation of these chemical groups in space. Optical isomerism is a form of stereoisomerism linked to optical activity of the compounds that have an asymmetric carbon atom. An asymmetric carbon atom is a carbon atom attached to four different groups, giving the possibility of two arrangements, mirror image to each other like a right and a left hand (chiral molecules). A solution of an optically active compound can rotate the plane-polarized light either to the right, being a dextrorotatory compound, or to the left, being a levorotatory one.

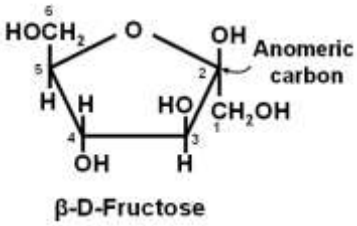
Sugars show the property of optical activity and optical isomerism. Glucose, galactose and mannose are optical isomers. Glucose and mannose differ only in the arrangement around carbon number 2, thus they are called 2-epimers. Glucose and galactose differ in the arrangement around carbon number 4, thus they are 4-epimers. All are D-sugars, having the carbon before the last with OH group to the right (in contrast to L-sugars, which are their mirror images)



Cyclic structure of sugars

A hemi-acetal linkage is formed between the aldehyde group and the alcohol group of another carbon of the same molecule, forming a five or six-atom cyclic structure. The same is true for the keto-sugars. The aldehyde or ketone carbon becomes an asymmetric carbon (anomeric carbon), having α- or β-orientation. This active carbon can undergo condensation reactions with other molecules, forming glycosides (glucosides, galactosides, etc).





Major monosaccharides

Glucose, the grape sugar, is found in fruits. It is also the main component of di- and polysaccharides, thus it is the major hydrolytic product of diet carbohydrate. It is the blood sugar and the main cell fuel. Glucose is also called dextrose.

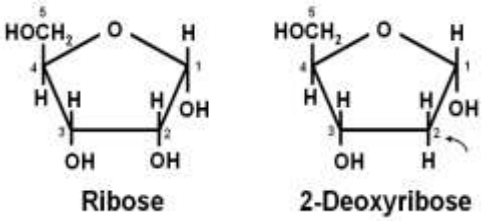
Galactose is an important constituent of glycoproteins and glycolipids. It constitutes half the hydrolytic product of lactose, thus it is half the carbohydrate of a baby diet.

Mannose, the manna sugar, is an important component of glycoproteins. By reduction, it is converted to mannitol, the sugar alcohol that is used in pharmaceutical preparations.

Fructose, the fruit sugar, constitutes half the hydrolytic product of sucrose. It is sweeter than glucose and used as a sweetener of various products. It is prepared in industry by isomerization of glucose resulting from hydrolysis of starch, forming high-fructose syrup. It is found naturally in honey. It is the semen sugar. A synonym for fructose is levulose.

Ribose and deoxyribose are two pentoses that are essential for nucleotide and nucleic acid synthesis.

Other pentoses include: arabinose and xylose, constituents of glycoproteins, and ribulose and xylulose, intermediates in metabolism.



Disaccharides

Disaccharide molecules can be hydrolyzed by specific enzymes or by boiling with strong acids, producing two monosaccharides. Major disaccharides include sucrose, lactose, and maltose.

Sucrose, the table sugar, is the most common sugar we know. It is a plant sugar obtained from sugar cane or beet. It is hydrolyzed by the intestinal sucrase enzyme to glucose and fructose.

Lactose, the milk sugar, is the main carbohydrate for babies. It is hydrolyzed by the intestinal lactase enzyme to glucose and galactose. It is a reducing sugar that can be found in the urine of lactating women. Many adults develop milk intolerance due to deficiency of intestinal lactase enzyme. Lactose is different from lactulose, the synthetic sugar (composed of galactose and fructose) that is used as an osmotic laxative and for treatment of hyper-ammonemia.

Maltose, the malt sugar, results mainly from the hydrolysis of starch by the amylase enzyme. It is hydrolyzed by the intestinal maltase enzyme to two glucose molecules.

Polysaccharides

Polysaccharides can be hydrolyzed producing many monosaccharide molecules. Starch is a poly-glucose, found in grains and tubers. It is formed by α-glucosidic linkages, forming straight and branched chains. It is the main carbohydrate taken in diet in rice, bread, potatoes and various products made from grain flour. As stated earlier, its complete hydrolysis results in glucose.

Glycogen is the animal starch, or the storage form of carbohydrate in the liver and muscles. It is a poly-glucose and can be hydrolyzed like starch.

Cellulose is the poly-glucose, formed by β-1$\rightarrow$4 glucosidic linkage, found in plant cell wall. It is insoluble and is not digested by human enzymes. It constitutes a diet fiber that makes the indigestible food bulk. It facilitates the movement of the intestine and prevents constipation. It is especially important in low-calorie diet, as it adds no calories. It is digested by the intestinal microorganisms of the herbivorous animals.

Dextrans are poly-glucose, produced by certain bacteria. They are used for the preparation of plasma substitutes.

Pectins are mixed polysaccharides present in fruits of some plants. It is made of galactose, arabinose, and galacturonic acid. It is a soluble gelatinous carbohydrate used in food preparations.

Inulin is a poly-fructose (fructosan) from plants. It is the reference material for measuring the glomerular filtration rate (inulin clearance test).

Glycosaminoglycans

Glycosaminoglycans (GAGs) are mixed polysaccharides, formerly known as mucopolysaccharides. They are formed of repeating disaccharide units containing aminosugars and sugar acids in addition to acetyl and sulfate groups. They act as structural polysaccharides of the extracellular matrix of animal tissues, and when linked to proteins, they form proteoglycans. Glycosaminoglycans include hyaluronic acid, chondroitin sulfate, heparin sulfate, heparan sulfate, keratan sulfate, and dermatan sulfate.

Heparin is an anticoagulant released by mast cells. It contains glucosamine as the aminosugar while the sugar acids are glucuronic acid and its 5-epimer: iduronic acid. It is highly sulfated and contains acetyl groups as well.

Hyaluronic acid is found in various tissues including synovial fluid, vitreous body of the eye, connective tissue and wall of the ovum. It is formed of glucuronic acid and N-acetyl glucosamine, but no sulfate. It is hydrolyzed by hyaluronidase enzyme (spreading factor) secreted by some bacteria and by spermatozoa.

Chondroitin sulfate is found in cartilage, bone, skin, tendons and cornea. It is formed of glucuronic acid and N-acetyl galactosamine (N-acetyl chondrosamine). It is highly sulfated at position 4 or 6 of N-acetyl galactosamine.

Glycoproteins

Glycoproteins (mucoproteins) are proteins to which is attached a carbohydrate branched or non-branched chain, up to 15 units. Hexoses attached include mannose, galactose, and L-fucose (6-deoxy-L-galactose). Also included are N-acetyl-galactosamine and N-acetyl-glucosamine. Pentoses include xylose and arabinose. Sialic acids (acyl-derivatives of the nine-carbon sugar acid neuraminic acid) are also present, mainly N-acetyl-neuraminic acid (NANA). No uronic acids are found in glycoproteins. Carbohydrate chain is attached to the protein through an N-glycosidic linkage to an asparagine residue, or an O-glycosidic linkage to a serine residue.

Most proteins are glycoproteins. Of these are structural proteins, e.g., collagen, many enzymes, plasma globulin, and some hormones, e.g., FSH, LH, TSH, and HCG.

Nucleosides and nucleotides

Pentose sugars form glycosidic bonds with nitrogenous bases. The resulting molecules are called nucleosides. Phosphorylation of the pentose in a nucleoside leads to the formation of a nucleotide, i.e., a nucleotide is a nucleoside phosphate. Nucleotides perform diverse functions. They are important for formation of coenzymes and activated intermediates of metabolism, for signal transduction, and in energy transformations. Nucleotides are the building blocks of nucleic acids, from which their name is derived.

Carbohydrate in diet – lactose intolerance

The major carbohydrates of adult diet are the polysaccharides: starch and cellulose and the disaccharide: sucrose, in addition to varying quantities of lactose, glucose and fructose. Digestion of carbohydrates starts at the mouth by the action of salivary amylase on starch. Pancreatic amylase then completes the digestion of starch to maltose in the intestine. The intestinal disaccharidases: maltase, sucrase and lactase digest the disaccharides to their constituent monosaccharides.

The major end-products of carbohydrate digestion are glucose, fructose and galactose, in addition to the indigestible cellulose.

Deficiency of lactase results in lactose, or milk, intolerance. This is a common condition in adults. Retention of lactose in intestinal lumen causes an osmotic effect, producing diarrhea. Gas production by bacterial action on lactose adds to the complaint of the patient. Lactose-free dairy products, e.g., yogurt, are recommended in such cases. Some patients get relieved upon replacing milk with yogurt in their diet.

Intestinal mucosal disaccharidase deficiency can result from mechanical disruption of the surface layer of intestinal mucosa following enteritis. This may necessitate withholding milk and other disaccharide-containing food. Low FODMAPs (fermentable oligo-, di-, and mono-saccharides and polyols) diet is recommended for the management of irritable bowel syndrome.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is a monosaccharide?
(A) Fructose. (C) Sucrose.
(B) Lactose. (D) Cellulose.

2- Dextrose is which of the following?
(A) Fructose. (C) Sucrose.
(B) Glucose. (D) Dextran.

3- Levulose is which of the following?
(A) Fructose. (C) Lactose.
(B) Glucose. (D) Cellulose.

4- Sucrose hydrolysis gives which of the following?
(A) Fructose. (C) Glucose and fructose.
(B) Glucose. (D) Glucose and galactose.

5- What is the milk sugar?
(A) Sucrose. (C) Glucose.
(B) Lactose. (D) Galactose.

6- Complete hydrolysis of starch results in which of the following?
(A) Sucrose. (C) Glucose.
(B) Maltose. (D) Fructose and glucose.

7- Which of the following is not a recognized function of diet carbohydrates?
(A) Major energy source.
(B) Food sweetening.
(C) Food bulk.
(D) Major source of nitrogen.

8- What are the major end-products of carbohydrate digestion?
(A) Glucose, fructose, galactose & starch.
(B) Glucose, fructose, galactose & glycogen.
(C) Glucose, galactose, sucrose & cellulose.
(D) Glucose, fructose, galactose & cellulose.

9- Which of the following may not be recognized as a glycoprotein?
(A) Albumin.
(B) Globulin.
(C) Collagen.
(D) Luteinizing hormone.

10- Which of the following is hydrolyzed by beta-galactosidase?
(A) Galactose.
(B) Sucrose.
(C) Lactose.
(D) Cellulose.

11- Heparin is characterized by which of the following?
(A) Being a glycoprotein.
(B) Containing N-acetyl-neuraminic acid.
(C) Carrying multiple positive charges.
(D) Activating antithrombin III.

12- Starch can by hydrolyzed to glucose by which of the following?
(A) Amylase.
(B) Maltase.
(C) β-Glucosidase.
(D) Acid hydrolysis.

13- Glucose is isomerized to fructose in food industry because fructose
(A) has a higher nutritional value.
(B) is sweeter than glucose.
(C) has zero calories.
(D) is not absorbed in the intestine.

14- The enzyme glucuronidase hydrolyzes which of the following?
(A) Glycoproteins.
(B) Glycosaminoglycans.
(C) Cellulose.
(D) β-Galactosides.

15- Fehling test may give a false positive result if the urine contains which of the following?
(A) Glucose.
(B) Lactose.
(C) Sucrose.
(D) Any oxidizing substance.

16- What is the sugar we usually add to tea?
(A) Glucose.
(B) Lactose.
(C) Sucrose.
(D) mannose.

17- Sorbitol is a sugar alcohol (polyol) used as a nonabsorbable sweetener. It results from
(A) oxidation of glucose.
(B) oxidation of fructose.
(C) reduction of glucose.
(D) reduction of mannose.

18- Invert sugar is
(A) the hydrolysate of sucrose.
(B) a mixture of glucose and fructose.
(C) levorotatory.
(D) all the above.

19- Carbohydrate may be attached to which aminoacid of the protein?
(A) Alanine.
(B) Proline.
(C) Serine or asparagine.
(D) Glycine or proline.

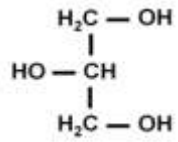
Lipids

Composition

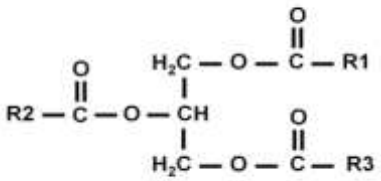
Lipids are a major component of tissues. With proteins, they constitute the bulk of organic matter. Lipids are also an important diet component. Chemically, lipids are heterogeneous. A shared property of most lipids is the solubility in organic solvents, a physical rather than a chemical property. The most common form of lipids is triacylglycerols (triglycerides).

Triacylglycerols

Triacylglycerols are esters of fatty acids and glycerol. They are found as fats and oils and as tissue depot fat. Depot fat is a fuel store, besides other functions. Lipolysis (hydrolysis of ester bonds of fat), catalyzed by lipase enzymes, yields free fatty acids. Fatty acids are an important cell fuel, in addition to their other uses.



Glycerol

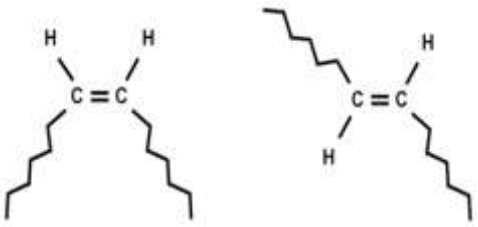


Triacylglycerol

Fatty acids

Fatty acids are usually long chain carboxylic acids. Saturated fatty acids have no double bonds in their hydrocarbon chain, hence cannot accept addition of more hydrogen. The presence of one or more double bonds in the hydrocarbon chain makes the fatty acid unsaturated, meaning that it can accept more hydrogen. Natural fats contain a mixture of fatty acids, saturated and unsaturated.

Naturally occurring unsaturated fatty acids have mostly a *cis*-configuration. The *cis*/*trans* configuration is a type of stereoisomerism that accompanies the double bond. A molecule in the *cis*-configuration is kinked and is difficult to pack in a solid crystal. Therefore, triacylglycerols with a high ratio of unsaturated fatty acids have a low melting point and are usually liquid at room temperature, e.g., corn oil, olive oil and other vegetable oils compared to butter oil (samna).



cis *trans*

Cold-water fish, e.g., tuna and mackerel, have lipids with highly unsaturated fatty acids. Plants growing in hot tropical areas have a high content of saturated fatty acids in their oils, e.g., palm and coconut oils. Palm oil, though a plant oil, may be solid in a temperate climate due to its high content of saturated fatty acids. *Trans*-fatty acids are formed as a side product during hydrogenation of plant oil to produce a solid plant fat (artificial samna). Margarine (artificial butter) may have a high *trans*-fatty acid content.

The following table lists some common fatty acids, showing their length (number of carbon atoms) and number of double bonds. The positions of the double bonds, starting the count from the carboxyl carbon, are shown between brackets.

Name	Length:Double bonds	Omega	Common Occurrence
Palmitic acid	16:0	Saturated	Animal fat, palm oil.
Stearic acid	18:0		
Palmitoleic	16:1(9)	ω-7	most fats and oils
Oleic acid	18:1(9)	ω-9	Olive oil, most common.
Linoleic acid	18:2(9,12)	ω-6	Corn oil, sunflower oil.
Linolenic acid	18:3(9,12,15)	ω-3	Linseed, less in soybean oil.
Arachidonic acid	20:4(5,8,11,14)	ω-6	Peanut.
Eicosapentaenoic acid (EPA)	20:5(5,8,11,14,17)	ω-3	Fish oil.
Docosahexaenoic acid (DHA)	22:6(4,7,10,13,16,19)		

Palmitic acid is synthesized in human cells. Fatty acids can be elongated, two carbons at a time, at the carboxyl side. Desaturase system inserts a double bond at carbon 4, 5, 6 or 9. Therefore, oleic acid can be synthesized from palmitic acid. By elongation and desaturation, the position of the last double bond from the end of the hydrocarbon chain, omega (ω) end, remains the same. In other words, by elongation and more desaturation, an ω-6 fatty acid remains ω-6 and an ω-3 acid remains ω-3. No new double bond can be inserted before ω-7.

Polyunsaturated fatty acids (having more than one double bond) that cannot be synthesized by our cells are called essential fatty acids. They should be taken in food. These include linoleic acid (18:2, ω-6) and linolenic acid (18:3, ω-3). They are important for membrane phospholipids. They also act as precursors for longer, more unsaturated fatty acids. Arachidonic acid, for example, can be synthesized from linoleic acid, since both belong to ω-6 series.

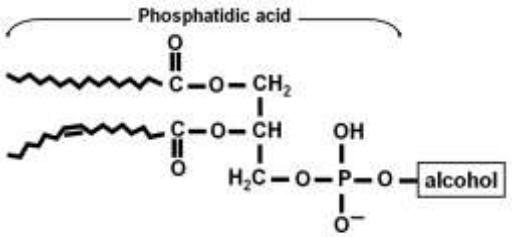
Unsaturated fatty acids are important in the diet for their structural function (membrane phospholipids), eicosanoid production, and their protecting effect against hypercholesterolemia and atherosclerosis. EPA and DHA of fish oil are important for nervous tissue membranes, and EPA is important to guard against platelet aggregation and coronary heart disease.

Structural Lipids

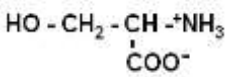
Structural lipids are not metabolically active fuel stores, but serve primarily a structural function. Their chemical composition suits well their function. These include phospholipids, glycolipids and cholesterol.

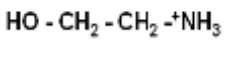
Phospholipids

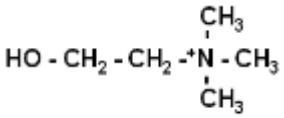
As their name indicates, phospholipids contain phosphate groups. Glycero-phospholipid is a class of these molecules that includes phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin), phosphatidyl serine, and phosphatidyl inositol. Cardiolipin is diphosphatidyl glycerol.

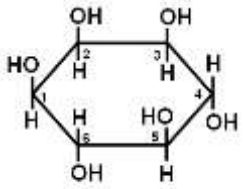


A typical glycero-phospholipid

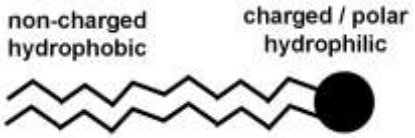

serine


ethanolamine


choline


myo-inositol

The fatty acid esterified to carbon 2 of glycerol is usually an unsaturated one. Ether phospholipids contain an ether bond instead of the ester bond at carbon 1 of glycerol. These include plasmalogens and platelet activating factor (PAF).



The structure of a phospholipid molecule can be symbolized by a hairpin shape. The head represents the phosphate group and the alcohol attached to it, the charged or polar part of the molecule. The two arms represent the hydrocarbon chains of the fatty acids, the hydrophobic part of the molecule. This amphipathic structure of the phospholipid molecule makes it an excellent biological membrane component.

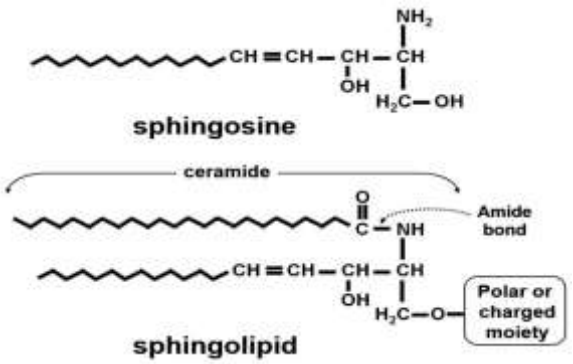
Membrane phospholipids are important for blood coagulation reactions. They are also the source of arachidonic acid for eicosanoid production. Phosphatidyl inositol bisphosphate is important for production of the second messengers: inositol triphosphate and diacylglycerol. The amphipathic nature of phospholipids is also important for the structure of plasma lipoproteins and for lung surfactant. Lung surfactant is primarily dipalmitoyl phosphatidyl choline in a complex with a number of small proteins. It decreases the surface tension of the aqueous lining of the alveolar epithelium and prevents the lung collapse.

Antiphospholipid syndrome (Hughes syndrome) is characterized by autoantibodies against phospholipids and protein/phospholipid complexes, mainly anticardiolipin. Disease manifestations include repeated miscarriages and episodes of deep vein thrombosis.

Lysophospholipids, e.g., lysolecithin result from hydrolysis of the ester bond at carbon 2 of glycerol by the action of the enzyme phospholipase A_2. A high activity of this enzyme, e.g., from snake venom can lead to disruption of membrane structure and hemolysis.

Sphingolipids

Sphingolipids do not contain glycerol, but the long chain alcohol, sphingosine. Sphingosine is synthesized from serine and palmitic acid. Linking of a long chain fatty acid by an amide bond to sphingosine forms ceramide. Linking of a polar or a charged moiety to the primary alcohol group of ceramide forms sphingolipids.



- If the charged moiety is phospho-choline, the molecule is a sphingomyelin (a sphingo-phospholipid).
- If the polar moiety is carbohydrate, the molecule is a glycolipid. According to the type of carbohydrate, glycolipids may be:
  - Glucose cerebrosides, with one glucose attached.
  - Galactose cerebrosides, with one galactose.
  - Gangliosides, with oligosaccharide that contains one or more sialic acids, e.g., N-acetyl-neuraminic acid (NANA).
  - Sulfatides, with sulfated galactose.

Sphingolipids are important constituents of cell membranes. Although sphingolipids contain no glycerol, they are similar in structure to the glycerophospholipids in that they have a hydrophilic region and two fatty acid-derived hydrophobic tails.

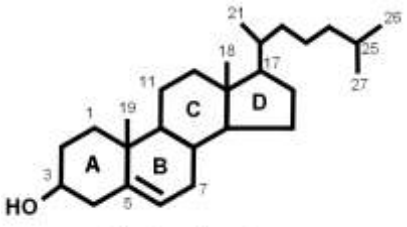
Sphingolipidosis (lipid storage diseases)

Sphingolipids released with membrane degradation are digested in endosomes after fusion with lysosomes. Lysosomes contain many enzymes, each of which removes specific groups from individual sphingolipids. Genetic deficiencies of many of these enzymes are known, which cause lipid storage diseases (sphingolipidoses) characterized by cellular inclusion bodies.

Gaucher disease is due to deficiency of glucocerebrosidase enzyme and is characterized by accumulation of glucocerebrosides, with hepatosplenomegaly and erosion of bones. Niemann-Pick disease is due to sphingomyelinase deficiency leading to accumulation of sphingomyelins, with hepatosplenomegaly, microcephaly, and early death. Tay-Sachs is due to deficiency of hexosaminidase A, with accumulation of ganglioside G_{M2}, leading to mental retardation, blindness, muscular weakness, and early death. Other sphingolipidoses show variable clinical pictures and accumulate lipids corresponding to the deficient enzyme. Treatment of these diseases is by enzyme replacement therapy and gene therapy trials.

Cholesterol

Cholesterol is the parent steroid. From it, the bile salts, vitamin D_3, and steroid hormones are synthesized. Its most important function however is being a constituent of biological membranes. It is a bulky hydrocarbon molecule with only one hydroxyl group that acts as its hydrophilic part. This structure fits it well in the membrane organization.



Cholesterol

The storage form of cholesterol is the esterified form, which is completely hydrophobic. Cholesterol is usually esterified with a long chain unsaturated fatty acid. It is to be stressed here that cholesterol is an animal sterol. Plant oils, whether in their natural form or hydrogenated, have virtually no cholesterol. Cholesterol is infamous for its link to atherosclerosis and coronary heart disease.

Study Questions

Choose one best answer for every question of the following:

1- What is the most common form of lipids?
(A) Free fatty acids.
(B) Free cholesterol.
(C) Cholesterol esters.
(D) Triacylglycerols.

2- Which of the following best describes essential fatty acids?
(A) Free fatty acids.
(B) Saturated fatty acids.
(C) Monounsaturated fatty acids.
(D) Polyunsaturated fatty acids.

3- Why are essential fatty acids called essential?
(A) They should be synthesized by our cells.
(B) They should be supplied by the diet.
(C) They are important in phospholipid structure.
(D) They are used for cholesterol esterification.

4- Deficiency of essential fatty acids
(A) is a common medical problem.
(B) occurs in infants taking bottle milk instead of breast milk.
(C) leads to retarded growth and skin manifestations.
(D) can be treated by giving prostaglandins.

5- Polyunsaturated fatty acids can be found more in which of the following?
(A) Plant oil and fish oil.
(B) Hydrogenated plant oil.
(C) Animal fat.
(D) Margarine (artificial butter).

6- “Omega-3 fatty acids” means which of the following?
(A) Fatty acids with three double bonds.
(B) A double bond every third carbon.
(C) The last double bond is carbon 3 from the end.
(D) Three unsaturated fatty acids esterified with glycerol.

7- Linolenic acid
(A) is an omega-6 fatty acid.
(B) is found particularly in linseed (flax) oil, and to a less extent in soybean oil.
(C) can be synthesized in mammalian cells from palmitic acid by chain elongation and desaturation.
(D) can be converted to arachidonic acid.

8- Fish oil is famous for its content of
(A) oleic acid and linoleic acid.
(B) palmitic acid and stearic acid.
(C) arachidonic acid.
(D) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

9- Oils of tropical trees, e.g., palm and coconut oils are characterized by a higher content of
(A) phosphorus.
(B) saturated fatty acids.
(C) unsaturated fatty acids.
(D) cholesterol.

10- Phospholipids are excellent for membrane structure because they are
(A) Amphipathic.
(B) High molecular weight.
(C) Osmotically active.
(D) Non-charged.

11- Lung surfactant is a complex of proteins with
(A) a lecithin (dipalmitoyl phosphatidyl choline).
(B) a sphingomyelin.
(C) a glucocerebroside.
(D) an ether phospholipid.

12- Ceramide is an essential component of all the following <u>except</u>
(A) cerebrosides.
(B) gangliosides.
(C) cardiolipins.
(D) sphingomyelins.

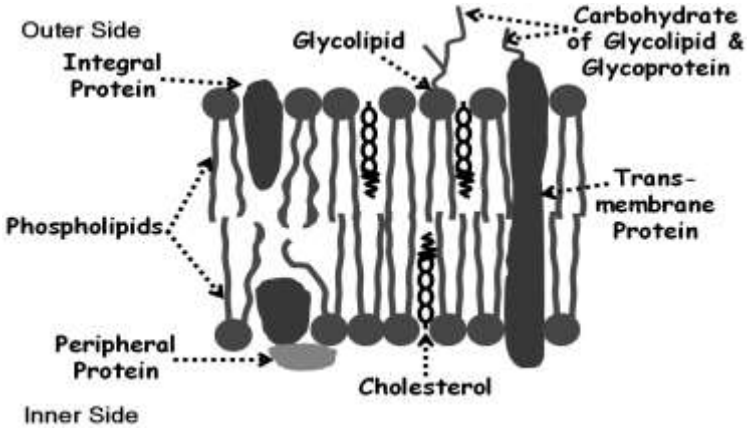
13- Sterols
(A) are steroid alcohols.
(B) include cholesterol in plant cells and ergosterol in animal cells.
(C) are present only in a free, non-esterified state.
(D) are precursors of phospholipids.

14- Cholesterol
(A) is a 26-carbon sterol.
(B) has the hydroxyl group attached to carbon 3.
(C) has no double bonds.
(D) has an eight-carbon branched side-chain attached to carbon 15.

15- What food is rich in cholesterol?
(A) Margarine (artificial butter).
(B) Palm oil.
(C) Animal fat.
(D) Hydrogenated vegetable oil.

Membrane Chemistry

Membranes are essential for compartmentation. A biological membrane separates two compartments; both are aqueous. Cell membrane, for example, separates the intracellular and extracellular fluids. Membranes are composed of lipids and proteins, plus little carbohydrate. Membrane lipids are: phospholipids, cholesterol and glycolipids. Lipid/protein ratio varies. In erythrocyte membrane, it is about 1/1 (w/w). Nerve cell membrane has a higher lipid content. Inner mitochondrial membrane has more proteins.



The fluid mosaic model for cell membrane structure that was confirmed by electron microscopy arranges the lipid component in a bilayer. The hydrophilic parts of the amphipathic lipid molecules make the two sides of the membrane. The unsaturated fatty acid hydrocarbon chains make the membrane fluid. Proteins are bathed in this lipid bilayer. Cholesterol modulates membrane fluidity. Carbohydrate is about 10% of cell membrane, in the form of glycolipids and glycoproteins lying on the outer leaflet of the membrane. They act as cell markers as well as antigenic and receptor determinant.

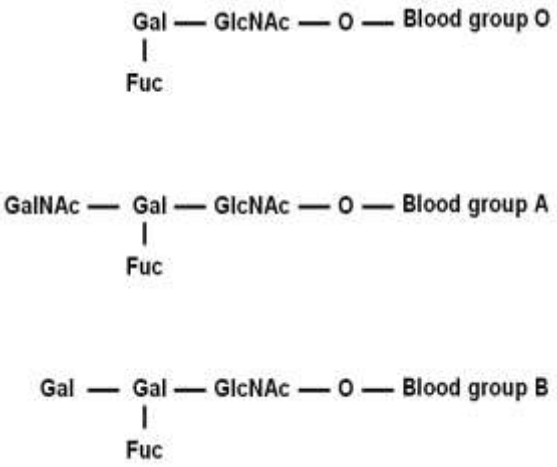
Phospholipids are asymmetrically distributed. Phosphatidyl serine and phosphatidyl inositol both carry a net negative charge and are located in the inner membrane leaflet. This asymmetry is important for marking the inside and outside of the cell. Exposure of the inner surface of platelet membrane activates the blood coagulation complexes. The presence of phosphatidyl inositol on the inner surface is important for the production of the intracellular second messengers: inositol triphosphate and diacylglycerol.

Peripheral membrane proteins can be separated from the membrane by mild treatment, e.g., salt, while integral proteins need a detergent for their separation. The integral proteins may be transmembrane (polytopic) or linked to only one leaflet of the membrane (monotopic). Peripheral membrane proteins link the membrane to the cytoskeleton.

Spectrin, a peripheral protein of the inner side of the erythrocyte membrane, is deficient in hereditary spherocytosis. Guillain-Barré syndrome is an inflammatory autoimmune neuritis wherein T-cells formed in response to viral illness attack the myelin sheath of peripheral nerves causing their demyelination. Multiple sclerosis (MS) is another disease that is characterized by degeneration of the myelin sheath around nerves, eventually interfering with nerve conduction. Hughes syndrome (Antiphospholipid syndrome) results from autoantibodies against acidic phospholipids and causes recurrent thrombosis and repeated fetal losses.

Blood group antigens

Membrane glycoproteins are essential membrane components, which act as receptors and antigenic determinant. The blood groups are determined by the specific carbohydrate residues of the membrane glycoprotein. The A antigen contains a terminal N-acetyl-galactosamine, while the B antigen has galactose instead, and the O group has neither one.



Experimental trials have been made, using specific enzymes from different species, to split off the sugar moieties defining the A and B antigens. The aim of these experiments is to convert blood group A or B red blood cells to group O, thus making a universal donor blood for transfusion.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following may <u>not</u> be a membrane component?
(A) An amphipathic protein. (C) Glycolipid.
(B) Cholesterol ester. (D) Phosphatidyl choline.

2- The fluidity of biological membranes is due to
(A) long saturated fatty acyl residues. (C) the phosphate groups.
(B) unsaturated fatty acyl residues. (D) cholesterol.

3- Water and ion channels may be provided by which membrane component?
(A) Glycolipids. (C) Peripheral proteins.
(B) Cholesterol. (D) Transmembrane proteins.

4- What are glycoproteins (mucoproteins)?
(A) Proteoglycans. (C) Mucopolysaccharides.
(B) Glycosaminoglycans. (D) Proteins that contain some carbohydrate.

5- Difference between blood group antigens is due to which of the following?
(A) Different glycosyl transferases.
(B) Different genes coding for the specific membrane protein.
(C) Difference in utilization of aminoacids to make the membrane protein.
(D) Difference in the rate of catabolism of the blood group antigens.

6- A group B blood may be hopefully used for transfusion to a group O patient after treating the donor blood with
(A) a galactosidase.
(B) a glucosidase.
(C) a glucosyl transferase.
(D) inosine and citrate.

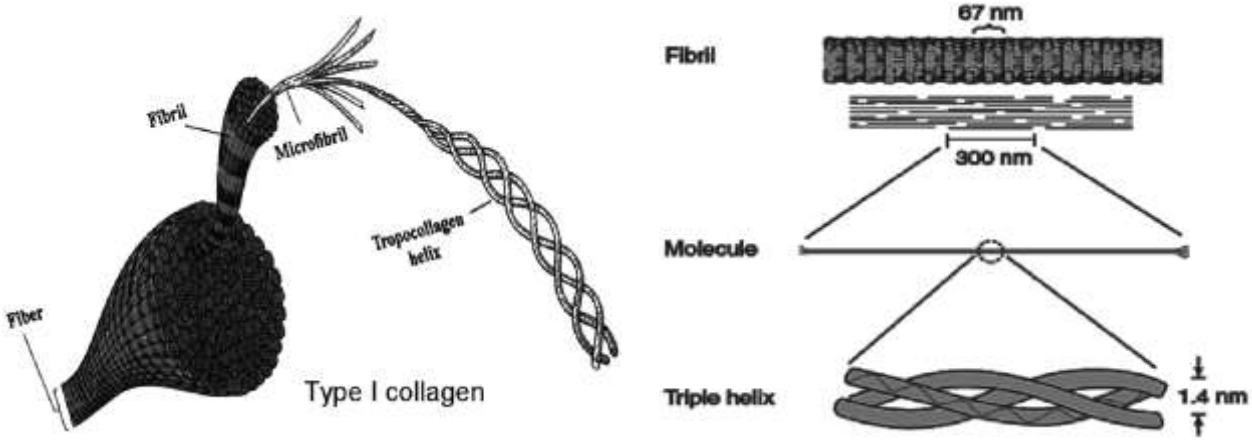
Extracellular Matrix

The extracellular matrix (ECM), or intercellular substance, is often referred to as the connective tissue. The ECM is composed of three major classes of biomolecules:

- Structural proteins**:** collagen, elastin, and keratin.
- Specialized proteins, e.g., fibrillin, fibronectin, and laminin. Laminin anchors cell surfaces to the basal lamina. Fibronectins connect extracellular matrix to the cell membrane integrins, and through which to the cellular cytoskeleton. At least 20 different fibronectins arise by alternative RNA splicing of the primary transcript from a single gene.
- Proteoglycans, composed of a protein and glycosaminoglycans (GAGs): hyaluronic acid, chondroitin sulfate, heparan sulfate, keratan sulfate, and dermatan sulfate.

Collagen

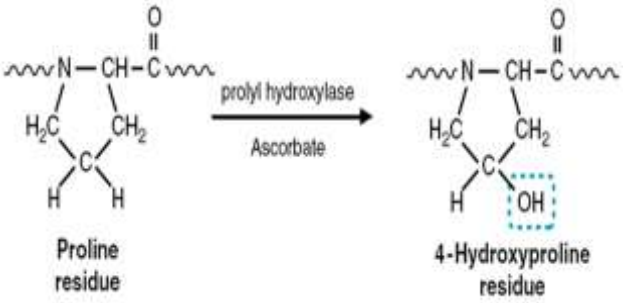
Collagen is a fibrous protein that represents one third of total body proteins. It is the major component of connective tissue. There are more than 30 different types of collagen. In tendons, which attach muscles to bones, collagen fibrils are aligned parallel to the long axis of the tendon, giving the tendon tremendous tensile strength. In bones, the fibers are arranged at an angle to each other to resist mechanical shear from any direction. In Skin, collagen forms loosely woven, flexible fibers. In cornea, collagen is stacked so as to transmit light with a minimum scattering. In extracellular matrix and vitreous humor of the eye, collagen is dispersed as a gel that gives support to the structure.



Tropocollagen is the basic structural unit of collagen. Tropocollagen molecule is a triple helix, formed of three α-chains twisted around each other in a right-handed direction forming a rope-like structure. The three chains are linked by inter-chain hydrogen bonds. Each single chain makes a left-handed twist, 3 aminoacids/turn.

The α-chain contains approximately 1,000 aminoacid residues. Collagen I contains approximately 33% glycine and 21% proline and hydroxyproline, with the sequence (Gly-X-Y)n. Every third amino acid is glycine. X is frequently proline and Y is often hydroxyproline or hydroxylysine. Glycine with its small size suits well the helix formation. Proline does not disrupt this helix, as it does with the alpha-helix secondary structure of other proteins. There are no cysteine residues, and no disulfide bonds, in mature collagen; they are found in procollagen in the part that is cleaved off. Tryptophan is also absent in collagen. Therefore, collagen and gelatin (produced by boiling of collagen) are proteins of low biological value.

Hydroxyproline and hydroxylysine are produced as post-translational modification of procollagen in the rough endoplasmic reticulum (RER), before triple helix formation. The hydorxylases involved require ascorbic acid (vitamin C) as a cofactor. Hydroxylysine residues are the sites of glycosylation (attachment of sugar moieties: galactose-glucose). Hydroxyproline, hydroxylysine and the added sugars are involved in hydrogen bonds that stabilize the triple helix.



Polymerization of tropocollagen molecules forms fibrils, which provide great tensile strength. Covalent cross-links occur both between and within the triple helical units. Cross-linking involves lysyl oxidase, an enzyme that requires O_2 and copper and oxidatively deaminates lysine (ε-amino group) to allysine (aldehyde lysine). Allysine shares in Schiff base and aldol condensations. Fibrils aggregate and cross-link to form collagen fibers.

Degradation of collagen

Collagen molecules are highly stable, with a half-life as long as several years. Collagen is degraded by collagenase, and further by matrix proteinases. This is most active during connective tissue remodeling in case of growth or injury.

Diseases of collagen

Vitamin C deficiency leads to defective hydroxylation of proline and lysine, resulting in weak collagen clinically manifested as scurvy. The disease is characterized by bone aches and fractures, delayed wound healing, loose teeth and bleeding gums.

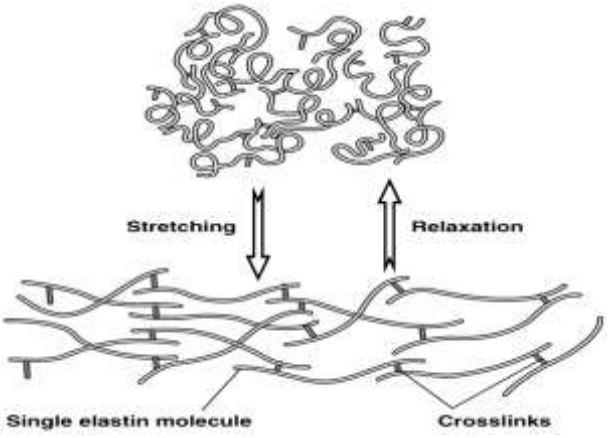
Mutation, most commonly, glycine to a bulkier aminoacid, e.g., cysteine leads to disturbance of the triple helix conformation, clinically manifested as osteogenesis imperfecta (brittle bone syndrome). The disease is characterized by soft bones, fractures, humped back, and blue sclera.

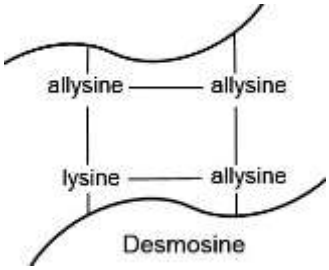
Ehlers-Danlos syndrome is due to a mutation in collagen gene or a gene of a procollagen processing enzyme, e.g., procollagen peptidase or lysine hydroxylase. Different gene defects lead to similar symptoms. There is hyperextensible, fragile skin, hypermobile joints, dislocations, varicose veins, ecchymoses, and arterial and intestinal ruptures.

In Menkes disease, an X-linked recessive disease, there is deficient cross-linking secondary to functional copper deficiency which leads to deficient lysyl oxidase reaction. It is caused by mutations in the gene encoding a Cu^{2+} efflux protein. Copper absorbed from the intestine becomes trapped in the intestinal epithelial cells and delivery to other tissues is inadequate. There is depigmented (steely) hair, arterial tortuosity and rupture, cerebral degeneration, osteoporosis, and anemia.

Elastin

Elastin is a connective tissue fibrous protein with rubber-like properties. It is present in tendons, arteries, and lungs, where stretch and recoil is important for the proper function. Elastin is rich in glycine, alanine, proline and lysine, with little hydroxyproline and no hydroxylysine. The basic structural unit is tropoelastin, a linear polypeptide ~700 aminoacids.





Elasticity of elastin is due to the presence of desmosine cross-links. Desmosine is a heterocyclic structure formed from four lysine side chains covalently linked. To be covalently linked, three lysine residues are oxidatively deaminated by lysyl oxidase enzyme and converted to allysine (aldehyde lysine) that condense with the fourth lysine (i.e., desmosine = 3 allysine + 1 lysine).

α_1-Antritrypsin deficiency

α_1-Antritrypsin (α_1-antiproteinase) inhibits a number of proteolytic enzymes present in blood and other body fluids. It comprises more than 90% of α_1-globulin fraction of normal plasma. Its main function is as anti-elastase in the lungs. The lung alveoli are chronically exposed to low levels of neutrophil elastase. In absence of α_1-antiprotease (quantitative or qualitative), elastase acts unopposed and destroys the connective tissue of alveolar walls. This causes emphysema. α_1-Antritrypsin deficiency may result from a mutation leading to misfolding of the protein and its aggregation in the liver cells (site of synthesis) causing liver cirrhosis. Smoking causes the oxidation and subsequent inactivation of a specific α_1–antitrypsin methionine residue required for binding to the target protease. Smokers have a higher rate of lung destruction and emphysema development than nonsmokers do.

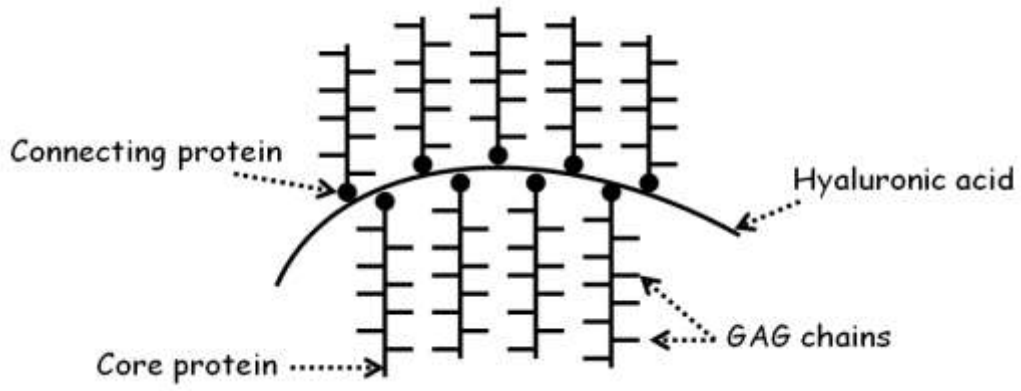
Keratin

Keratin is a scleroprotein, rich in cysteine. It is present in hair and nails. Hair is formed from dead cells, each packed with keratin macrofibrils. Keratin of hair is formed almost exclusively of α-helices. Alpha-helices are arranged together forming protofibrils, which contribute to the formation of hair fibers. Inter-chain disulfide bonds stabilize the keratin structure. Waving of hair entitles that inter-chain disulfide bonds of keratin are initially broken by reduction. The hair is made into curls with repositioning of the amino acid chains relative to each other. Oxidation makes new S-S bonds and causes permanent waving of hair.

Proteoglycans

Glycosaminoglycans (GAGs) are mixed polysaccharides, formerly known as mucopolysaccharides. They are formed of repeating disaccharide units: aminosugar-sugar acid. The aminosugar is glucosamine or galactosamine, which may be acetylated and sulfated. The sugar acid is glucuronic acid or iduronic acid.

A proteoglycan monomer is composed of a protein core to which is attached long chains of glycosaminoglycans: hyaluronic acid, chondroitin sulfate, heparan sulfate, keratan sulfate, and dermatan sulfate. It forms a highly negative, water-absorbing, bottlebrush structure, which attracts cations and water.



Proteoglycan Aggregate

Association of proteoglycan monomers with a molecule of hyaluronic acid forms huge macromolecular aggregates, which give the tissue its turgor. They impart high viscosity to a solution and they are ideal as lubricators and shock absorbers in the joints.

Mucopolysaccharidoses

These are lysosomal storage diseases, resulting from defects in the lysosomal enzymes responsible for the catabolism of GAGs. There are at least 14 known types. All, excepting Hunter's syndrome (X-linked), are inherited in an autosomal recessive manner. These disorders give variable manifestations, including organomegaly, corneal clouding, and heart disease, with variable mental affection and life span.

Other medical problems related to extracellular matrix

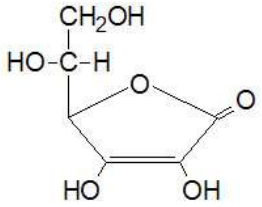
Marfan syndrome is due to mutation in fibrillin, inhibiting the formation of functional microfibrils. There is impaired structural integrity in the skeleton, the eye, and the cardiovascular system.

Spreading of bacterial infection in a tissue is helped by hyaluronidase (spreading factor) produced by the invading bacteria. This enzyme breaks down the normal glycosaminoglycan structure. Hyaluronidase may be used as an adjuvant to increase drug infiltration.

Metastasis of cancer is due to malignant cells escaping the adhesion mechanism imparted by the extracellular matrix.

Achondroplasia is caused by a mutation in a fibroblast growth factor receptor gene that leads to a number of skeletal abnormalities.

Vitamin C



Ascorbic acid (vitamin C) is synthesized from glucose via the uronic acid pathway in all animals except primates and guinea pig. The active form of vitamin C is ascorbic acid itself. Its main function is as a reducing agent in a number of reactions, and as an antioxidant. Ascorbic acid becomes oxidized to dehydroascorbate, which is reduced spontaneously by glutathione, as well as enzymatically in reactions using glutathione or NADPH.

The most important reaction requiring ascorbate as a cofactor is probably the hydroxylation of proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue, bones, and wound healing. Other metabolic reactions requiring vitamin C include the synthesis of norepinephrine from dopamine and the synthesis of the bile acids. It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C, which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland. Vitamin C, by reducing ferric to ferrous, also helps in iron absorption.

Daily requirement of vitamin C is about 100 mg/day. Requirement is higher with pregnancy and lactation and in smokers. The main source is fresh fruits and vegetables, especially citreous fruits. It is rapidly oxidized by air, especially in presence of metals like copper and iron, and it is destroyed by cooking. Deficiency in vitamin C leads to scurvy due to the role of the vitamin in the post-translational modification of collagen. Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement. The primary physiological state leading to an increased requirement for vitamin C is severe stress (or trauma), due to a rapid depletion in the adrenal stores of the vitamin. Vitamin C readily passes in the urine and does not accumulate in the body.

Study Questions

Choose one best answer for every question of the following:

1- To compare different tissues as regards their collagen content, which aminoacid is compared in their hydrolysates?
(A) Glycine.
(B) Tryptophan.
(C) Lysine.
(D) Hydroxyproline.

2- Copper is needed for modification of which aminoacid during synthesis of collagen and elastin?
(A) glycine.
(B) Lysine.
(C) Proline.
(D) Hydroxyproline.

3- Which of the following is the most common aminoacid in collagen?
(A) Proline.
(B) Hydroxyproline.
(C) Lysine.
(D) Glycine.

4- α1-Antritrypsin derives its name from
(A) being the major constituent of α1-globulin band of plasma proteins.
(B) binding to α-chain of collagen.
(C) both (A) and (B).
(D) neither (A) nor (B).

5- The main function of α1-antritrypsin is
(A) inhibiting pancreatic trypsin in the small intestine.
(B) inhibiting neutrophil elastase in the lung.
(C) both (A) and (B).
(D) neither (A) nor (B).

6- Glycosaminoglycans do not include
(A) heparin and heparan sulfate.
(B) hyaluronic acid and chondroitin sulfate.
(C) keratan sulfate and dermatan sulfate.
(D) keratin and creatine.

7- Hyaluronic acid is hydrolyzed by hyaluronidase enzyme (spreading factor) secreted by
(A) some bacteria.
(B) spermatozoa.
(C) both (A) and (B).
(D) neither (A) nor (B).

8- Glucuronic acid is
(A) a reducing mucopolysaccharide.
(B) oxidized glucose.
(C) an important constituent of glycoproteins.
(D) none of the above.

9- Hoping to promote formation of GAGs, patients with joint injury may be given which of the following?
(A) Trypsin.
(B) α1-Antritrypsin.
(C) Glucosamine.
(D) Hyaluronidase.

10- A mucopolysaccharidosis may be due to deficiency of which of the following?
(A) β-Glucuronidase.
(B) α-Glucosidase.
(C) Lactase.
(D) Sucrase.

11- Which of the following is not true about vitamin C?
(A) It is a water-soluble vitamin.
(B) It passes in the urine causing it to reduce Fehling's and Benedict's reagent.
(C) It can be used as an acidifier of the urine.
(D) It is a major contributing factor for hyperoxaluria.

12- Vitamin C is biologically important as
(A) an antioxidant.
(B) a reducing agent which facilitates the absorption of iron from the intestine.
(C) a cofactor for hydroxylation of proline and lysine of procollagen.
(D) all the above.

13- Scurvy is a disease not characterized by
(A) swollen gums, which readily bleed, and loosening of teeth.
(B) poor healing of wounds and anemia.
(C) swollen joints and easily fractured bones.
(D) resulting if one takes a diet deficient in ascorbic acid for three days.

Bone Chemistry

Organic matter constitutes about 30% of the bone mass. Osteoblasts produce the organic part of the bone matrix, an array of proteins collectively termed osteoid. Three functionally important proteins are:

- Collagen (type I, with tropocollagen triple helix units containing two α1-chains and one α2 chain) represents about 90% of the osteoid. It is formed in the endoplasmic reticulum after the vitamin C-dependent post-translational hydroxylation of proline and lysine residues of individual chains. It is secreted as procollagen, followed by proteolytic removal of C- and N-terminal peptides. The resulting collagen monomers spontaneously aggregate in a staggered fashion, forming long fibrils that are subsequently covalently cross linked using the oxidized lysine as explained before.
- Osteocalcin is a small protein (49 aminoacids) that is carboxylated on glutamic acid residues with the help of vitamin K, like coagulation factors. The two adjacent negative charges of gamma-carboxyglutamate residues are ideal docking sites for double positive Ca^{2+} ions. Osteocalcin binds hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, and may thus function as a shock-absorber between organic and inorganic matrix components. Deficiency of vitamin K results in bleeding disorder long before effects on bone might cause problems. A second vitamin is important for osteocalcin: the transcription of its gene is induced by activated vitamin D receptor. Circulating osteocalcin (probably the uncarboxylated form) has been reported to have a hormonal action, controlling several physiological processes such as glucose homeo-stasis, brain development, cognition, and male fertility.
- Osteonectin is an osteoid component that makes contact to type I collagen as well as to hydroxyapatite, forming a link between organic and inorganic bone matrix.

In addition, osteoblasts engage in inducing local super saturation of Ca^{2+} and phosphate to mineralize the freshly produced osteoid. For this process, alkaline phosphatase on the outside of the osteoblast plasma membrane may increase extracellular phosphate concentration by dephosphorylating organic molecules or cleaving pyrophosphate. Bone structure may be compared to armored concrete. The matrix proteins provide the scaffolding for the deposition of hydroxyapatite.

Throughout life, bone remodeling occurs continuously. It allows the adaptation to biomechanical forces, maintaining the integrity of bone and homeostasis of calcium and phosphates. Mechanical strain constantly results in micro fissures in the bone matrix, microtraumas to be repaired. Bone formation and bone resorption have to go hand in hand. Osteoclasts break down bone tissue much like macrophages break down phagocytosed material; only the process is shifted to the extracellular space. Osteoclasts seal off a certain matrix area, which they acidify with the help of a proton pump. To maintain intracellular pH, they release HCO_3^- at their back side. Hydroxyapatite dissolves in the acidic environment, then acid proteases like cathepsin K hydrolyze the remaining matrix proteins. After that, osteoblasts fill the gap with the scaffold material, which is then mineralized. The whole process is orchestrated employing different growth factors and cytokines.

Calcium (Ca^{2+})

Calcium is required for bone mineralization, cardiac function, muscle contraction, and secretory processes. In addition, Ca^{2+} is necessary for blood coagulation. About 99% of body Ca^{2+} resides in bone matrix. Blood and extracellular fluid contain only 0.1% of total body Ca^{2+}.

Plasma Ca^{2+} concentration is physiologically maintained in a small window between 2.1 and 2.6 mM. This measured Ca^{2+} is the sum of three forms: Ca^{2+} bound to plasma proteins (about 45%), Ca^{2+} complexed with small organic anions (10%) and free ionized Ca^{2+} (about 45%). Hence, total Ca^{2+} depends on plasma protein concentration. The biologically relevant, regulated parameter is free Ca^{2+}.

Ca^{2+} balance is basically maintained by two hormones: parathyroid hormone (PTH) and calcitriol (1,25-dihydroxyvitamin D). PTH regulates short-term plasma Ca^{2+} concentrations by dipping into bone reserves. Vitamin D strategically maintains the total Ca^{2+} pool of the body. A third hormone, fibroblast growth factor-23 (FGF23), regulates elimination of phosphate via the kidneys, which directly impacts on the calcium balance. A fourth hormone, calcitonin, which inhibits osteoclastic activity and stimulates calcium deposition in bone is of minor importance in humans.

Calcium supplementation is recommended, with or without vitamin D, for the elderly, especially women to guard against osteoporosis. Its role in development of cardiovascular problems is questionable. Dairy products are the main source of calcium in the diet. The recommended intake is 1000 mg/day. This amount is covered by about 1 L of milk, which is not tolerated by many people. Other sources include molasses, vegetables, fruits, nuts, and fish. Note that calcium carbonate supplement contains only 40% calcium. Calcium absorption is decreased by diet components that bind Ca^{2+} in an insoluble form, e.g., oxalate, phytic acid (inositol hexa-phosphate), and fatty acids. Excess dietary fat may be blamed for low binding of oxalate in the intestine by Ca^{2+}, which leads to increased oxalate absorption, hyperoxaluria and urinary stones.

Phosphorus

Besides its role in bone mineralization, phosphorus is necessary for energy utilization and formation of phosphate-containing compounds, e.g., nucleotides and nucleic acids. Phosphate buffer: $H_2PO_4^-/HPO_4^{2-}$ is an important blood buffer.

Parathyroid hormone (PTH)

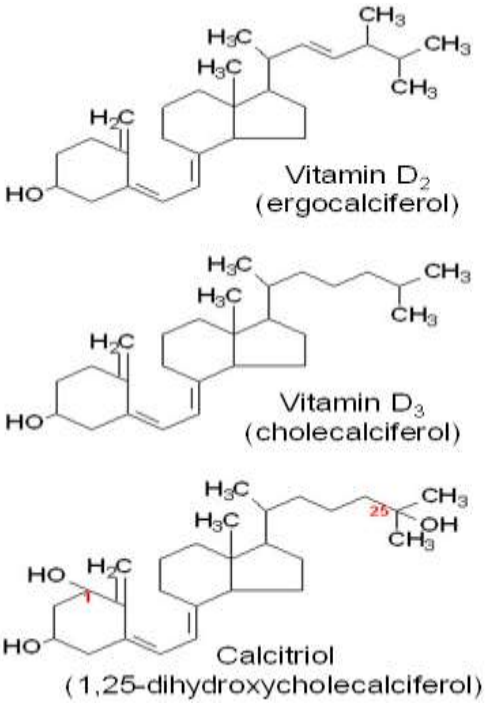
Parathyroid hormone (PTH) is an 84-aminoacid peptide and has an extremely short half-life of about four minutes. PTH increases Ca^{2+} concentration via two main mechanisms: by liberating it from bone and by influencing the kidneys. PTH's net effect in bone is an increase in resorption by activation of osteoclasts. PTH is sensed by osteoblasts, which react by producing cytokines to activate osteoclasts and to induce differentiation and proliferation of more osteoclasts. In the kidney, PTH increases reabsorption of Ca^{2+} in the distal tubule. Simultaneously, it inhibits phosphate reabsorption in both the proximal and distal tubule by down regulation of Na^+/phosphate cotransporter, inducing phosphaturia, thus keeping a high soluble Ca^{2+} that maintains the solubility product of Ca^{2+} and phosphate (Lecture 1). Its third renal function is to stimulate hydroxylation of carbon 1 of vitamin D, the last and rate-limiting step in the vitamin activation. The resulting calcitriol then increases the Ca^{2+} pool by increasing Ca^{2+} absorption in the intestine and kidney. PTH secretion is feedback inhibited by increased Ca^{2+} and calcitriol.

Vitamin D

Vitamin D_2 (ergocalciferol) is formed by ultraviolet (UV) irradiation of the fungal sterol: ergosterol. Vitamin D_3 (cholecalciferol) is formed by UV irradiation of 7-dehydrocholesterol in the skin. It can be taken from animal sources: fish, cod liver oil, liver, and egg yolk. Milk is fortified with vitamin D to guard against vitamin D deficiency, especially in cold countries and in black colored children.

Vitamin D_1 is not in use. It is a mixture of vitamin D_2 and lumisterol (a stereoisomer of ergosterol formed from it upon exposure to UV)

Vitamin D_2 and D_3 are processed to D_2-calcitriol and D_3-calcitriol, respectively. The vitamin is hydroxylated in the liver at carbon 25, then in the kidney at position 1α, producing the active vitamin (hormone): 1,25-dihydroxy-vitamin D. The three hydroxyl groups give it the name “calcitriol”. The main circulating form is the 25-hydroxylated vitamin (calcidiol), which is the one assayed in the plasma.



Vitamin D is a steroid pro-hormone. The active vitamin D, from the kidney, acts through a specific receptor in intestinal mucosal cells, like a steroid hormone, to increase the expression of a calcium binding protein, which facilitates the absorption of calcium. The increased absorption of calcium ions requires concomitant absorption of a negatively charged counter ion to maintain electrical neutrality. The predominant counter ion is phosphate. Calcitriol also stimulates renal reabsorption of calcium and phosphate. The increase in calcium and phosphate leads to their deposition in bones due to their low solubility product (Lecture 1).

The enzyme 1α-hydroxylase in the kidney proximal tubule is activated by the parathyroid hormone (PTH), which is released in response to low plasma calcium. A decrease in plasma phosphate increases the 1α-hydroxylase activity. Growth hormone, estrogen, and prolactin stimulate 1α-hydroxylase, thus increasing calcium absorption in the physiological states of growth, pregnancy, and lactation. Reduced levels of PTH stimulate synthesis of the inactive 24,25-dihydroxy-vitamin D. Elevated plasma levels of calcitriol and phosphate inhibit the 1α-hydroxylase enzyme, a feedback control.

Calcitriol is rapidly metabolized by further oxidation to calcitroic acid and other metabolites. These are mainly excreted in the bile.

The daily requirement of vitamin D is 5 µg or 200 IU (international units). With adequate exposure to sunlight, there may be no need to take vitamin D in the diet. The required UV rays are absorbed by glass. Therefore, indoor sun is not good for vitamin D production. More vitamin is recommended for the elderly (10 µg/day for age above 50 years). Adequate calcium intake need not be emphasized.

Vitamin D deficiency may result from inadequate exposure to sunlight (low vitamin synthesis), fat malabsorption (low vitamin absorption), or severe kidney disease (low vitamin activation). The main manifestation of vitamin D deficiency in children is rickets and in adults is osteomalacia. To overcome a low 1α-hydroxylase function (renal failure, renal rickets), 1α-cholecalciferol (Alfacalcidol) may be given, which is easily transformed to calcitriol by the liver. Hypoparathyroidism too may lead to functional vitamin D deficiency. Some drugs like barbiturates and other anticonvulsants induce the vitamin catabolism. It is advisable to look at all the medications a patient takes for a possible interaction with vitamin D or an effect on the bones by other ways.

Vitamin D toxicity results from excessive administration of the vitamin. It can lead to premature closure of sutures and fontanelles causing microcephaly. Deposition of calcium in many tissues causes renal stones, nephrocalcinosis, and calcification of arteries. An upper limit of 4000 IU/day has been recommended.

Apart from its role in calcium metabolism, vitamin D was found to have other actions (pleiotropic effects). It may play a role in regulation of immune system and may guard against hypertension by decreasing expression of renin. Its claimed benefit in prevention of respiratory infection was not substantiated.

Fibroblast growth factor-23 (FGF23)

FGF23 is produced by osteocytes and osteoblasts in response to 1,25-dihydroxyvitamin D and dietary phosphate loading. It increases renal phosphate excretion by reducing the number of Na^+/phosphate cotransporters in the apical membrane of the proximal tubule. In this function, it acts similar to PTH. Yet, it counters PTH by inhibiting 1α-hydroxylation of vitamin D. It thus lowers active vitamin D, which in turn reduces uptake of Ca^{2+} via the intestine.

CKD-MBD (chronic kidney disease- mineral and bone disorder): We take up all available phosphate from the intestine and balance that by eliminating the surplus via the kidneys. In many old people, a problem arises from the decline in glomerular filtration rate. As filtrated volume comes down, a progressively higher percentage of filtrated phosphate needs to be excreted, so FGF23 levels steadily rise higher and higher. FGF23 keeps calcitriol down, meaning plasma Ca^{2+} concentration can only be maintained by parathyroid hormone. Over time, secondary hyperparathyroidism results in bone disorder.

X-linked hypophosphatemia: The X-chromosomal PHEX gene (phosphate-regulating neutral endopeptidase, X-linked) encodes a peptidase which inhibits FGF23. Deficiency of this peptidase causes FGF23 hyperactivity resulting in renal phosphate losses. The disease is reminiscent of rickets, yet does not respond well to vitamin D.

Other hormones

Growth hormone (GH) has a role in activating vitamin D, and is essential for longitudinal bone growth. It exerts its growth promoting effects indirectly through insulin-like growth factor-1 (IGF-1). GH stimulates hepatocytes to secrete IGF-1 into the blood. Many cells, including chondrocytes and osteoblasts, are induced to produce IGF-1 that acts in a paracrine fashion. In bone, part of it is embedded by mineralization, together with other growth factors like TGFβ (transforming growth factor β) and PDGF (platelet-derived growth factor). This creates a reservoir of growth factors that is activated in case of bone resorption (one reason why metastasizing tumor cells frequently find a fertile soil in bone). IGF-1 acts back on the cells in a paracrine fashion, stimulating chondrocytes in the epiphyseal plate and osteoblasts to divide.

Thyroid hormone, estrogens and androgens are important for bone growth. Their cell receptors are ligand-activated transcription factors, which activate transcription of several genes. With hyperthyroidism, osteoclast activation may lead to osteoporosis. The effect of androgens in preventing osteoporosis may be indirect through conversion to estrogens by aromatization.

Glucocorticoids decrease calcium absorption in the intestine through a decrease in binding proteins. They inhibit transcription of collagen and osteocalcin genes and reduce the life span of osteoblasts. On the other hand they increase the number and activity of osteoclasts. Therefore, they strongly promote osteoporosis.

Biochemical markers of bone metabolism

Many substances can be laboratory indicators of bone turnover. These include plasma calcium, phosphates, parathormone, calcitonin, vitamin D, estrogens, glucocorticoids, etc. Besides these, there are also specific markers of bone metabolism. Blood (serum) sampling is usually preferred to 24-hour urine being easier and avoiding a major source of preanalytical variation.

Bone formation markers include: osteocalcin, bone-specific alkaline phosphatase (BSAP), and the propeptides released during collagen polymerization. Specifically, these are procollagen I C-terminal propeptide (PICP) and procollagen I N-terminal propeptide (PINP). Although produced by the osteoblasts, osteocalcin may be defined as a bone turnover marker reflecting both bone formation and bone resorption, since it is also released from the bone matrix during bone resorption. Osteocalcin measurement has a limited value in patients with reduced renal function since it is mainly cleared by the kidneys. Commercial assays available for measuring BSAP show cross-reactivity with liver alkaline phosphatase; therefore, in patients with liver disease BSAP measurements have limited applicability. Assays measure the monomeric and trimeric forms (total PICP) or only the trimeric form (intact PICP). Being dependent on renal clearance, the monomeric form of PICP accumulates in chronic kidney disease.

Bone resorption markers include: bone isoenzyme of acid phosphatase (tartrate-resistant), hydroxyproline, and collagen cross-link structures: pyridinoline and deoxypyridinoline. Serum CrossLaps® (CTx-I) assay is now the one of choice to examine osteoclastic bone resorption activity and to monitor anti-osteoclast therapy. This immunoassay measures the C-terminal cross-linked ("x") fragments of collagen I, formed by degradation of mature collagen fibers. As bone ages, the alpha-form of aspartic acid present in CTx isomerizes to the beta-form, hence the name “β-CTx”. In patients taking antiresorptive agents, a decrease of 25% or more from baseline β-CTx levels (i.e., prior to the start of therapy) 3 to 6 months after initiation of therapy indicates an adequate therapeutic response. CTx and its counterpart, the N-terminal telopeptide NTx, are both cleared by the kidneys, therefor, their clinical usefulness in chronic kidney disease is significantly limited.

Biomarker levels vary considerably due to quite a few endogenous factors, therefore, one needs to take into consideration the circadian rhythm, the phase of the menstrual period, seasonal variation, physical exercise and diet.

Osteoporosis

Osteoporosis is a systemic bone disease characterized by low bone density and altered bone microarchitecture with consequent increase in fragility. The loss of inorganic and organic bone matter leads to increased fracture risk. Primary or idiopathic osteoporosis starts earlier in women, as estrogens in women decrease earlier than androgens in men. We reach our peak bone mass in our twenties. From then on, the net effect of the many factors affecting bone metabolism is slightly negative. Several factors contribute to the negative net effect: decrease in estrogen and androgen concentrations, reduced physical activity as mechanical load is essential to maintain bone mass, insufficient vitamin D and calcium intake, and reduced UV exposure. Reduced renal function secondary to diabetes, arteriosclerosis, or analgesics abuse, results in insufficient 1-hydroxylation of vitamin D. Interestingly, an excess of weight to a certain degree protects from osteoporosis. Whether this is due to increased load, or to enhanced estrogen synthesis from androgens by fatty tissue aromatase remains to be elucidated.

Dual energy x-ray absorptiometry (DEXA) till date is the gold standard methodology to measure bone mineral density (BMD). DEXA is based on the fact that the density of tissue affects its differential absorption properties for low energy versus high energy X rays. The result is expressed as a multiple of the standard deviation from mean bone density of 30 year-olds of the same sex (T-score). Osteoporosis is defined by a negative T-score below −2.5. The response to treatment shows on DEXA very slowly; therefore, it is better to use the biomarkers for monitoring therapy.

Several treatment choices exist for osteoporosis. These include estrogens, raloxifene (a selective estrogen receptor modulator), bisphosphonates, and the monoclonal antibody: denosumab (Prolia®), which inhibits the signal pathway for osteoclast activation. The parathyroid hormone analogue: teriparatide (Forteo®), given intermittently (once daily), activates osteoblasts more than osteoclasts. Other choices include calcitonin, fluoride, and strontium salt. Calcium, vitamin D, and physical activity need not be emphasized.

Study Questions

Choose one best answer for every question of the following:

1- The major protein of the bone matrix is
(A) Collagen.
(B) Elastin.
(C) Osteocalcin.
(D) Osteonectin.

2- The mineral of bone matrix is
(A) Calcium.
(B) Calcium and sodium phosphate.
(C) Fluoroapatite.
(D) Hydroxyapatite.

3- Which of the following is not true about calcium?
(A) Calcium constitutes about 1.5 kg of a 70 kg healthy adult body.
(B) 99% of body calcium is present in bones and teeth, with phosphate, as hydroxyapatite crystals.
(C) Calcium concentration in the cytosolic fluid is higher than in extracellular fluid or plasma.
(D) Calcium ions are essential for nerve and muscle function, secretion, blood clotting, and control of various enzyme activities and metabolic pathways.

4- Which of the following is not true about plasma calcium?
(A) Plasma calcium concentration is 8.5-10.5 mg/dL.
(B) About half the plasma calcium is bound to albumin.
(C) Alkalosis increases binding of calcium to albumin and decreases ionized calcium, e.g., with hyperventilation.
(D) Plasma calcium concentration is controlled by calcium intake in the diet.

5- Which of the following is not true about calcium supplementation?
(A) Calcium supplementation, with or without vitamin D, is recommended for the elderly, especially women to guard against osteoporosis.
(B) Calcium carbonate supplement contains only 40% calcium.
(C) Excessive calcium and vitamin D intake may lead to abnormal calcium deposition, mainly as urinary stones.
(D) Over-intake of calcium is proved to cause cardiovascular disease.

6- Vitamin D is considered a
(A) water-soluble vitamin.
(B) coenzyme.
(C) proenzyme.
(D) prohormone.

7- Which of the following is correct?
(A) Vitamin D is formed when the B ring of its precursor's steroid nucleus is opened by ultraviolet irradiation.
(B) The most widely used vitamin D is vitamin D_1.
(C) Vitamin D_2 is produced by the action of UV rays on 7-dehydrocholesterol.
(D) Vitamin D_3 is produced by the action of UV rays on ergosterol.

8- Calcitriol is which of the following?
(A) Vitamin D_3.
(B) Vitamin D synthesized by UV rays.
(C) Active form of vitamin D.
(D) 25-hydroxycholecalciferol.

9- The form of vitamin D that is measured in the blood is which of the following?
(A) Cholecalciferol.
(B) 1α-Hydroxycholecalciferol.
(C) 25-Hydroxycholecalciferol.
(D) 1,25-Dihydroxycholecalciferol.

10- Calcitriol is produced by
(A) action of UV rays on ergosterol.
(B) reduction of vitamin D_3 in the kidney.
(C) isomerization of cholecalciferol.
(D) oxidation of calcidiol.

11- Which of the following is given to renal failure patients?
(A) Cholecalciferol.
(B) Ergocalciferol.
(C) 1α-Hydroxycholecalciferol.
(D) 25-Hydroxycholecalciferol.

12- With vitamin D therapy, which of the following is essential to give to the patient?
(A) Adequate exposure to sun.
(B) Calcium.
(C) 7-Dehydrocholesterol.
(D) Saturated fat.

13- What is the effect of growth hormone on calcium metabolism?
(A) Increased calcium absorption from the intestine by activation of vitamin D.
(B) Increased excretion of calcium by the kidney.
(C) Antagonizing the effect of parathyroid hormone on plasma calcium concentration.
(D) Inhibition of calcitriol production by the kidney.

14- Which of the following is an effect of glucocorticoids on bone metabolism?
(A) Induction of calcium binding protein in the intestine.
(B) Increased transcription of collagen and osteocalcin.
(C) Increased number and activity of osteoclasts.
(D) Increased calcium absorption by the intestine.

15- The drug of first choice for treating osteoporosis is
(A) Estradiol.
(B) Bisphosphonate.
(C) Calcitonin.
(D) Cortisol.

16- Which biomarker may be used to monitor anti-resorptive bone therapy?
(A) Bone-specific alkaline phosphatase.
(B) Procollagen I C-terminal propeptide (PICP).
(C) Procollagen I N-terminal propeptide (PINP).
(D) Serum CrossLaps (β-CTx-1)

17- With chronic kidney disease, which bone resorption biomarker may be used?
(A) CTx.
(B) NTx.
(C) Osteocalcin.
(D) Tartrate-resistant acid phosphatase.

Metabolism – Enzymes

Definitions

We are made of molecules, atoms, and ions and we function by interaction of these components. All functions of living tissues depend on chemical reactions. The sum of all chemical reactions is called "Metabolism". Anabolism is the building up of large molecules from small units, while catabolism is the breakdown of large molecules to small ones. A metabolic pathway is a series of chemical reactions that serves a certain function.

One-way and two-way reactions

Most biochemical reactions are two-way reactions, proceeding in either direction. Chemical reactions proceed in the direction of loss of free energy, meaning that ΔG (delta G) should be negative (exergonic reaction). At equilibrium, ΔG equals zero. An endergonic reaction (with positive ΔG) can proceed only if coupled to an exergonic one with a higher magnitude of free energy change. Actual ΔG depends on the relative concentrations of reactants and products and on ΔG_O (the standard free energy change, the free energy change when the concentrations of all reactants and products equal 1 mol/L). If ΔG_O is highly negative, ΔG will always be negative, meaning that the reaction is one-way. It is to be noted here that ΔG gives no clue to the speed of the reaction or to the time a reaction is to proceed.

Enzymes

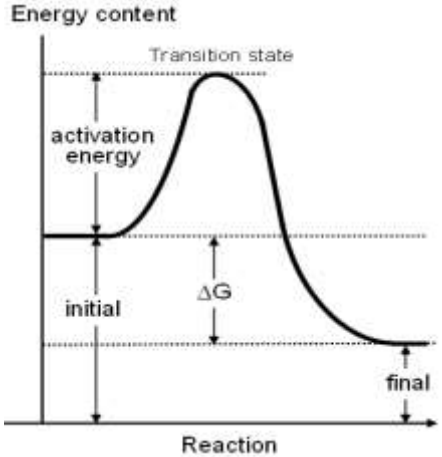
Enzymes are biological catalysts. A catalyst is a substance that speeds a reaction. Thus, enzymes speed the chemical reactions in living systems. As catalysts, they are required in very small amounts, and they are not consumed by the chemical reactions.

Do we need catalysts?

A protein or starch can be hydrolyzed in the lab by adding a strong concentrated acid and boiling on a flame. Certainly, we cannot do that in our bodies to digest our food. We surely need special catalysts. Thanks to enzymes, protein and starch can be hydrolyzed in our guts in few minutes, at 37°C, with no need for concentrated acid or alkali. A car engine gets energy by burning a fuel, a combustion reaction. Our cells clearly cannot do the same. Thanks to enzymes, all our biological reactions proceed at 37°C, within our tissue environment, at the required rate.

How do enzymes work?

An enzyme brings reacting molecules together, or the enzyme alters the molecules to bring them to transition state. Thus, enzymes decrease the activation energy of the reaction. Enzymes do not change the free energy change (ΔG). Since reactions proceed in the direction of loss of free energy (negative ΔG), enzymes do not dictate the direction of the reaction.



Enzyme specificity

Enzymes are highly specific, interacting with one or a few substrates, and usually catalyze only one type of reaction.

Nature of enzymes and cofactors

Enzymes are proteins. A cofactor, another substance other than the enzyme protein, may be needed for the enzyme to work. This cofactor may be a metal like calcium, magnesium, or zinc ion. The metal may be required for the proper conformation of the enzyme. It may bridge the enzyme and substrate or it may participate in catalysis by donating or accepting electrons.

Organic cofactors are called co-enzymes. A coenzyme tightly bound to the enzyme is called a prosthetic group. A coenzyme may act as a temporary carrier of a chemical group in the re-action being catalyzed or it may undergo a change antiparallel to the change in the substrate. In the latter case, it may be considered a cosubstrate. Co-enzymes are commonly derived from vitamins, which therefore are essential in our diet.

Vitamin	*Coenzyme*	*Coenzyme Function*
Thiamin Vit B_1	Thiamin pyro-phosphate (TPP)	Oxidative decarboxylation
Riboflavine Vit B_2	FMN & FAD	Hydrogen carriers
Niacin	NAD^+ & $NADP^+$	Hydrogen carriers
Pyridoxine Vit B_6	Pyridoxal phosphate	Aminoacid metabolism
Lipoic acid	Lipoic acid	Oxidative decarboxylation
Pantothenic acid	CoA	Carbohydrate, fat & protein metabolism
Biotin	Biotin	Carboxylation reactions
Folic acid	Tetrahydrofolate	Carrier of one carbon unit

Enzyme Nomenclature

Most commonly used names have the suffix “ase”, e.g., urease and sucrase, which act on urea and sucrose respectively. The name may describe the enzyme function, e.g., lactate dehydrogenase, which catalyzes an oxidation-reduction reaction involving lactate. Some old enzyme names however give no indication to the substrate or the action of the enzyme, e.g., pepsin, the proteolytic enzyme of the stomach.

Enzyme classification code

Every enzyme is given a four-number enzyme commission code (*E.C. X.X.X.X*) that fully characterizes the enzyme. The first digit denotes one of the six enzyme classes: 1-oxido-reductases, 2-transferases, 3-hydrolases, 4-lyases, 5-isomerases, 6-ligases. This code describes the reaction catalyzed by the enzyme.

Study Questions

Choose one best answer:

1- Enzymes accelerate reactions by which of the following?
(A) Decreasing free energy change.
(B) Decreasing activation energy.
(C) Increasing free energy change.
(D) Increasing the equilibrium constant.

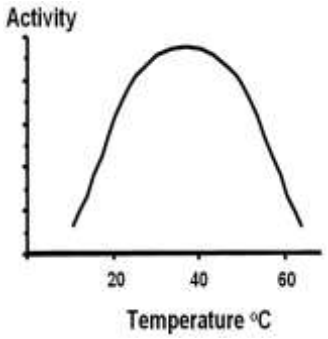
Enzyme activity

Enzyme activity is measured as the rate (velocity, speed) of the reaction catalyzed by the enzyme. Since enzymes are present at low concentration amongst high protein media, it is difficult to measure the amount of an enzyme. Activity is measured instead. One unit of enzyme activity is equal to one micromole of the reaction product in one minute, under specified conditions. The initial velocity, before product accumulation, is measured for this purpose. Specific activity is the activity per mass unit of protein.

Factors that affect enzyme activity

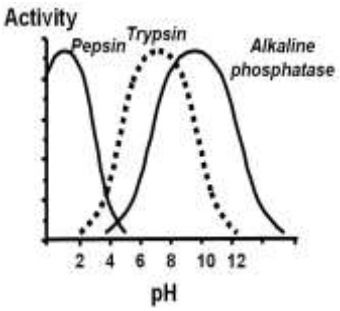
Enzymatic activity depends on the conformation of the enzyme and its ability to bind the reacting substance, the substrate. Thus, the activity of the enzyme is affected by factors that affect its conformation or the rate of its binding to the substrate. To study any factor, all other factors should be fixed and only the studied factor is varied.

A rise in temperature increases the kinetic energy of reacting molecules, hence the rate of binding of the enzyme and substrate. Thus, enzyme activity increases by rise in temperature. At a high temperature however, the enzyme protein is denatured and the enzymatic activity declines. Hence, there is an optimum temperature for the enzyme action, at which the velocity of an enzyme catalyzed reaction is maximum. Below or above this optimum temperature, the reaction velocity declines.



For human enzymes, the optimum temperature is about 37°C. Some organisms, e.g., *Thermus aquaticus* bacteria, which live near hot water springs have enzymes of a higher optimum temperature.

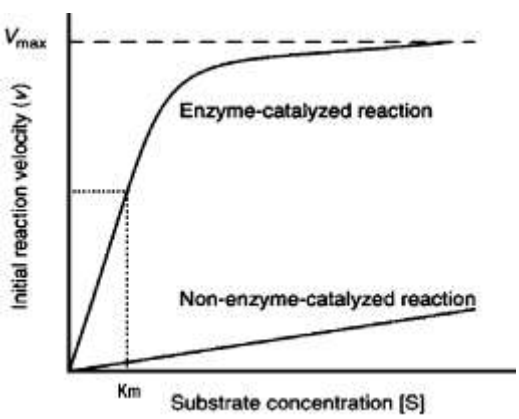
The pH of the medium affects the charges on the enzyme, hence the enzyme conformation, and the substrate, hence the enzyme-substrate affinity. Therefore, the activity of an enzyme is pH-dependent. Each Enzyme has its optimum pH, above or below which the enzyme activity decreases. At extreme pH, the enzyme is denatured and loses its function completely. The opposite figure shows optimum pH for some enzymes.



The effect of substrate concentration on enzyme activity is summarized by Michaelis-Menten equation, and plotted as hyperbolic curve as shown. The hyperbolic curve reflects the saturation of the enzyme with increasing the substrate concentration.

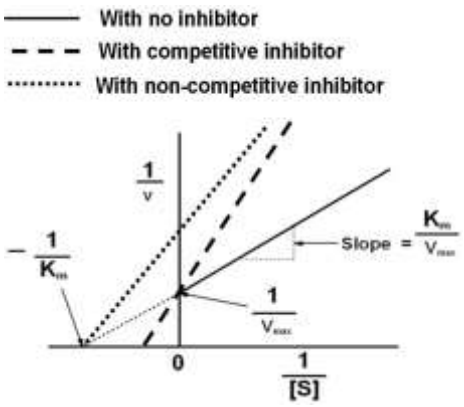
$$\frac{v_o}{V_{max}} = \frac{[S]}{K_m + [S]}$$

Maximal velocity (V_{max}) is attained by full enzyme saturation. At half the maximal velocity (half enzyme saturation), the substrate concentration [S] equals the equation constant K_m. This constant reflects the affinity of the enzyme to the substrate. A low K_m means a high affinity, and vice versa.



Enzyme inhibition

Some substances can bind the enzyme and prevent the natural enzyme-substrate binding. They decrease the rate of the reaction. Therefore, they are called enzyme inhibitors. These are competitive or non-competitive. The difference between the effect of these inhibitors is best illustrated on the Lineweaver-Burk plot derived from Michaelis-Menten equation.



Competitive inhibitors are substrate analogues, which compete with the substrate for the active site of the enzyme. These are reversible, and can be overcome by increasing the substrate concentration. Increasing the substrate concentration can still fully saturate the enzyme. Therefore, V_{max} remains unaffected. To reach half saturation needs more substrate than usual. Thus, K_m is increased.

Sulfonamides (para-aminobenzoic acid analogues) are used to inhibit folic acid synthesis by bacteria. Methotrexate (folate analogue) inhibits dihydrofolate reductase, and is used for treatment of cancer. Statins are competitive inhibitors of the enzyme HMG CoA reductase of cholesterol synthesis.

Non-competitive inhibitors may or may not resemble the substrate. They bind the free enzyme or the enzyme-substrate complex. They are not overcome by increasing substrate concentration, and are usually irreversible. The inhibited enzyme molecules are no longer available for catalysis. Therefore, V_{max} decreases. The remaining enzyme molecules have normal affinity to the substrate. Thus, K_m is not affected. This pattern is seen with enzyme poisons and suicidal inhibitors.

Enzyme poisons bind to the active site or destroy an active group, causing irreversible inhibition. Lead binds SH of cysteine of ferrochelatase important for heme synthesis. Cyanide inhibits cytochrome oxidase. Penicillin inhibits transpeptidase necessary for synthesis of bacterial cell wall. Organophosphorus compounds (war gas) bind covalently to serine residue of cholinesterase. Aspirin (acetyl salicylic acid) inhibits cyclooxygenase responsible for synthesis of prostaglandins and thromboxane. Suicidal inhibitors are substrate analogues, which bind to the active site, and are acted upon by the enzyme but do not leave it, thus causing irreversible inhibition. Allopurinol (hypoxanthine analogue) used to treat gout binds xanthine oxidase and is converted to alloxanthine that is covalently linked to the enzyme, inhibiting it.

Study Questions

Choose one best answer:

2- K_m equals which of the following?
(A) Half maximal velocity.
(B) Velocity at half saturation.
(C) Substrate concentration at half saturation.
(D) Half substrate concentration at maximal velocity.

3- Aspirin leads to which of the following changes in cyclooxygenase?
(A) Decreased K_m and V_{max}.
(B) Increased K_m and V_{max}.
(C) Increased K_m only
(D) Decreased V_{max} only.

Plasma enzymes

All enzymes are synthesized intracellularly. Cellular enzymes catalyze energy production, synthetic pathways, and other important reactions. Some enzymes act outside the cells, e.g., digestive enzymes work in gastric and intestinal lumen. Blood coagulation and fibrinolysis are also extracellular reactions catalyzed by plasma enzymes. Other enzymes are present in the plasma with no function. These are called non-functional plasma enzymes. They result from cell leakage or normal cell turnover. They originate from different tissues, and their plasma concentration is lower than their concentration inside the cells.

Enzymes in diagnosis

The concentration of non-functional plasma enzymes is low, but increases with tissue damage. Hence, it reflects the state of cell destruction. Measuring the plasma enzyme activities is used in diagnosis of various diseases. Some enzymes are specific to specific tissue cells, e.g., alanine transaminase, ALT (glutamate pyruvate transaminase, GPT) is elevated with active liver disease. Also, elevated prostatic acid phosphatase is indicative of prostate cancer. Some enzymes are not specific, e.g., the elevated lactate dehydrogenase, LDH, may indicate a disease of the liver, the heart, blood cells, etc.

Isozymes provide a more powerful diagnostic tool. Isozymes are different molecular forms of the same enzyme, which can be separated by electrophoresis. They are usually oligomeric proteins, formed of more than one subunit. Creatine kinase for example is formed of two subunits: M or B, thus it is three isozymes. Creatine kinase-MB is useful for diagnosis of heart injury. Lactate dehydrogenase is formed of four subunits: H or M, thus it is five isozymes.

Some intra-cellular enzymes are also used for diagnosis of some diseases, e.g., glucose 6-phsphate dehydrogenase in red blood cells, which is deficient in cases of favism.

Enzymes as lab tools

Enzymes may be used as laboratory tools. The fungal enzyme: glucose oxidase is used for measuring blood glucose. *Taq* polymerase, from *Thermus aquaticus*, is used in polymerase chain reaction (PCR). In enzyme immuno-assays (ELISA), an enzyme is used to label the immune complex of the assay.

Enzymes in therapy

Enzymes may also be used in therapy, e.g., streptokinase is used for treatment of coronary thrombosis. It activates plasminogen (pro-fibrinolysin) to dissolve the blood clot in the coronary artery.

Study Questions

Choose one best answer:

4- Glucose oxidase is used for measuring blood glucose because:
(A) It is a human enzyme.
(B) It hydrolyzes glucose at a fast rate.
(C) It is specific to glucose.
(D) It is oxidized by blood glucose.

Control of metabolism

Cellular metabolism is strictly controlled. Every cell has a main task, which is to keep alive, then be ready to do other functions in the most efficient way. For this purpose, cellular reactions and pathways are finely controlled to provide the cell with its needs and conserve its resources at the same time. Reactions in our bodies are distinguished from non-biological reactions by being controlled by body needs. Our reactions are best studied as specific pathways. Each chemical pathway has specific functions, e.g., synthesis of fatty acids and degradation of fatty acids, which are two separate pathways. These should be strictly regulated so that only one of the opposing pathways is active at a time, according to body needs.

Compartmentation of pathways is one method of controlling them. Fatty acid oxidation for example takes place inside the mitochondria. Therefore, to be oxidized, fatty acids should enter the mitochondria, which is subject to control.

Control of pathways in general is exerted through the control of key enzymes of these pathways. Enzyme regulation is either by regulating the enzyme concentration (by induction or repression) or by modification (allosteric or covalent) of the present enzyme according to body needs. Regulatory mechanisms respond to either local changes, or signals coming from neighboring or distant cells.

Allosteric regulation ensures fine control of metabolic pathways according to the intracellular milieu. An allosteric regulator (modifier: activator or inhibitor) binds to an allosteric site of the enzyme, which is distinct from its active site. This causes a conformational change of the enzyme, which affects its binding to the substrate. An enzyme may be allosterically activated by its substrate, or by another substance that signifies the cell need for this enzyme action. A final product of a pathway can inhibit an early step (feedback inhibition). Allosteric enzymes do not show the hyperbolic curve of Michaelis-Menten kinetics, but rather have a sigmoid curve. Allosteric inhibitors shift the curve to the right.

Enzymes may be activated by proteolytic cleavage, e.g., gastrointestinal enzymes and blood clotting enzymes. By this mechanism, the tissue that secretes the enzyme in an inactive form is protected from the action of the enzyme. When needed, the enzyme is already present at adequate amounts, only waiting to be activated.

Covalent modification of an enzyme may be achieved by adding or removing a phosphate or another chemical group. This may lead to activation or inactivation of enzymes in a finely orchestrated pattern to serve the function of the cell and the whole body.

Study Questions

Choose one best answer:

5- Phosphorylation of an enzyme occurs at which aminoacid residue?

(A) Seine, threonine, or tyrosine.
(B) Alanine or phenylalanine.
(C) Lysine, histidine, or arginine.
(D) Aspartic acid or glutamic acid.

Study Questions

Choose one best answer for every question of the following:

6- Reactions in our bodies are distinguished from non-biological reactions by which of the following?
(A) They are catalyzed.
(B) They are controlled by body needs.
(C) They go at a faster rate.
(D) They proceed between chemicals not present outside the body.

7- A metal ion may act in enzyme catalysis by
(A) changing the conformation of the enzyme or neutralizing a charge on the substrate .
(B) bridging the enzyme and substrate.
(C) giving or accepting electrons.
(D) any of the above.

8- A coenzyme is which of the following?
(A) An organic compound.
(B) An apoenzyme.
(C) An inactive enzyme.
(D) An isoenzyme.

9- A prosthetic group is
(A) a tightly bound coenzyme.
(B) the group transferred by the enzyme.
(C) an inorganic cofactor.
(D) combination of the enzyme and coenzyme.

10- An apoenzyme is
(A) the prosthetic group of the enzyme.
(B) the protein part of the holoenzyme.
(C) the non-protein part of the holoenzyme.
(D) the protein part plus the prosthetic group.

11- The term "cosubstrate" may describe
(A) a second substrate in the chemical reaction.
(B) a coenzyme which changes in the reaction as the substrate is converted to a product.
(C) either (A) or (B).
(D) neither (A) nor (B).

12- Binding of the substrate to the enzyme depends on
(A) complementarity in shape between the substrate and the binding site.
(B) special chemical groups in the enzyme and substrate.
(C) both (A) and (B).
(D) neither (A) nor (B).

13- How can lemon juice or vinegar prevent darkening of sliced food such as potatoes?
(A) Bleaching the dark color formed.
(B) Breaking down the dark compounds formed.
(C) Acting as enzymes that prevent darkening of food.
(D) Changing the pH away from the optimum pH for enzymatic browning.

14- On studying reaction kinetics, we measure the initial velocity, which is the velocity
(A) before adding the enzyme.
(B) when there is no substrate.
(C) at the beginning, before products accumulate and slow down the reaction.
(D) at $0^{O}C$ and pH 0.

15- Which of the following is an example of competitive enzyme inhibition?
(A) Methotrexate in cancer therapy.
(B) Allopurinol for treatment of gout.
(C) Aspirin as antiplatelet.
(D) Toxicity by an organophosphate.

16- Antimetabolites
(A) are substrate analogues which can bind the active site of an enzyme.
(B) interfere with the synthesis or utilization of a substance essential for the cell.
(C) both (A) and (B).
(D) neither (A) nor (B).

17- Examples of antimetabolites are
(A) folate analogues, e.g., methotrexate.
(B) pyrimidine analogues, e.g., fluorouracil.
(C) purine analogues, e.g., azathioprine and mercaptopurine.
(D) all the above.

18- An enzyme whose concentration is constant may be called
(A) an inducible enzyme.
(B) a constitutive enzyme.
(C) a key enzyme.
(D) any of the above.

19- Phenobarbitone may be used to treat neonatal jaundice because it
(A) inhibits an enzyme that catalyzes synthesis of bilirubin.
(B) induces an enzyme that catalyzes conjugation, hence excretion of bilirubin.
(C) both (A) and (B).
(D) neither (A) nor (B).

20- A specific isoenzyme activity may be identified by the use of
(A) electrophoresis.
(B) specific inhibitors.
(C) either (A) or (B).
(D) neither (A) nor (B).

21- Serum amylase may be elevated in cases of
(A) acute pancreatitis.
(B) acute parotitis.
(C) both (A) and (B).
(D) neither (A) nor (B).

22- Plasma acid phosphatase may be elevated in cases of
(A) prostatic carcinoma.
(B) obstructive liver disease.
(C) coronary heart disease.
(D) hemolysis.

23- Which of the following enzymes may be used to kill leukemia cells, which need a lot of asparagine?
(A) Asparagine synthase.
(B) Asparaginase.
(C) Streptokinase.
(D) Amylase.

24- The first number of an enzyme commission "*EC"* code may be
(A) 0-6
(B) 0-9
(C) 1-6
(D) 1-9

25- Urease enzyme is used for determination of blood urea because it
(A) produces ammonia
(B) is specific to urea.
(C) is a human enzyme.
(D) may decrease urea concentration.

26- Plasma amylase may be elevated in which case?
(A) Acute enteritis.
(B) Hyperglycemia.
(C) High carbohydrate diet.
(D) Chronic kidney disease.

Signal Molecules

Cell-cell communication is important for any multicellular organism. A cell may be designed to serve other cells of the body. The metabolism of a cell then responds to changing needs of other cells. Therefore, the whole body metabolism is under neuro-endocrine control. A chemical signal, a hormone, a neurotransmitter, or a cytokine reaches the cell to initiate a signal transduction process that usually leads to a key enzyme or protein. Covalent modification by phosphorylation or dephosphorylation of enzymes or other proteins is usually an event in the sequence leading to the required response.

Signal molecules are mostly proteins, peptides, or aminoacid derivatives. Other signal molecules include steroid hormones of the adrenal cortex, gonads and the placenta, prostaglandins and other eicosanoids, and the vitamin-derived hormones: calcitriol and retinoic acid.

Classical hormones are secreted by defined endocrine (ductless) glands and released in the blood. Oligopeptide hormones include hypothalamic releasing hormones and the posterior pituitary hormones: oxytocin and vasopressin. Longer peptide hormones include insulin, glucagon, calcitonin, parathormone, prolactin and growth hormone. Peptide hormones also include gastrointestinal peptides and angiotensin.

The two-subunit glycoprotein hormones include: thyrotropin (thyroid stimulating hormone, TSH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) of the anterior pituitary, in addition to chorionic gonadotropin (hCG, h=human) from the placenta. The alpha subunit is common to all, but the beta subunit differs. The immunoassays for hCG (e.g., pregnancy test) target the beta subunit.

A number of hormones are formed by specific cleavage of a large polypeptide: proopio-melanocortin, produced by the anterior and intermediate lobes of the pituitary gland. These hormones include corticotropin (adrenocorticotropic hormone, ACTH), melanotropins (melanocyte stimulating hormones, MSH), and beta-endorphin. This may explain the pigmentation seen with increased ACTH secretion, e.g., Addison's disease and Cushing's disease.

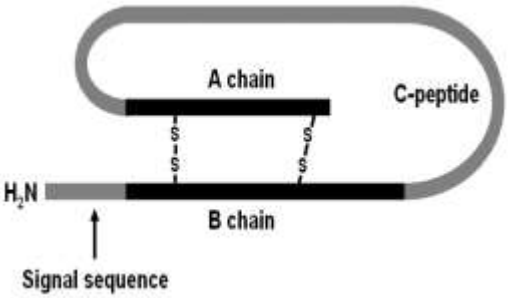
A number of hormones and neurotransmitters are nitrogenous compounds formed from aminoacids. Hormones synthesized from tyrosine include the catecholamines: dopamine, nor-epinephrine, and epinephrine, and the thyroid hormones: triiodothyronine (T_3) and thyroxine (T_4). Serotonin and melatonin are formed from tryptophan, nitric oxide is formed from arginine, and histamine is formed from histidine.

Neurotransmitters are produced by nerve cells and released in an intercellular space. Therefore, they can be considered to have a paracrine function, and some with an autocrine function. Some neurotransmitters are peptide in nature; some are smaller nitrogenous molecules. Acetylcholine is formed by acetylation of choline, which may be derived from serine and methionine. Some aminoacids act as neurotransmitters, e.g., glutamic acid, aspartic acid, glycine, and gamma-aminobutyric acid (GABA). Glycine and GABA are inhibitory while aspartic acid and glutamic acid are excitatory neurotransmitters. Note that mono-sodium glutamate (MSG) is a widely used food additive that gives the umami taste.

Cytokines (Greek *cyto-*, cell; and *-kinos*, movement) are small molecules used for cell signaling. They are peptides, proteins, or glycoproteins. They encompass a large and diverse family of regulators produced throughout the body by cells of diverse embryological origin. The term "cytokine" has been used to refer to the immunomodulating agents, such as interleukins and interferons. Interleukins were first seen to be expressed by white blood cells, but they are now known to be produced by a wide variety of body cells. Interferons are made and released by host cells in response to pathogens such as viruses, bacteria, parasites or tumor cells. They act to trigger the protective defenses of the immune system that eradicate pathogens or tumors.

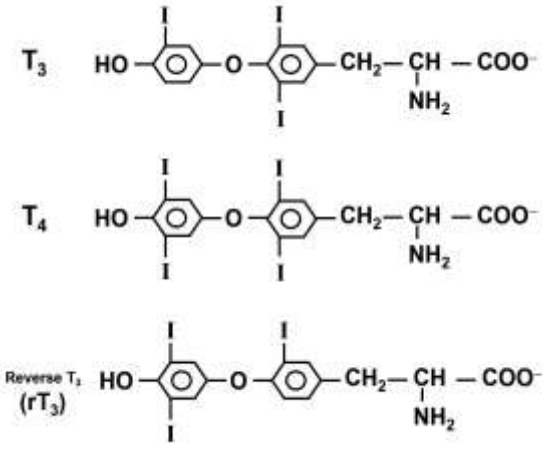
Bradykinin, a nonapeptide that acts on the B_2 receptor and slightly on B_1, is produced when kallikrein releases it from high molecular weight kininogen produced by the liver together with prekallikrein. It acts mainly as a cofactor in coagulation and inflammation. Angiotensin converting enzyme (ACE) while activating angiotensin I inactivates a number of peptide mediators, including bradykinin. ACE inhibitors lead to decreased conversion of angiotensin I to angiotensin II (a vasoconstrictor) but also to an increase in bradykinin due to decreased degradation.

peptide and protein hormones are synthesized by ribosomes as pre-prohormones, then post-translationally processed. The figure to the right shows the parts of pre-proinsulin that is processed to the insulin hormone formed of A and B chains (21 and 30 aminoacids). The split-off C-peptide concentration is a measure of endogenous insulin production and is virtually absent in type 1 diabetes. It can be a tumor marker of insulinoma.



Thyroid hormones

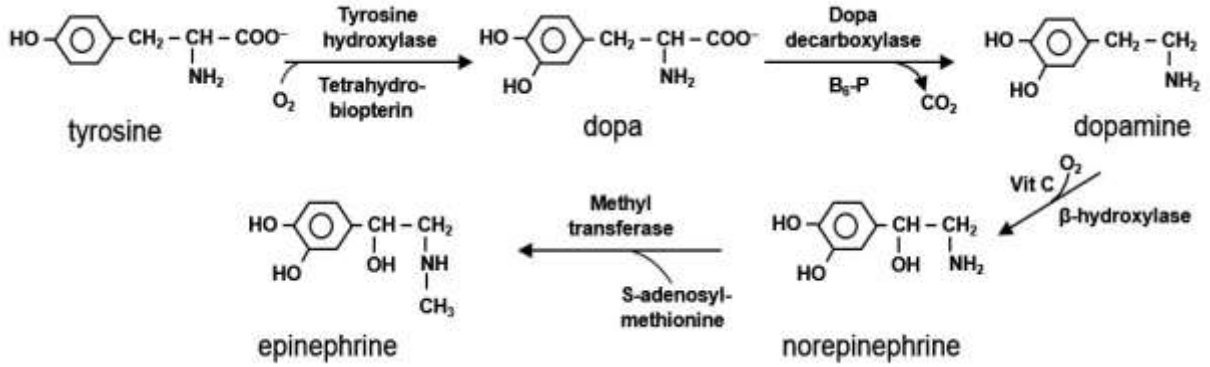
Thyroxine (T_4) and triiodothyronine (T_3) are derived from tyrosine. Iodine comprises 65% of T_4's weight, and 58% of T_3's. The thyroid hormones are the only iodine-containing compounds with established physiologic significance. Iodide is concentrated in the thyroid, where it is oxidized, and incorporated into thyroglobulin to form monoiodo- and diiodotyrosine (MIT & DIT). Coupling of iodotyrosines forms T_4 and T_3. Proteolytic cleavage of thyroglobulin releases T_4 and T_3. T_4 and T_3 are transported in the plasma mainly by thyroxine binding globulin (TBG). Plasma albumin contributes to the transport of T_4 and T_3 (low affinity but high concentration).



Thyroxine production is exclusively thyroidal, while 70-90% of T_3 and 95-98% of rT_3 are produced by peripheral de-iodination mostly in the liver. T_3 accounts for most of the thyroid hormone activity in peripheral tissues since it is 3-4 times more potent than T_4. Some researchers have questioned whether T_4 has any intrinsic biological activity. Reverse T_3 is biologically inactive.

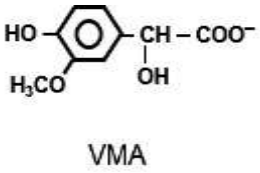
Catecholamines

Dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) are derived from tyrosine. They all have the catechol group (3,4-dihydroxyphenyl).



Dopa decarboxylase can be inhibited by the drug alpha-methyl dopa. L-dopa is a drug that can cross the blood-brain barrier to be converted to catecholamines in the brain, hence its usefulness in Parkinsonism.

Catabolism of epinephrine and norepinephrine is catalyzed by two enzymes: COMT (Catechol O-methyl transferase), which produces metanephrine and normetanephrine, and MAO (Monoamine oxidase). The final product is Vanillo-mandelic acid (Vanillyl-mandelic acid, VMA), which passes in the urine. Plasma metanephrines and 24-hour urine VMA indicate the amount of epinephrine and nor-epinephrine production and increase in cases of an epinephrine-secreting tumor (pheochromocytoma).



Tyramine

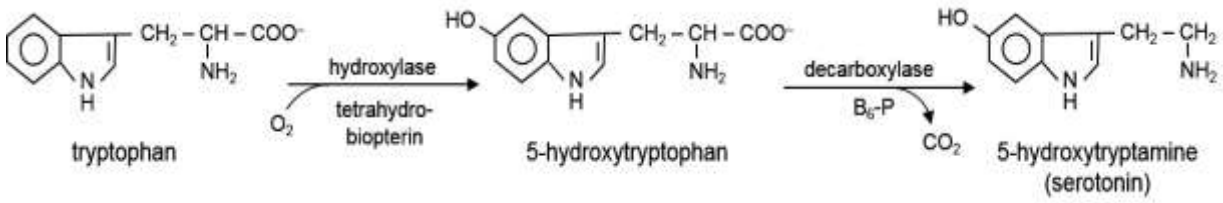
Tyramine is present in aged foods. It is derived from tyrosine by decarboxylation. It can displace catecholamines from their stores, which is dangerous in patients taking MAO inhibitors as antidepressant.

Note: L-aminoacid decarboxylases require, as a coenzyme, pyridoxal phosphate derived from vitamin B_6 (pyridoxine) and produce important amines, e.g., dopamine, serotonin, histamine and GABA. This shows the importance of vitamin B_6 for the function of the nervous system.

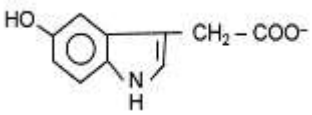
Note: Brain uptake of aminoacids depends on their plasma concentration. Aminoacids compete for brain transporters, e.g., branched chain aminoacids decrease tryptophan uptake and tryptophan decreases glutamic acid uptake. Trials have been made to induce sleep or control the mood by diet, and also to enhance performance and delay fatigue in athletic competitions by preferential aminoacid supply.

Serotonin

Serotonin (5-hydroxytryptamine) is derived from tryptophan.

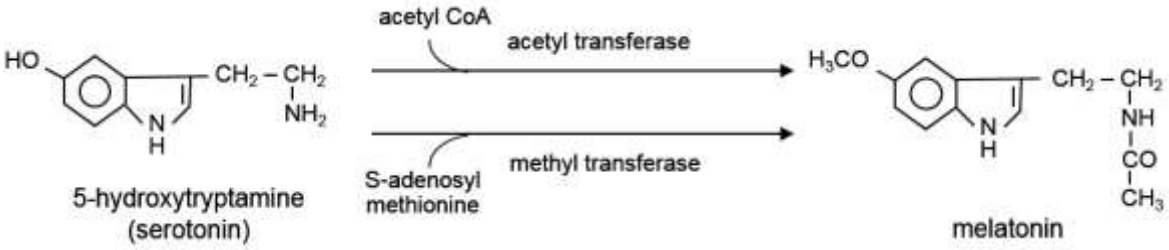


Catabolism of serotonin is catalyzed by monoamine oxidase (MAO). The product is 5-hydroxy-indole-acetic acid (5-HIAA), which passes in the urine. It increases in 24-hour urine in cases of carcinoid disease (argentaffinoma, serotonin-secreting tumor).



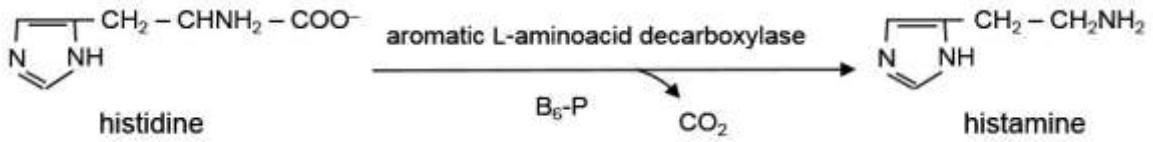
Melatonin

Melatonin is produced in the pineal body by N-acetylation and O-methylation of serotonin.



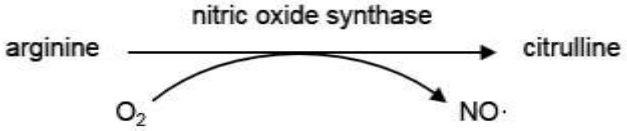
Histamine

Histamine is produced by decarboxylation of histidine.



Nitric oxide

Nitric oxide ($NO^{\cdot}$) is a free radical, a hormone, and a neurotransmitter. It is produced from arginine. Nitric oxide synthase is three isozymes: neuronal, endothelial, and phagocytic. The last one is an inducible isozyme, which generates nitric oxide for killing invading bacteria.



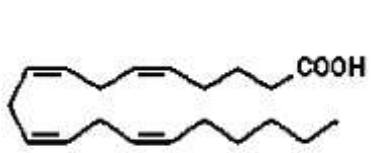
Eicosanoids

Eicosanoids are metabolites of arachidonic acid (C20:4, eicosatetraenoic acid). These are hormone-like substances with a localized action due to their very short half-life. Eicosanoids occur at nanomolar concentrations in tissues, but they have profound biological activities.

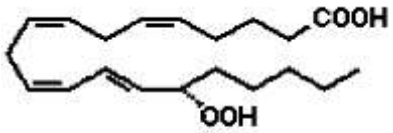
Arachidonic acid is released from position 2 of glycerophospholipids of the membrane by the action of phospholipase A_2. Arachidonic acid takes then either one of two main routes: cyclooxygenase pathway, which leads to formation of prostanoids (prostaglandins and thromboxane), or lipoxygenase pathway, which leads to formation of leukotrienes and lipoxins. Cyclooxygenase is inhibited by aspirin and non-steroidal anti-inflammatory drugs. Phospholipase A_2 is inhibited by corticosteroids, which block the formation of all eicosanoids. The production of eicosanoids is not limited to the prostate or leukocytes. Their names were derived with their early discovery. Their production is widely distributed.

Lipoxygenase Products

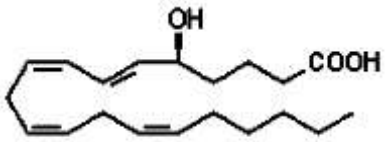
Lipoxygenase enzymes introduce a hydroperoxyl group next to any of the four double bonds of arachidonic acid, forming hydroperoxyeicosatetraenoic acid (HPETE). By peroxidase action, the corresponding hydroxyeicosatetraenoic acid (HETE) is produced. Many of these compounds have been identified as inflammatory mediators.



Eicosatetraenoic acid
(Arachidonic acid)

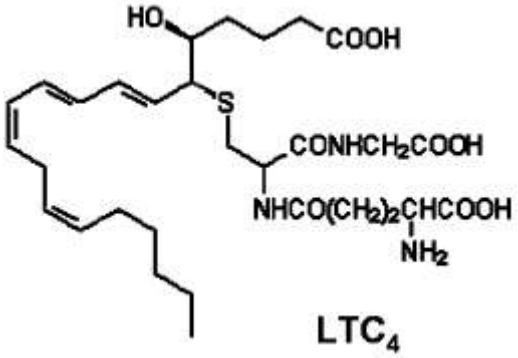


15-HPETE



5-HETE

Leukotrienes are derived from 5-HPETE. Leukotriene C_4 (LTC_4) has a conjugated glutathione. LTD_4 and LTE_4 are produces by removal of glutamate, then glycine. They are termed trienes because of the three conjugated double bonds (alternating double and single bonds). The subscript denotes the total number of double bonds.

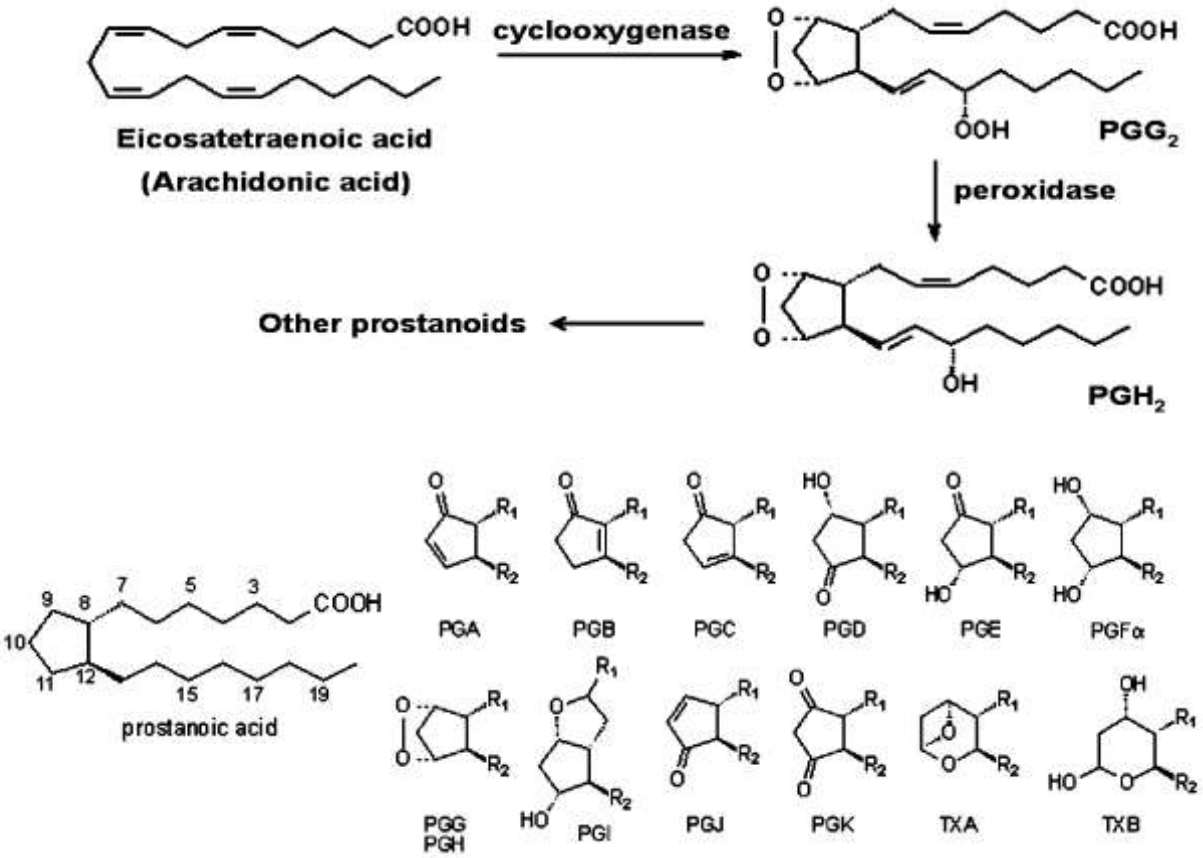


LTC_4

A mixture of leukotrienes (LTC_4, LTD_4, and LTE_4) constitutes what was known as slow reacting substance of anaphylaxis involved in hypersensitivity. Leukotriene antagonists have been used for the management of bronchial asthma, e.g., montelukast (trade names: Singulair and Montelo-10) and zafirlukast (Accolate) the leukotriene receptor antagonists and the 5-lipoxygenase inhibitor zileuton (Zyflo) used for the maintenance treatment of asthma.

Cyclooxygenase products

Cyclooxygenase enzyme initiates the production of prostanoids. Prostanoids have a ring structure and two hydrocarbon tails. The alphabet numbering distinguishes one member from the others by the shape of the ring structure. The subscript 2 refers to the two double bonds remaining in the hydrocarbon tails.



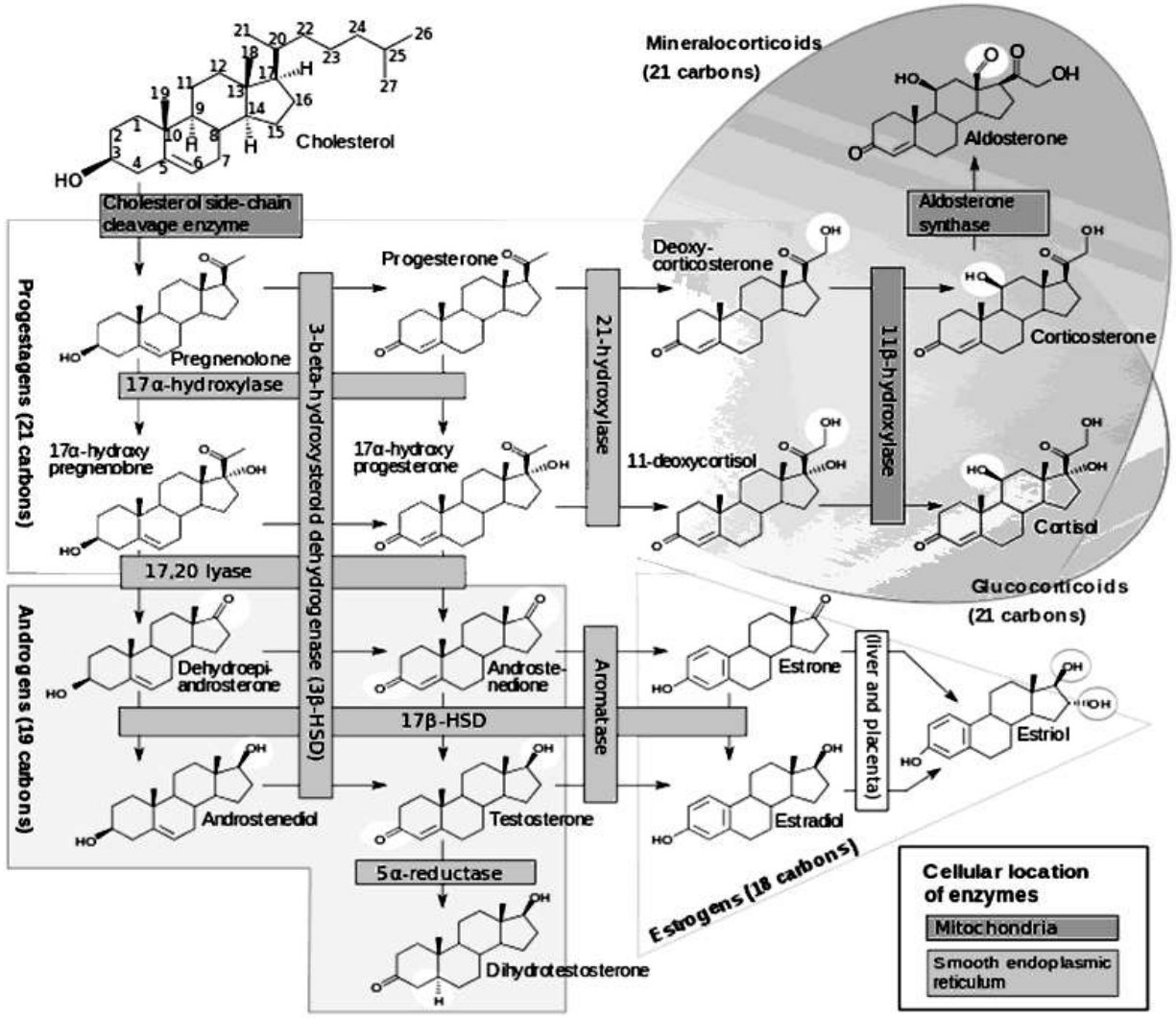
Prostaglandins have diverse biologic activities. Their role in inflammation and pain production has led to the use of cyclooxygenase inhibitors as anti-inflammatory. Thromboxane A_2 (TXA_2) is a powerful platelet aggregator and vasoconstrictor. Thromboxane A_2 produced by platelets is antagonized by prostacyclin (PGI_2) produced by the endothelium. Aspirin is useful as anti-aggregator due to its inhibition of cyclooxygenase and prevention of thromboxane synthesis.

The 3-series prostanoids are derived from eicosapentaenoic acid (EPA, C20:5). They have much lower biological activities than those of the 2-series. Thromboxane A_3 produced from EPA is a less potent aggregator than thromboxane A_2 produced from arachidonic acid. This may explain the protective action of fish oil against coronary thrombosis.

Two isozymes have been identified for cyclooxygenase. COX-1 isozyme is constitutive, and it is essential for healthy gastric tissue, renal homeostasis, and platelet aggregation. COX-2 is inducible by cytokines and bacterial endotoxin, and is inhibited by corticosteroids and selective COX-2 inhibitors. Aspirin and non-steroidal anti-inflammatory drugs inhibit both isozymes. COX-3 is a splice variant of COX-1, which is said to be the target of paracetamol action. However, its function in humans is highly disputed.

Steroid hormones

Steroid hormones are synthesized from cholesterol in the adrenal cortex, gonads, and placenta. The main glucocorticoid in man is cortisol. The main mineralocorticoid is aldosterone. The main adrenal androgens are dehydroepiandrosterone and androstenedione. The main androgen, which is formed by the testis, is testosterone. The main estrogen, which is formed by the ovary, is estradiol.



Deficiency of 21-hydroxylase leads to deficient cortisol production and accumulation of 17-hydroxyprogesterone. The latter shifts to androgen synthesis. The condition is worsened by the lack of feedback inhibition of ACTH production by cortisol. Urinary 17-ketosteroids increase.

Estradiol may be produced by peripheral aromatization of testosterone in the liver and adipose tissue. Aromatase inhibitors may be used for hormonal therapy, e.g., with estrogen receptor-positive breast cancer.

Testosterone reductase is important for production of the active metabolite, dihydro-testosterone, responsible for secondary sex characters.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following hormones may be secreted concomitantly with ACTH?
(A) Cortisol.
(B) Melanocyte stimulating hormone.
(C) Cortictropin releasing hormone.
(D) Growth hormone.

2- Which of the following may be a marker for an epinephrine-secreting tumor?
(A) Vanillylmandelic acid (VMA).
(B) Homovanillic acid.
(C) Phenyl acetic acid.
(D) Phenyl pyruvic acid.

3- To increase the synthesis of catecholamines in the brain, a patient with Parkinsonism may be given
(A) L-dopa.
(B) alpha-methyl dopa.
(C) MAO inhibitors.
(D) tyramine.

4- Which of the following may be a urinary marker for a serotonin-secreting tumor?
(A) 5-Hydroxytryptamine.
(B) 5-Hydroxyindole-acetic acid (5-HIAA).
(C) Vanillylmandelic acid (VMA).
(D) Phenylacetyl-glutamine.

5- Prostaglandin E differs from prostaglandin F in which of the following?
(A) The number of carbon atoms.
(B) The number of double bonds in the two chains.
(C) The attachments to the cyclopentane structure.
(D) Absence of the 15-OH group in prostaglandin F.

6- Which of the following enzymes produces peroxides?
(A) Cyclooxygenase.
(B) Lipoxygenase.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

7- Vitamin C is needed for which of the following?
A) Lysyl oxidase.
B) Dopamine hydroxylase.
C) L-Aminoacid decarboxylase.
D) Monoamine oxidase.

8- Tyramine is which of the following?
(A) A product from tyrosine.
(B) A hormone produced by adrenal medulla.
(C) A drug for treatment of hypertension.
(D) A drug used for treatment of Parkinsonism.

9- Which of the following is the correct order in the steroid hormone synthetic pathway?
(A) testosterone $\rightarrow$ estradiol $\rightarrow$ progesterone.
(B) progesterone $\rightarrow$ estradiol $\rightarrow$ testosterone.
(C) progesterone $\rightarrow$ testosterone $\rightarrow$ estradiol.
(D) estradiol $\rightarrow$ progesterone $\rightarrow$ testosterone.

Signal Transduction

The response of a cell to a chemical signal is mediated by opening or closure of an ion channel and/or cell protein modification. Signal transduction starts by binding of the signal molecule to its receptor. Hormone receptors are proteins; they are located in the cell membrane or intracellularly. Intracellular receptors are for lipid-soluble hormones, retinoic acid, and gases like nitric oxide and carbon monoxide.

Lipid-soluble (hydrophobic) hormones include steroid hormones, thyroid hormones: thyroxine and triiodothyronine (T_4 and T_3), and calcitriol (active vitamin D). These, in addition to retinoic acid, need a transporter protein in the blood. They cross the cell membrane and bind to intracellular receptors. The hormone-receptor complex binds to a specific hormone-responsive element of DNA, thus enhancing or inhibiting the transcription of certain messenger RNA. The final effect of the hormone is therefore induction (increased synthesis) or repression (decreased synthesis) of a certain protein or enzyme. Cellular endogenous substances may use a similar mechanism, e.g., cholesterol inhibition of its own synthesis. This is a slow mechanism, suitable for long-term control.

Hydrophilic hormones, on the other hand, need no blood transporters and have a short half-life due to rapid degradation or excretion. They bind to cell membrane receptors. Membrane receptors for hormones like insulin and growth factors, which stimulate cell growth and proliferation, have intracellular domains linked to a protein tyrosine kinase or they have tyrosine kinase activity themselves. Other hydrophilic hormones like pituitary glycoproteins and catecholamines have serpentine membrane receptors, linked through G proteins to an enzyme that produces an intracellular second messenger. The receptor may have enzyme activity itself that produces the second messenger, e.g., atrial natriuretic factor receptor, which produces cGMP.

Tyrosine kinases phosphorylate different proteins at their tyrosine residues, which activates them to start a cascade of reactions leading to cell growth and division. Insulin receptor is a glycoprotein hetero-tetramer. It has two α-subunits protruding on the outer surface of the cell membrane, forming an insulin-binding site, and two transmembrane β-subunits having intracellular domains with tyrosine kinase activity. Upon binding insulin, the receptor undergoes conformational change that activates the two intracellular domains, which tyrosine-phosphorylate each other. The phosphorylated receptor then phosphorylates insulin receptor substrates causing several enzyme cascades to be activated leading to the different effects of insulin.

JAK-STAT pathway is activated by different cytokines, growth factors, growth hormone, and prolactin. Upon binding the ligand, the receptors dimerize and bind to Janus kinases (JAKs), named after the two-headed Roman god. JAKs phosphorylate each other, the receptors, and STATs (Signal Transducer and Activator of Transcription), which are gene-specific transcription factors. Activated STATs enter the nucleus and bind to specific enhancer sequences of target genes, thus regulating their transcription. Alterations in JAK-STAT signaling can result in serious diseases like cancer and diseases affecting the immune system, such as severe combined immunodeficiency disorder (SCID).

Serpentine membrane receptors traverse the cell membrane seven times and are linked to G proteins. G proteins are membrane proteins, which link the hormone receptor to an enzyme that produces an intracellular second messenger. G protein is formed of three subunits: alpha (α), beta (β), and gamma (γ). Upon binding the hormone, the receptor undergoes conformational change and activates the specific G protein. The α-subunit then separates from the other two subunits, binds GTP (guanosine triphosphate) and activates or inhibits the enzyme that produces the second messenger. The α-subunit is then self-deactivated by its GTPase activity that hydrolyzes GTP to GDP (guanosine diphosphate). The subunits of G proteins then reassemble. This cascade amplifies the signal: one receptor activates several G proteins, and one enzyme produces several copies of the second messenger.

Enzymes linked to G proteins include adenylate cyclase, which is activated or inhibited by α-subunit (α_s or α_i) of G_s or G_i. This enzyme produces the second messenger cAMP (3',5'-cyclic adenosine monophosphate) from ATP (adenosine triphosphate). Cyclic AMP activates protein kinase A (cAMP-dependent protein kinase), which phosphorylates different enzymes at their serine, and possibly threonine residues, thus activating or inhibiting them. Hormones that act by increasing cAMP include glucagon and epinephrine (through β-receptors). Epinephrine, through α_2-receptors and G_i proteins, decreases cAMP and inhibits insulin release from pancreatic cells. Cyclic AMP is inactivated by phosphodiesterase, which converts it to 5'-AMP. Phosphorylated proteins are dephosphorylated by protein phosphatases, which are generally activated by insulin. Thus, insulin causes phosphorylation at tyrosine and dephosphorylation at serine residues.

Another enzyme linked to G_q protein is phospholipase C. This enzyme acts on membrane phosphatidyl inositol 4,5-bisphosphate producing two second messengers: diacylglycerol (DAG), which activates protein kinase C, and inositol 1,4,5-trisphosphate (IP_3), which stimulates the release of calcium ions from their intracellular stores. Both protein kinase C and calcium-calmodulin complex phosphorylate different proteins and enzymes. An example of this action is α_1-adrenergic stimulation.

Another second messenger is cGMP (3',5'-cyclic guanosine monophosphate) produced from GTP by either a membrane bound or a soluble (cytosolic) guanylate cyclase. The soluble enzyme is activated directly by nitric oxide, and the membrane enzyme by atrial natriuretic factor (the receptor has enzyme activity). Cyclic GMP activates protein kinase G (cGMP-dependent protein kinase), and is deactivated by phosphodiesterase.

Various drugs and natural toxins exert their effect by interfering with the production or the elimination of second messengers. Cholera toxin and the toxin produced by some strains of *E.coli*, for example, ADP-ribosylate the α_s-subunit of the intestinal mucosal cells (using NAD^+ as an ADP-ribose donor). This inhibits the GTPase of α_s-subunit and its inactivation, leading to excessive production of cAMP. Pertussis toxin inhibits the α_i-subunit, causing an increase in cAMP. Xanthine drugs inhibit phosphodiesterase, leading to increased intracellular cAMP. Lithium, used as antidepressant, inhibits dephosphorylation of inositol phosphate and blocks the phosphoinositide cycle. Inhibitors of cGMP phosphodiesterase are used for maintaining smooth muscle relaxation and vasodilatation to treat erectile dysfunction. Nitric oxide is unstable and degraded in seconds, but nitroglycerine and other nitro-vasodilators used for angina yield a steady stream of nitric oxide.

Study Questions

Choose one best answer for every question of the following:

10- Which of the following has an intracellular receptor?
(A) Insulin.
(B) Glucagon.
(C) Growth hormone.
(D) Nitric oxide.

11- Serpentine membrane receptors have their ligand binding site on the
(A) outer surface of the membrane.
(B) inner surface of the membrane.
(C) domain linked to G protein.
(D) domain linked to adenylate cyclase.

12- What is the function of G proteins?
(A) Activating the hormone receptor.
(B) Catalyzing the production of cAMP.
(C) Joining the hormone receptor to ATP for production of cAMP.
(D) Activating or inhibiting the production of the second messenger.

13- G proteins are
(A) membrane proteins.
(B) hormone receptors.
(C) both (A) and (B).
(D) neither (A) nor (B).

14- G proteins are activated by which of the following?
(A) Activated receptors.
(B) Adenylate cyclase.
(C) Cyclic AMP.
(D) GTPase activity of alpha-subunit.

15- Which of the following enzymes is linked to G protein?
(A) Phospholipase C.
(B) Protein kinase A.
(C) Guanylate cyclase.
(D) Protein kinase C.

16- Which of the following enzymes produces two second messengers?
(A) Phospholipase C.
(B) Adenylate cyclase.
(C) Guanylate cyclase.
(D) Phosphodiesterase.

17- Which of the following enzymes catalyzes the formation of a phosphate ester bond?
(A) Phospholipase C.
(B) Adenylate cyclase.
(C) Phosphodiesterase.
(D) Protein phosphatase.

18- Which of the following is a membrane enzyme?
(A) Adenylate cyclase.
(B) Phospholipase C.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

19- How is the G protein action terminated?
(A) Dissociation of the ligand from the receptor.
(B) Dissociation of the α-subunit from the rest of the protein.
(C) GTPase activity of the α-subunit.
(D) Phosphodiesterase activity of insulin.

20- Which of the following hormones causes phosphorylation of serine residues of proteins?
(A) Insulin.
(B) Insulin-like growth factor.
(C) Epinephrine.
(D) Calcitriol.

21- How is the hormone action terminated?
(A) Dissociation of the hormone from the receptor.
(B) GTPase activity of the α-subunit.
(C) Protein phosphatase and phosphodiesterase activities.
(D) All the above combined.

22- A hormone may have different effects on different tissues. Why?
(A) Presence or absence of a receptor for the hormone.
(B) The type of the hormone receptors.
(C) Multiplicity of the pathways from the activated receptor.
(D) All the above.

23- Lithium may disturb the functions that depend on which of the following enzymes?
(A) Protein kinase A.
(B) Protein kinase C.
(C) Protein kinase G.
(D) Protein tyrosine kinase.

24- Which of the following enzymes act on a membrane component?
(A) Phospholipase C and phospholipase A_2.
(B) Protein kinase A and protein kinase G.
(C) Adenylate cyclase and guanylate cyclase.
(D) Cyclooxygenase and lipoxygenase.

25- Sildenafil (Viagra®) exerts its effect through which mechanism?
(A) Activation of guanylate cyclase.
(B) Inhibition of guanylate cyclase.
(C) Activation of phosphodiesterase.
(D) Inhibition of phosphodiesterase.

26- Caffeine increases intracellular cAMP via which mechanism?
(A) Activation of adenylate cyclase.
(B) Inhibition of adenylate cyclase.
(C) Activation of phosphodiesterase.
(D) Inhibition of phosphodiesterase.

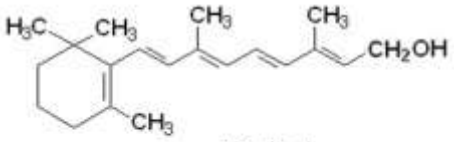
27- The hormone-responsive element of DNA is activated by which of the following?
(A) Cortisol and aldosterone.
(B) Testosterone and estradiol.
(C) The hormone receptor.
(D) The hormone-receptor complex.

28- Thyrotropin and thyroxine have which feature of signal transduction?
(A) They activate adenylate cyclase through receptor-linked G protein.
(B) They bind to their receptors and act as transcription factors.
(C) Thyrotropin binds to a cell surface receptor but thyroxine binds to an intracellular one.
(D) Thyroxine binds to a cell surface receptor but thyrotropin binds to an intracellular one.

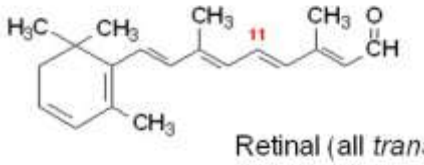
29- Which of the following have receptors with catalytic activity?
(A) Insulin and atrial natriuretic factor.
(B) Glucagon and epinephrine.
(C) Cortisol and estradiol.
(D) Growth hormone and insulin-like growth factor.

Vitamin A

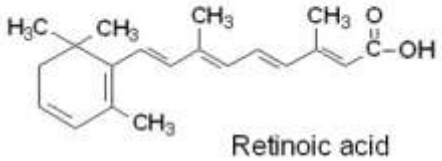
Vitamin A consists of three biologically active molecules, retinol, retinal (retinaldehyde) and retinoic acid. The term “retinoids” refers to all compounds, natural and synthetic, with similar structure and retinol-like activity. Vitamin A, in the form of retinyl ester is obtained from animal sources: cod liver oil, liver, kidney, butter, and eggs. Vitamin A is light-sensitive, and may be destroyed by light.



Retinol



Retinal (all *trans*)



Retinoic acid

Beta-carotene (β-carotene, a member of a family of plant molecules known as carotenoids) is a provitamin A. It is cleaved in the intestine by β-carotene dioxygenase to yield two retinal molecules. Retinal is reduced to retinol and esterified to retinyl ester. Carotenoids are obtained from fruits and vegetables containing yellow, orange and dark green pigments. They are only partially converted to vitamin A in the intestine, and mostly absorbed intact.

The recommended daily allowance of vitamin A is 900 retinol equivalents (RE) for adult male and 700 RE for adult female. For children it is 300-500 RE. Requirement increases in lactating women. One RE equals 1 µg of retinol or 3.3 International Units of vitamin A. It is equivalent to 6 µg of β-carotene, or in recent estimates double this amount, or even higher. The recommended upper limit for retinol intake is 3000 µg/day for adults.

Vitamin A is stored in the liver as retinyl ester. It is exported to other tissues carried on retinol binding protein (RBP). Carotenoids reaching the liver in chylomicron remnants are exported in VLDL and stored in adipose tissue. Plasma transport of retinoic acid is accomplished by binding to albumin.

Beta-carotene acts as a free radical scavenger. It is an important antioxidant component of a healthy diet. It is used for this purpose in some skin-care preparations.

Retinol functions in the synthesis of certain glycoproteins and mucopolysaccharides necessary for mucous production and normal growth regulation, acting like dolichol. Retinol is important for spermatogenesis and prevention of fetal resorption in rats.

Retinal is important for vision. The photoreceptor of rod cells, rhodopsin or visual purple, contains 11-*cis*-retinal coupled at three of the transmembrane domains of this protein. Rhodopsin is coupled to a specific G-protein called transducin. When rhodopsin is exposed to light, it is bleached, releasing all-*trans*-retinal. The conformational change activates transducin. The active α-subunit-GTP activates cGMP phosphodiesterase. The concentration of cGMP drops resulting in complete closure of the Na^+ channels. This leads to hyper-polarization of the cell membrane, which leads to a sequence of events with propagation of nerve impulses to the brain. The all-*trans*-retinal is processed by retinal epithelial cells: reduced to retinol, isomerized to 11-*cis*-retinol, then oxidized back to 11-*cis*-retinal that is incorporated in rhodopsin in the rod cells.

Retinoic acid results from irreversible oxidation of retinal. Retinoic acid is important for cell growth and differentiation and maintenance of a healthy epithelium. It has an intracellular receptor and acts on the cell genome like a steroid hormone. Expression of several genes is altered by retinoic acid, involving the earliest processes of embryogenesis including the differentiation of the three germ layers, organogenesis and limb development. More than 130 genes have been shown to be directly regulated (either positive or negative) via interaction with retinoic acid receptor. Retinoic acid is used therapeutically for dermatologic conditions and promyelocytic leukemia.

Vitamin A deficiency occurs only after prolonged lack of dietary intake. Biliary obstruction, fat malabsorption, and advanced liver disease may also cause vitamin A deficiency. Night blindness is an early symptom of vitamin A deficiency. Prolonged lack of vitamin A leads to deterioration of the eye tissue through xerophthalmia and progressive keratinization of the cornea. It is reported that approximately 250,000 to 500,000 malnourished children in the developing world go blind each year from a deficiency of vitamin A. Additional symptoms of the vitamin deficiency include xeroderma, increased susceptibility to infection (due to skin fissures and unhealthy mucosa, in addition to impaired immune system), anemia and cancer.

Over intake of the vitamin may lead to toxicity, which manifests as bone pain, hepatosplenomegaly, nausea, diarrhea, blurred vision, fatigue, weight-loss, menstrual abnormalities, and birth defects. If eaten in one meal, 30-90 g of polar bear liver is enough to kill a human being. It is advised to limit liver intake during pregnancy. It is safer to take the provitamin. Excessive carotene intake can cause yellowing of the skin, but no serious side effects.

Study Questions

Choose one best answer for every question of the following:

30- Transducin of rod cells is
(A) a membrane receptor.
(B) a G protein.
(C) an enzyme of phosphodiesterase activity.
(D) called the visual purple.

31- Nerve impulses are generated by light via
(A) increased cAMP.
(B) increased cGMP.
(C) cell membrane depolarization.
(D) complete closure of the Na^+ channels.

32- Which of the following compounds is directly involved in the visual cycle?
(A) Beta-carotene.
(B) Retinol.
(C) Retinal.
(D) Retinoic acid.

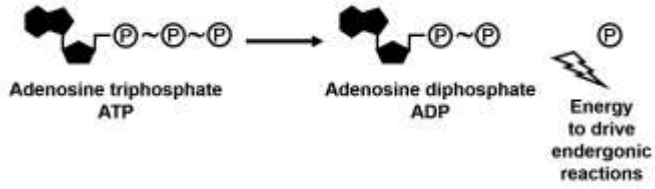
33- Which of the following compounds has a hormone-like action?
(A) Beta-carotene.
(B) Retinol.
(C) Retinal.
(D) Retinoic acid.

34- Which vitamin is the most toxic?
(A) A.
(B) C.
(C) D.
(D) E.

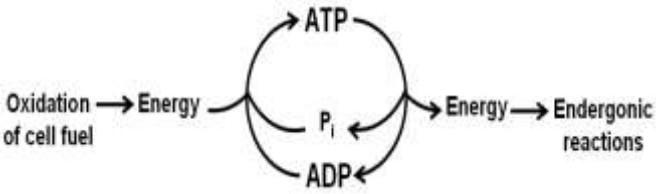
Energy Transformation

Exergonic reactions are accompanied by release of energy. Endergonic reactions need energy to go. Catabolic reactions are usually exergonic, while anabolic reactions are endergonic.

Endergonic reactions usually get energy from the hydrolysis of the nucleotide ATP (adenosine triphosphate). Hydrolysis of ATP is an exergonic reaction.



ATP is regenerated by phosphorylation of adenosine diphosphate (ADP). This is an endergonic reaction that gets the energy from the oxidation of cell fuel. Thus, ATP acts as the "energy currency" of the cell. A part of the energy resulting from fuel oxidation is saved in the form of ATP, and then ATP is consumed for driving various endergonic activities of the cell.



Cell fuel

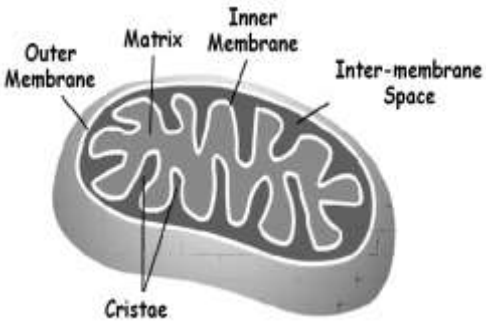
Cell fuel comprises molecules derived from dietary components:

- Carbohydrate fuel includes the monosaccharides: glucose, fructose, and galactose in addition to glycogen stored in the muscle and lactic acid produced by muscles and red blood cells.
- From lipids: fatty acids and glycerol are the fuel resulting from lipolysis (hydrolysis of triacylglycerols), and the ketone bodies: acetoacetate and beta-hydroxy-butyrate are produced by liver cells from fatty acids.
- Protein hydrolysis (proteolysis) produces aminoacids that can also act as a cell fuel.

Outline of cell fuel oxidation

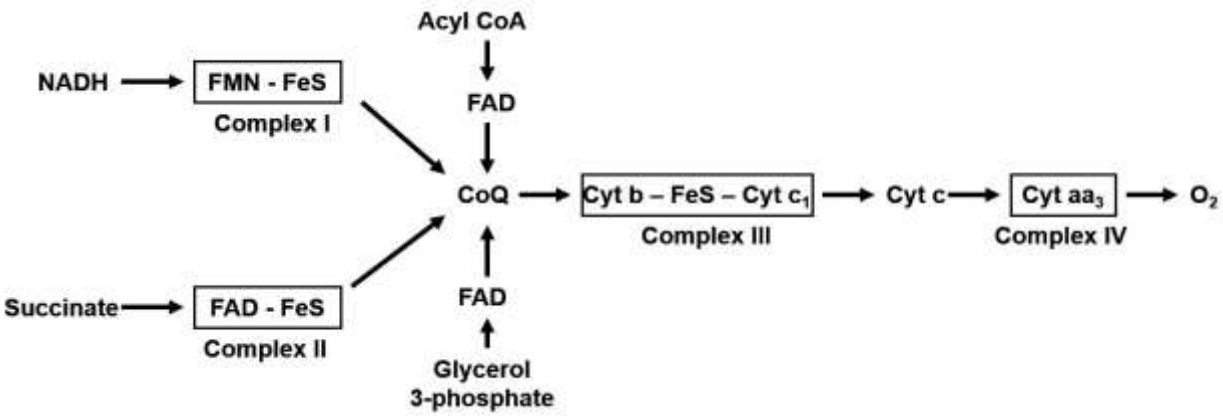
Oxidation of cell fuel is not a combustion process, but rather a slow, stepwise, and controlled procedure. A cell fuel is oxidized step by step in a pathway of chemical reactions, special for every type of fuel, to produce the common intermediate: acetyl coenzyme A (acetyl CoA, active acetate). This common intermediate is then oxidized by the tricarboxylic acid cycle (citric acid cycle, Krebs' cycle) in the cell mitochondria. During these oxidation steps, a little ATP is produced in what is called "substrate level phosphorylation" (two reactions in glycolysis and one in citrate cycle). Meanwhile, the coenzymes NAD^+ and FAD are reduced to NADH and $FADH_2$. NAD = Nicotinamide Adenine Dinucleotide, derived from niacin (nicotinic acid), a member of vitamin B complex. FAD = Flavin Adenine Dinucleotide, derived from riboflavin, another member of vitamin B complex. These reduced coenzymes are then oxidized by the respiratory chain, the electron transport chain, located in the inner mitochondrial membrane. This pathway is known as "oxidative phosphorylation", since it is coupled to ATP generation. In fact, this is the main ATP generator.

The respiratory chain

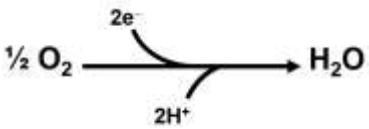


Respiratory chain components are a group of inner mitochondrial membrane proteins with their coenzymes or prosthetic groups that form an electron transport chain. Electrons flow from a component of low reduction (or redox) potential to one of higher reduction potential, and so on. The final electron acceptor is oxygen, which is reduced to water. During this step by step oxidation, most of the cellular ATP is generated by capturing the energy of oxidation to phosphorylate ADP. Mitochondria are considered the power plant of the cell since they contain the respiratory chain, in addition to enzymes of tricarboxylic acid cycle, fatty acid oxidation, and ketone body metabolism in the mitochondrial matrix.

Respiratory chain components include flavoproteins, iron-sulfur proteins, ubiquinone (coenzyme Q), and cytochromes. Cytochromes are heme-proteins whose iron oscillates between Fe^{3+} and Fe^{2+} by oxidation and reduction.



The electron transport chain is studied as four complexes, I to IV. Complex I oxidizes NADH and reduces ubiquinone (Co Q). Complex II oxidizes succinate, through FAD/$FADH_2$, and reduces coenzyme Q. Thus, coenzyme Q collects electrons from NADH and reduced flavoproteins. Complex III oxidizes ubiquinone and reduces cytochrome c. Complex IV (cytochrome aa_3), also called cytochrome oxidase, oxidizes cytochrome c and reduces molecular oxygen to water.



Energy from oxidative steps is used to pump protons out across the inner mitochondrial membrane against concentration gradient. Protons pass back into the mitochondria through complex V (ATP synthase), which drives the phosphorylation of ADP.

P:O ratio is the ratio of phosphorus atoms incorporated in ATP to oxygen atoms reduced to water by the mitochondria. Three ATP are produced when NADH is oxidized, but only two ATP by oxidation of $FADH_2$.

Control of energy production

The control of energy production is exerted by the concentration of ADP, which reflects the cell needs for energy (state IV of mitochondrial electron transport chain). A high concentration of ADP means a high cell activity that consumes much ATP. A low concentration of ADP means a low cell activity, which leads to lowered rate of oxidative phosphorylation. This leads to increased $NADH/NAD^+$ which inhibits the tricarboxylic acid cycle and oxidation of cell fuel. Thus, the oxidation of cell fuel and the production of energy proceed according to cellular needs. Excess fuel passes to storage pathways.

In a muscle undergoing strenuous exercise, the energy production by the respiratory chain is limited by oxygen supply (state V). After exercise, energy production is going at a maximal rate to restore energy store, limited only by the capacity of the respiratory chain (state III).

Inhibitors of the respiratory chain

Many substances can inhibit the respiratory chain. These can be used in the lab to study the chain reactions. Many of these inhibitors are of medical importance.

Inhibitors of the oxidative reactions: Complex I may be inhibited by rotenone (fish poison, insecticide) and some drugs like the barbiturate drug, amytal (hypnotic, sedative) and chlorpromazine (major tranquilizer). Succinate dehydrogenase (complex II) may be competitively inhibited by malonate. Complex III may be inhibited by some antibiotics like antimycin A. Cytochrome oxidase may be inhibited by carbon monoxide, cyanide, azide and hydrogen sulfide (poisons). These inhibitors stop the oxidative reactions and consequently ADP phosphorylation.

Inhibitors of phosphorylation: Some antibiotics like oligomycin block phosphorylation rather than oxidation. Since oxidation is coupled to phosphorylation, it stops consequently. Adding an uncoupler in this case allows oxidation to go on without phosphorylation.

Uncouplers: Some compounds like dinitrophenol can uncouple oxidation and phosphorylation, probably through dissipating the H^+ gradient across the inner mitochondrial membrane. Oxidation goes on with energy released as heat. P:O ratio decreases and may reach zero. Naturally occurring uncouplers include thyroxine, bilirubin and some microbial toxins. Calcium ions are pumped into the mitochondria against concentration gradient, deriving energy from oxidative reactions of respiratory chain. Therefore, calcium causes oxidation to go on with no phosphorylation, and it may be considered an uncoupler.

Inhibitors of ATP/ADP transport: Substances like atractyloside can inhibit oxidative phosphorylation by inhibiting the ATP/ADP transporter across the inner mitochondrial membrane.

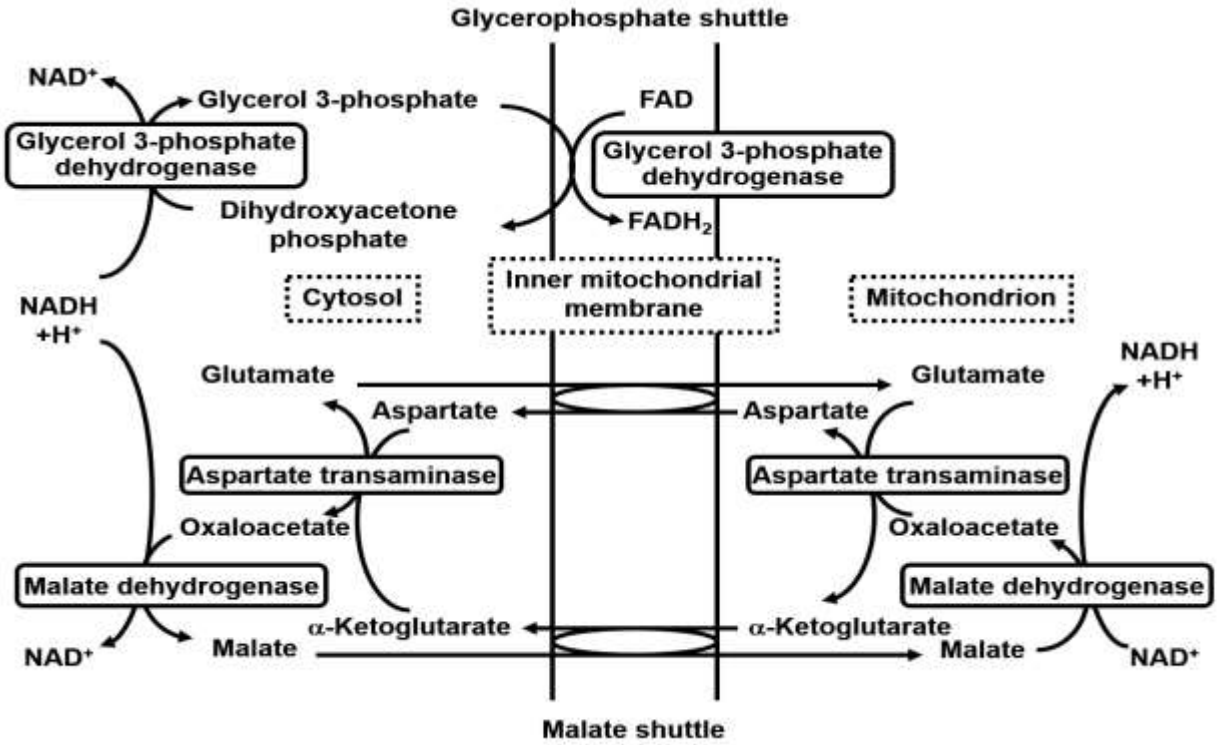
Heat production by the respiratory chain

Not all the free energy content of fuel molecules is trapped in ATP. Roughly speaking, about one half of the energy of fuel oxidation is liberated as heat. This is not wasted energy since warm-blooded organisms need heat production to compensate for heat loss to the environment and to keep a constant body temperature. When more heat is needed, shivering or voluntary activity ensures more free energy liberation as heat.

In certain cases, heat production needs to go on beyond the respiratory control by ADP. This is achieved by "brown adipose tissue", the site of "non-shivering thermogenesis". This tissue is present in humans in a small quantity, more in newborns. It is characterized by a well-developed blood supply and a high content of mitochondria. The inner mitochondrial membrane contains an uncoupling protein, "thermogenin", which acts as a proton conductance route. Its action is triggered by fatty acids released by the hormone sensitive lipase. It continually dissipates the proton gradient across the inner mitochondrial membrane, thus uncoupling oxidation and phosphorylation. Oxidation goes on, with production of heat. This tissue appears to be responsible for "diet induced thermogenesis", which may account for how some persons can "eat and not get fat". It is reduced or absent in obese persons.

Reducing equivalents from outside the mitochondria

Reducing equivalents generated in reactions outside the mitochondria (carried on NADH) may be transferred into the mitochondria through glycerol 3-phosphate/dihydroxyacetone phosphate shuttle or malate/aspartate shuttle. The latter is the main shuttle used.



Genetic defects of mitochondria

Due to the extreme importance of mitochondrial enzymes as power generators, genetic abnormalities of these enzymes lead to serious disease and are often fatal. Lack of energy supply is reflected in tissue and organ dysfunction like myopathy, encephalopathy and renal impairment. Lactic acidosis results from accumulation of NADH (with conversion of pyruvate to lactate by lactate dehydrogenase). MERRF (myoclonic epilepsy with ragged red fibers) and MELAS (myopathy, encephalopathy, lactic acidosis, and stroke) are due to mutations in mitochondrial tRNA. Leigh syndrome (subacute necrotizing encephalomyelopathy) is a neurometabolic disorder that results mainly from mutations of the respiratory chain. Inheritance pattern for mutated mitochondrial genes is from the mother to her infants, since mitochondria come from the ovum. For nuclear genes that produce mitochondrial proteins, the mutation is inherited in autosomal or X-linked patterns.

High energy compounds

ATP is a high-energy compound since it releases high energy on hydrolysis, enough to drive most endergonic reactions of the cell. Other high-energy phosphate compounds include ADP and other nucleoside di- and triphosphates, creatine phosphate, phosphoenol pyruvate, and 1,3-bisphosphoglycerate. ATP can be generated from these compounds.

$$ADP + GTP \rightleftharpoons ATP + GDP$$

$$ADP + ADP \rightleftharpoons ATP + AMP$$

$$ADP + Creatine\sim P \rightleftharpoons ATP + Creatine$$

Adenosine monophosphate (AMP) is present at low concentration in the cell. Its formation from ADP by adenylate kinase (the middle reaction above) is an indicator of the cell need for energy. It is an important allosteric modifier in the cell that facilitates energy producing pathways. Energy charge of the cell equals the ratio of ATP plus half ADP to total adenosine phosphate concentration, since two ADP can give one ATP.

Carbamoyl phosphate is another high-energy phosphate compound. Sulfur-containing high-energy compounds include acyl coenzyme A, e.g., acetyl CoA.

Functions of ATP:

- Energy currency of the cell.
- Phosphate donor.
- S-adenosyl methionine formation.
- Coenzyme synthesis.
- Allosteric regulator.
- Precursor of cAMP.
- Substrate for RNA synthesis.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is considered a catabolic pathway?
(A) Storing of excess fuel in adipose tissue.
(B) Conversion of glucose to glycogen.
(C) Oxidation of fatty acids for energy production.
(D) Synthesis of tissue proteins.

2- All cell fuel molecules are degraded to a common intermediate, which is
(A) Glucose.
(B) Citric acid.
(C) NAD^+.
(D) Acetyl CoA.

3- The rate of cellular oxidation for energy production is controlled by the concentration of which of the following?
(A) Fuel molecules.
(B) FAD.
(C) ADP.
(D) Acetyl CoA.

4- The respiratory chain oxidizes which of the following?
(A) Acetyl CoA.
(B) NADH.
(C) FAD
(D) Oxygen.

5- Which of the following is a mechanism of action of thermogenin?
(A) Inhibition of ATP/ADP transporter.
(B) Increasing production of thyroxine.
(C) Uncoupling of mitochondrial oxidation and phosphorylation.
(D) Activation of cytochrome oxidase.

6- Which of the following increases oxygen consumption by the mitochondria?
(A) 2,4-dinitrophenol.
(B) Oligomycin.
(C) Cyanide.
(D) Amytal.

7- Decreased energy charge of the cell leads to increased
(A) phosphorylation of creatine.
(B) rate of fuel oxidation.
(C) glycogen formation.
(D) all anabolic reactions.

8- The highest redox potential is that of
(A) NAD^+/NADH.
(B) oxygen/water ($½O_2/H_2O$) .
(C) pyruvate/lactate.
(D) cytochrome a: Fe^{3+}/Fe^{2+}.

9- Adding cyanide to a mitochondrial preparation causes
(A) inhibition of cytochrome oxidase.
(B) inhibition of ADP phosphorylation.
(C) decrease of the proton gradient across the inner mitochondrial membrane.
(D) all the above.

10- Why is the energy yield of glucose oxidation in the skeletal muscle and brain lower than in the liver, kidney and heart?
(A) Glycerol phosphate shuttle in the liver.
(B) Malate shuttle in the brain.
(C) Absence of malate shuttle in the liver.
(D) Production of $FADH_2$ by mitochondrial glycerol 3-phosphate dehydrogenase.

Oxidant Stress and Antioxidants

Oxygen has a high reduction potential. However, oxygen molecules in their ground state are quite stable. An oxygen molecule is reduced by four electrons to produce water, which is the normal process of mitochondrial electron transport chain. Reactive oxygen species (ROS) impose an oxidant stress that should be dealt with.

Sources and danger of reactive oxygen species (ROS)

The respiratory chain (electron transport chain) of the mitochondria, when working at maximum speed like in case of reperfusion after ischemia, may produce ROS. An oxygen molecule may be partially reduced producing a ROS as a byproduct. One-electron reduction of O_2 produces superoxide anion radical ($\cdot O_2^-$). Two-electron reduction of O_2 produces hydrogen peroxide (H_2O_2).

Superoxide is generated as an intermediate product of some oxidation-reduction reactions and as a defense mechanism by activated neutrophils. Superoxide, spontaneously or catalyzed by superoxide dismutase, produces H_2O_2.

Organic peroxides are formed by normal pathways of the cell, e.g., cyclooxygenase and lipoxygenase pathways of arachidonic acid. Peroxides can be the source of free radicals.

Hydroxyl radical can be generated by radiation and from the reaction of H_2O_2 with ferrous ions (Fenton reaction):

$$H_2O_2 + Fe^{2+} \longrightarrow OH^- + Fe^{3+} + \cdot OH$$

Singlet oxygen ($^1O_2$, a ROS) is generated by activation of ground state oxygen by sunlight and porphyrins. Singlet oxygen can attack sound molecules to produce peroxides.

Free radicals are chemically active, and to pair their single electrons, attack sound molecules thus forming new free radicals. This propagates a destructive chain reaction, commonly known as lipid peroxidation since unsaturated lipids are the most susceptible molecules for this process. This destructive process can affect all molecules including proteins and DNA. Some signs of aging, e.g., skin pigments are attributed to peroxidative reactions. Cutaneous lesions of porphyria may result from ROS. Hemolysis can be a consequence of decreased antioxidant capacity of erythrocytes. Altered LDL (low-density lipoprotein) may initiate the atherogenic process. Alteration of DNA may even lead to cancer.

Main antioxidant mechanisms

Antioxidants are compounds that act as free radical scavengers. Lipid-soluble antioxidants include vitamin E (tocopherol) and carotenes. Water-soluble antioxidants include vitamin C (ascorbic acid) and uric acid.

Plasma proteins have an antioxidant role: transferrin binds iron in the plasma and ceruloplasmin ferroxidase activity keeps it in the ferric state, thus preventing the generation of free radicals. These two proteins may provide the main antioxidant power of the plasma.

The cellular integrity is a safeguard against peroxidation. Once the tissue integrity is compromised, destructive peroxidative reactions proceed much faster.

Antioxidant enzymes

Superoxide dismutase is an enzyme, present in all aerobic organisms, which catalyzes the dismutation of superoxide:

$$\cdot O_2^- + \cdot O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Premature infants may not have a fully developed capacity to produce superoxide dismutase. Therefore, it may be dangerous to expose them to excessively oxygen enriched atmosphere.

Catalase helps getting rid of hydrogen peroxide:

$$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2$$

Glutathione peroxidase, a selenium-containing enzyme, catalyzes the reduction of hydrogen peroxide or organic peroxides on the expense of glutathione (G-SH). Glutathione is a tripeptide that owes its reducing power to the sulfhydryl group of cysteine:

$$2G\text{-}SH + H_2O_2 \longrightarrow G\text{-}S\text{-}S\text{-}G + 2H_2O$$

Glutathione is kept in the reduced state thanks to glutathione reductase enzyme:

$$G\text{-}S\text{-}S\text{-}G + NADPH + H^+ \longrightarrow 2G\text{-}SH + NADP^+$$

The main generators of NADPH are the dehydrogenases of the hexose monophosphate (HMP) pathway. Therefore, the antioxidant power of the cell is reduced in cases of glucose 6-phosphate dehydrogenase deficiency, manifested in hemolysis.

Study Questions

Choose one best answer for every question of the following:

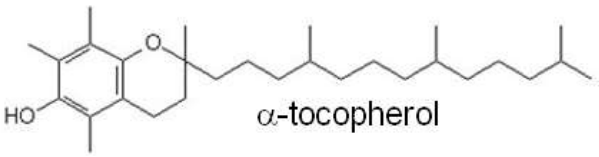
1- A free radical has
(A) an unpaired electron.
(B) the ability to attack sound molecules.
(C) both (A) and (B).
(D) neither (A) nor (B).

2- The free radical-initiated and propagated-chain reaction is called lipid peroxidation because
(A) only lipids are attacked.
(B) only lipids generate free radicals.
(C) unsaturated lipids are the most susceptible molecules.
(D) none of the above.

3- To increase the antioxidant power, one may take any of the following <u>except</u>:
(A) vitamin E and vitamin C.
(B) beta-carotene.
(C) selenium.
(D) iron.

4- Premature infants are not to be exposed to high-oxygen atmosphere because they have
(A) high lipid peroxides levels.
(B) high uric acid in their plasma.
(C) low ventilation capacity of their lungs.
(D) the capacity to produce superoxide dismutase not fully developed.

Vitamin E



Vitamin E is a mixture of tocopherols and tocotrienols. The latter contain three double bonds in the hydrocarbon chain. The tocopherols are the major sources of vitamin E in the diet. The tocopherols differ by the number and position of methyl ($-CH_3$) groups present on the ring system of the chemical structure. The different tocopherols are designated α-, β-, γ-, and δ-tocopherol. Most vitamin E in diet is in the form of γ-tocopherol from soybean, canola, corn, and other vegetable oils. The name "tocopherol" is derived from Greek words meaning "to carry a pregnancy" since this alcohol was found to be essential for rat fertility.

Vitamin E is absorbed from the intestine packaged in chylomicrons, and taken by the liver in chylomicron remnants. Within the liver, α-tocopherol transfer protein preferentially transfers α-tocopherol to VLDL, thus α-tocopherol is the most abundant tocopherol in tissues. Due to its lipophilic nature, vitamin E accumulates in cellular membranes, fat deposits and other circulating lipoproteins. The major site of vitamin E storage is in adipose tissue.

The major function of vitamin E is to act as a natural antioxidant, a free radical scavenger. It can donate the phenolic hydrogen and form rather stable radicals itself. Tocopherols can be regenerated from the tocopheroxyl radicals by reaction with vitamin C or glutathione.

The recommended daily allowance of vitamin E is 15 mg of α-tocopherol. The vitamin requirement is dependent on the consumption of polyunsaturated fatty acids, which are most prone to peroxidative damage. The requirement increases with lactation. The major source of the vitamin is plant oils and nuts. Therefore, vegetable fat is important in diet.

No major disease states have been found to be associated with vitamin E deficiency in humans. The major manifestation of the vitamin deficiency is an increase in red blood cell fragility. Neurological disorders have been associated with vitamin E deficiencies due to fat malabsorptive disorders. Increased intake of vitamin E is recommended in premature infants as well as in persons consuming a diet high in polyunsaturated fatty acids. Clinical trials showed no value of the vitamin supplement towards prevention of coronary heart disease. Results of the trials concerning different conditions related to oxidant stress, such as cancer, cataract, and Alzheimer's disease are non-conclusive or disappointing.

No sure toxicity has been related to vitamin E, although high doses are claimed to cause bleeding problems. Many agencies have set an upper tolerable intake level (UL) for vitamin E at 1,000 mg (1,500 IU) /day.

Study Questions

Choose one best answer for every question of the following:

5- Which of the following is an antioxidant that may be integrated in cell membrane?
(A) Tocopherol.
(B) Ascorbic acid.
(C) Uric acid.
(D) Hydrogen peroxide.

6- Vitamin E is
(A) a water-soluble vitamin.
(B) synthesized by intestinal flora.
(C) an antioxidant.
(D) a major energy source.

7- Rich sources of tocopherol include
(A) plant oils.
(B) fish liver oil.
(C) both (A) and (B).
(D) neither (A) nor (B).

8- Tocopherol can be regenerated from tocopheroxyl radicals by reaction with
(A) vitamin C.
(B) glutathione.
(C) either (A) or (B).
(D) neither (A) nor (B).

9- Vitamin E deficiency may be seen with
(A) obstructive jaundice.
(B) intake of mineral oils.
(C) both (A) and (B).
(D) neither (A) nor (B).

10- Vitamin E acts as an antioxidant by
(A) donating the phenolic hydrogen and forming rather stable radicals itself.
(B) catalyzing the dismutation of superoxide anion radicals.
(C) inhibiting Fenton reaction.
(D) increasing selenium absorption.

11- Photosensitivity of porphyria patients may be mitigated by the use of
(A) Iron-containing compounds.
(B) Carotene and vitamin E.
(C) Unsaturated oils.
(D) Organic peroxides.

12- Vitamin E supplements would be recommended for which of the following?
(A) Prevention of coronary heart disease.
(B) Management of glucose 6-phosphate dehydrogenase deficiency.
(C) Consumption of high-animal-fat diet.
(D) Low exposure to sunlight.

Carbohydrate Metabolism

Carbohydrate digestion and absorption

Digestion of diet carbohydrate starts in the mouth and is completed in the intestine. Polysaccharides (starch and glycogen), and disaccharides (sucrose and lactose) are enzymatically digested producing monosaccharides, mainly glucose in addition to fructose and galactose. These monosaccharides are absorbed from the intestine to the blood stream.

The main blood sugar is glucose, which acts as a universal cell fuel used by all cells. Glucose is actively absorbed by intestinal mucosal cells. It is co-transported with sodium ions by the transporter SGLT1, acting as a symport, across the luminal surface of the cells. Sodium pump uses ATP to pump sodium outside across the serosal side of the cells. This keeps the concentration gradient between the intestinal lumen and intracellular fluid that allows the absorption of more sodium and glucose. Glucose passes from the intestinal cells across the serosal side down its concentration gradient through a glucose uniport.

Galactose is absorbed the same way like glucose. Fructose is passively absorbed by GLUT5. Pentoses are passively absorbed.

Glucose uptake

Glucose is taken by different tissue cells by facilitated diffusion, down its concentration gradient. Some tissue cells like muscle cells can use fatty acids, ketone bodies, and even aminoacids as cell fuel. Insulin recruits glucose transporters (GLUT4) to the cell membrane of muscle and adipose tissue cells, allowing glucose utilization following meals. With low insulin level in the post-absorptive period, these cells cannot take glucose. However, increase of AMP in active muscle cells can recruit GLUT4 like insulin. Erythrocytes are entirely dependent on glucose for energy production by anaerobic degradation, since they lack mitochondria. Nerve cells are dependent on glucose and oxygen for energy production. Therefore, glucose transport in these cells is not insulin-dependent (GLUT1, GLUT3). Hepatic cell uptake of glucose is not insulin-dependent too. GLUT2 of hepatic cells has a high K_m for glucose, suitable for post-prandial portal blood glucose level. The same is true for pancreatic beta cells.

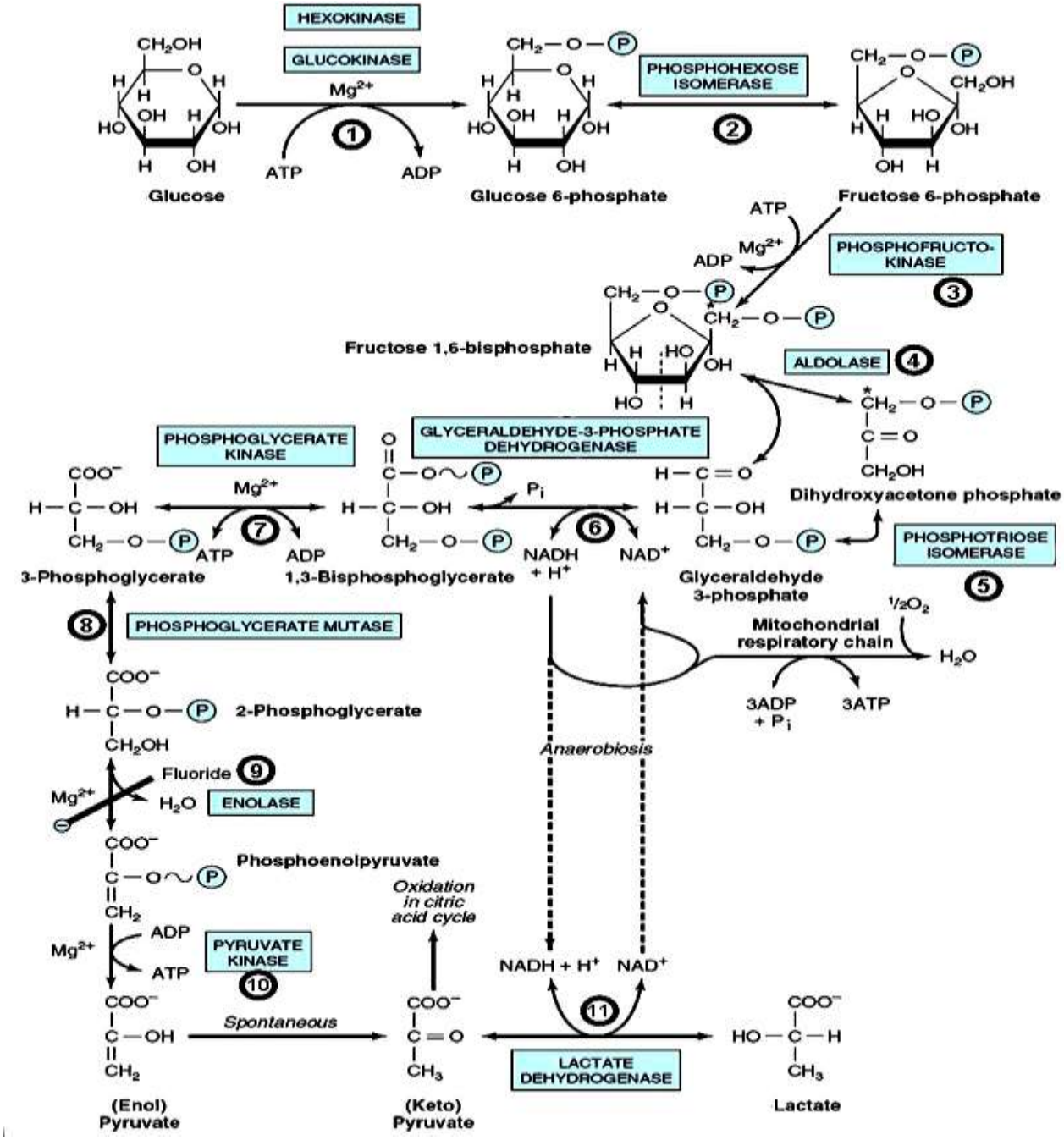
Glucose phosphorylation

Once inside the cell, glucose is trapped by phosphorylation to glucose 6-phosphate, using ATP as the phosphate and energy donor. This keeps the concentration gradient of glucose across the cell membrane in favor of glucose uptake. This reaction is catalyzed by glucokinase in liver cells, and hexokinase in other cells. Glucokinase has a high K_m for glucose (low affinity), suitable to deal with high glucose concentrations in the portal blood after meals. It is not allosterically inhibited by its product, glucose 6-phosphate, and it is induced in well-fed persons. Glucokinase in pancreatic beta cells acts as a glucose sensor. A mutation that increases its K_m decreases insulin secretion, and vice versa.

Oxidation of glucose

Glucose is a universal cell fuel. It is used by all cells. Glucose oxidation can be seen as two phases: anaerobic and aerobic phases. Glycolysis, or glycolytic pathway, is the anaerobic phase of glucose catabolism that takes place in all cells. In absence of oxygen or mitochondria, glucose is catabolized to lactic acid with little production of energy (2 ATP). In presence of oxygen and mitochondria, glycolysis ends in pyruvate, which is oxidized to acetyl CoA. The latter is oxidized by the citric acid cycle. Thanks to the respiratory chain, which oxidizes the produced NADH and $FADH_2$, glucose can yield 38 ATP.

Glycolysis



The glycolytic pathway is located in the cytosol of all cells. Lactate dehydrogenase (reaction 11) is driven by increased NADH. It is important for provision of NAD^+ needed for reaction 6 (glyceraldehyde 3-phosphate dehydrogenase) in anaerobic conditions. There are three one-way reactions: reactions 1, 3, and 10. This pathway can be inhibited by fluoride, which inhibits enolase enzyme (reaction 9). Fluoride is added to blood samples waiting for glucose estimation to prevent consumption of glucose by blood cells.

Energy yield

ATP is consumed in reactions 1 and 3 and is produced for each triose (half glucose) in reactions 7 and 10 (substrate level phosphorylation). This gives a net production of 2 ATP per each glucose molecule catabolized anaerobically. In case of aerobic glycolysis, 2 NADH from reaction 6 are oxidized by the respiratory chain giving 6 more ATP. The sum will thus be 8 ATP.

Control of glycolysis

The uptake of glucose by muscle and adipose cells is subject to control by insulin level in the blood, which increases by food intake. The three one-way reactions of glycolysis are site for control of the pathway by controlling their catalyzing enzymes.

Hexokinase (reaction 1) is allosterically inhibited by glucose 6-phosphate. It deals with low glucose concentrations (low K_m) of the systemic circulation, only to drive glucose utilization for the cellular needs. Glucokinase in the liver deals with high glucose concentrations of the portal circulation following meals. It is not inhibited by its product, but it is induced by the high glucose. It is to be noted here that glucose phosphorylation is not unique to the glycolytic pathway, but it is important for glucose utilization in general.

Phosphofructokinase reaction (reaction 3) is the key reaction of glycolysis. Its catalyzing enzyme, phosphofructokinase-1 (PFK-1), is allosterically activated by low ATP/ADP (low cellular energy supply). It is inhibited by citrate and fatty acids, which mean high energy status or ongoing energy production from other sources.

Phosphofructokinase-1 main allosteric activator is fructose 2,6-bisphosphate produced by phosphofructokinase-2 (PFK-2). The latter is a bi-functional enzyme, having also a 2,6-bisphosphatase activity that converts fructose 2,6-bisphosphate to fructose 6-phosphate. The activity of this bi-functional enzyme is regulated by covalent modification (phosphorylation-dephosphorylation) in response to insulin/glucagon ratio. At low glucose concentration, glucagon in liver cells triggers an increase in intracellular second messenger cAMP. The latter activates protein kinase A that phosphorylates the enzyme PFK-2, inhibiting its kinase activity and activating its fructose 2,6-bisphosphatase activity. This leads to drop in fructose 2,6-bisphosphate, which inhibits glycolysis and gives way to its opposing pathway: gluconeogenesis. At high glucose concentration, insulin causes dephosphorylation of the enzyme, activating its kinase activity. This leads to increase in fructose 2,6-bisphosphate, which activates the key enzyme of glycolysis. Epinephrine increases cAMP like glucagon and acts in liver and muscle cells. However, the kinase activity of PFK-2 in the muscle is active in the phosphorylated form, activating glycolysis, which is consistent with the action of epinephrine in emergencies.

Pyruvate kinase (reaction 10) is also controlled. It is allosterically activated by AMP and fructose 1,6-bisphosphate and inhibited by ATP, acetyl CoA, and fatty acids. It is inhibited by phosphorylation caused by glucagon and activated in the dephosphorylated form under the effect of insulin.

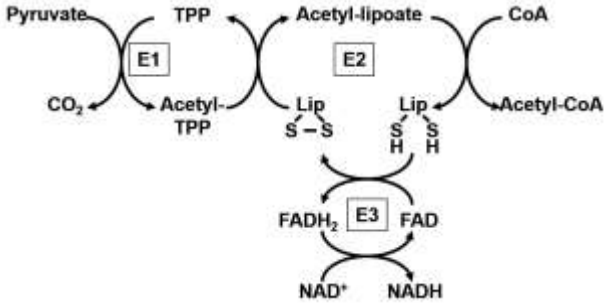
It is to be noted here that glucagon promotes the phosphorylation and insulin promotes the dephosphorylation of enzymes. Enzymes that work to lower glucose concentration are active in the dephosphorylated form, under the effect of insulin.

Pyruvate kinase deficiency

Deficiency of pyruvate kinase (red blood cell isoform) is the second enzyme deficiency, after glucose 6-phosphate dehydrogenase, that causes hemolytic anemia. Other enzymes whose deficiency causes hemolysis include hexokinase and phosphohexose isomerase.

Pyruvate oxidation

Pyruvate undergoes oxidative decarboxylation in the mitochondria, producing acetyl CoA. The reaction is catalyzed by the enzyme complex, pyruvate dehydrogenase (PDH), which needs five coenzymes: thiamin pyrophosphate (TPP), lipoic acid, coenzyme A, FAD, and NAD^+. The enzyme is formed of three subunits: E1, E2, and E3. The E1 subunit binds TPP and catalyzes the decarboxylation of pyruvate. Its gene is on the X chromosome. The E3 subunit, with dihydrolipoyl dehydrogenase activity, is common to pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and the branched-chain α-ketoacid dehydrogenase. NADH, produced in this reaction, can generate 3 ATP by the respiratory chain.

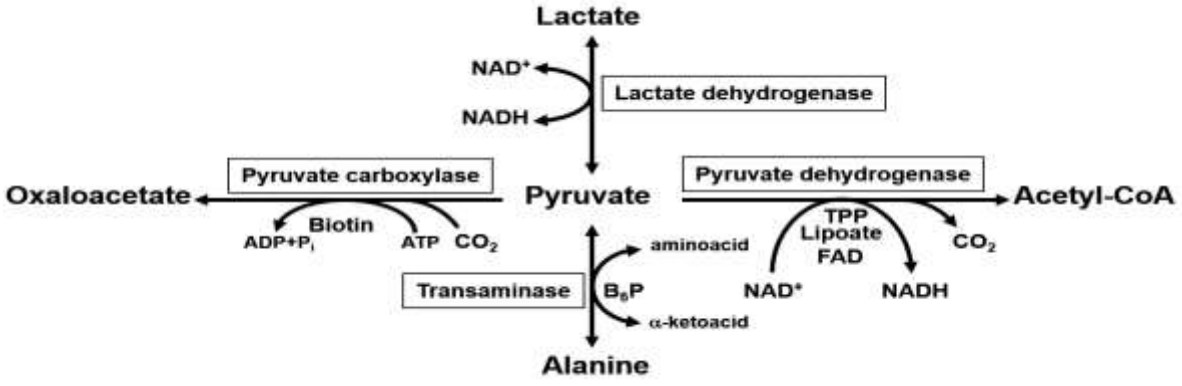


Thiamin deficiency leads to deficiency of this type of reaction, causing the disease beriberi or encephalopathy commonly seen in alcoholics. Arsenite, a lipoic acid poison, inhibits pyruvate dehydrogenase. Leigh syndrome (subacute necrotizing encephalomyelopathy) is a neurometabolic disorder that results mainly from mutations of the respiratory chain but can result also from pyruvate dehydrogenase deficiency. Lactic acidosis is a feature of pyruvate dehydrogenase deficiency. Some cases may respond to high doses of thiamin.

Pyruvate dehydrogenase is allosterically inhibited by its products: acetyl CoA and NADH. It is also inhibited by phosphorylation and activated by dephosphorylation (insulin).

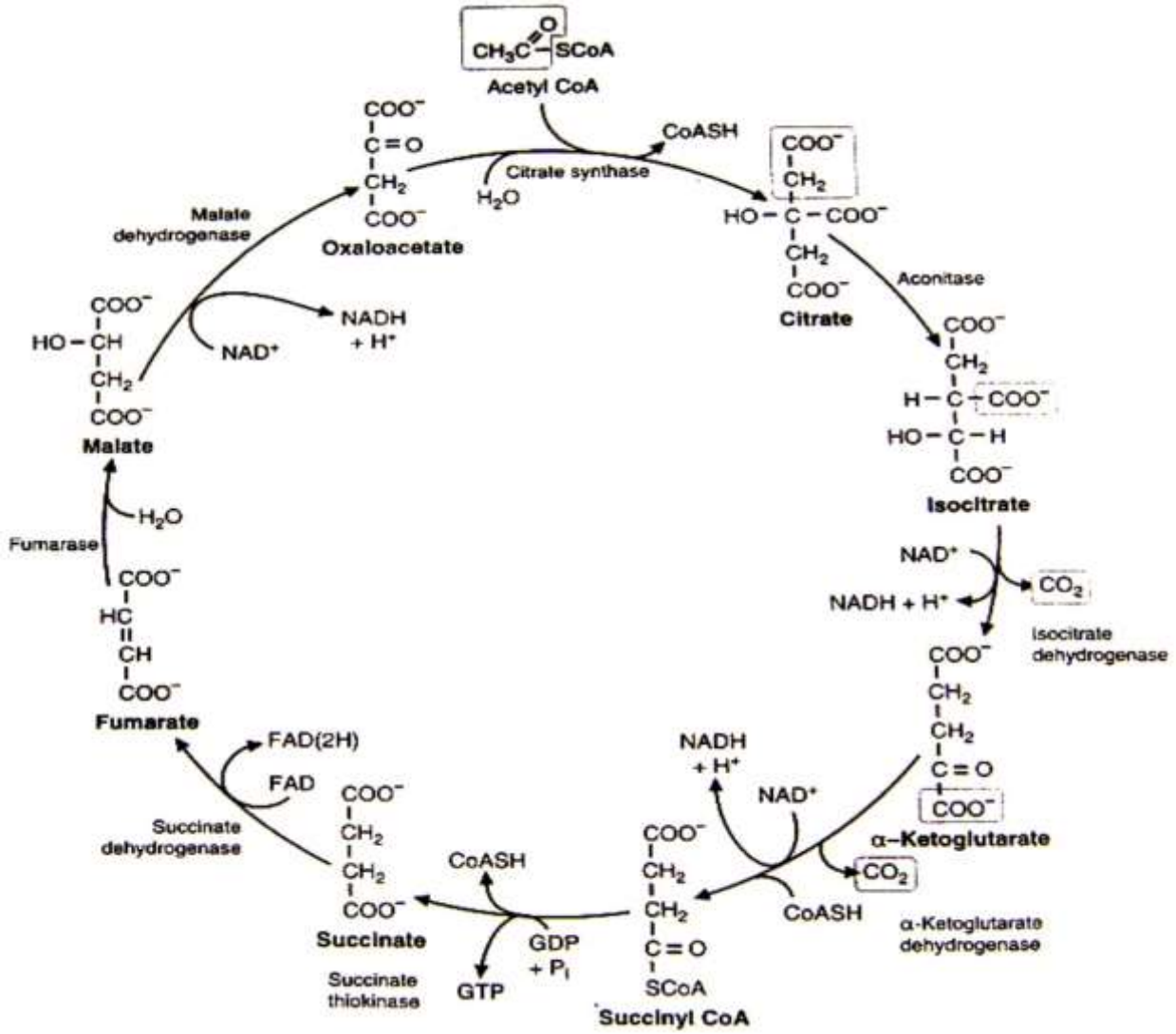
Pyruvate carboxylation

Pyruvate is carboxylated by pyruvate carboxylase that needs biotin coenzyme. This reaction is required for gluconeogenesis and is the main anaplerotic reaction that generates oxaloacetate to maintain the citrate cycle. Biotin deficiency results in fasting hypoglycemia due to defective gluconeogenesis. Ketosis follows the failure of oxidation of acetyl CoA from fatty acids in the citric acid cycle. Accumulation of pyruvate leads to lactic acidosis. Deficient carboxylation reactions also lead to deficient fatty acid synthesis and deficient metabolism of propionate.



Pyruvate carboxylase, as an enzyme of gluconeogenesis, is induced by glucagon and cortisol with low blood glucose concentration. It is allosterically activated by acetyl CoA. Thus, acetyl CoA inhibits its own synthesis from glucose (by inhibiting pyruvate dehydrogenase) and activates its oxidation in citrate cycle (by activating pyruvate carboxylase). By this mechanism of control, acetyl CoA from fatty acid degradation spares glucose and enhances the provision of energy (citrate cycle) and intermediates (oxaloacetate) for gluconeogenesis.

Tricarboxylic acid cycle



Tricarboxylic acid cycle (TCA cycle) is also called citric acid (citrate) cycle and Krebs' cycle. The enzymes are all in the mitochondrial matrix, except succinate dehydrogenase, which is a component of complex II of the respiratory chain in the inner mitochondrial membrane. In this cycle, there is net oxidation of two carbons to CO_2, corresponding to two carbon entry by active acetate (acetyl CoA).

One substrate level phosphorylation reaction, succinate thiokinase, is found in this cycle. It actually forms GTP, which can transfer its high-energy phosphate to ADP, forming ATP. Three NADH are produced, by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase, in addition to one $FADH_2$ by succinate dehydrogenase. Therefore, the net energy gain is 12 ATP per one active acetate.

This cycle is both catabolic and anabolic (amphibolic). It oxidizes active acetate derived from carbohydrate, fat, or protein. Citrate is the vehicle for transport of acetyl CoA to the cytosol for fatty acid synthesis. Oxaloacetate and α-ketoglutarate can be transaminated, giving aspartate and glutamate used for protein synthesis. Oxaloacetate is an intermediate for gluconeogenesis from pyruvate. Many aminoacids can give pyruvate or intermediates of the cycle, which are converted to oxaloacetate for gluconeogenesis. Succinyl CoA is used for heme synthesis and for activation of acetoacetate (ketolysis).

Control of citrate cycle is mainly respiratory. The rate of the cycle is dependent on the rate of reduction of the coenzymes: NADH and $FADH_2$ by the respiratory chain, which is dependent on the supply of oxygen and ADP.

Vitamins play an important role in Krebs' cycle by providing the coenzymes: NAD^+ from niacin, FAD from riboflavin, TPP from thiamin, and coenzyme A from pantothenic acid. Pyridoxal phosphate from vitamin B_6 is important for transamination reactions that may supply oxaloacetate and α-ketoglutarate. Biotin is important for providing oxaloacetate by pyruvate carboxylation. Lipoic acid is needed for α-ketoglutarate dehydrogenase.

Study Questions

Choose one best answer for every question of the following:

1- What are the major end-products of carbohydrate digestion?
(A) Glucose, fructose, galactose & starch.
(B) Glucose, fructose, galactose & glycogen.
(C) Glucose, galactose, sucrose & cellulose.
(D) Glucose, fructose, galactose & cellulose.

2- Glucose is absorbed from the intestinal lumen by
(A) active transport (secondary active).
(B) passive diffusion.
(C) facilitated diffusion.
(D) insulin-dependent transport.

3- Glycolysis is the anaerobic phase of glucose oxidation: it proceeds ……..
(A) inside the mitochondria.
(B) not needing oxygen.
(C) only in absence of oxygen.
(D) at a faster rate in presence of oxygen.

4- Which of the following is an oxidation-reduction reaction of aerobic glycolysis?
(A) Phosphofructokinase.
(B) Glyceraldehyde 3-phosphate dehydrogenase.
(C) Pyruvate kinase.
(D) Lactate dehydrogenase.

5- Lactate dehydrogenase in the glycolytic pathway is important for
(A) production of lactate.
(B) oxidation of NADH.
(C) getting rid of pyruvate.
(D) control of the glycolytic pathway.

6- How many carbon dioxide molecules are produced per one turn of citrate cycle?
(A) 1
(B) 2
(C) 3
(D) 4

7- What is the minimal number of oxaloacetate molecules required to oxidize 4 acetyl CoA?
(A) 1
(B) 2
(C) 4
(D) 8

8- Citrate cycle is inhibited by which of the following?
(A) High ADP.
(B) Low NADH.
(C) High FAD.
(D) Low oxygen.

Gluconeogenesis

This is a pathway by which liver (and kidney) cells can synthesize glucose from non-carbohydrate sources. These sources are mainly:

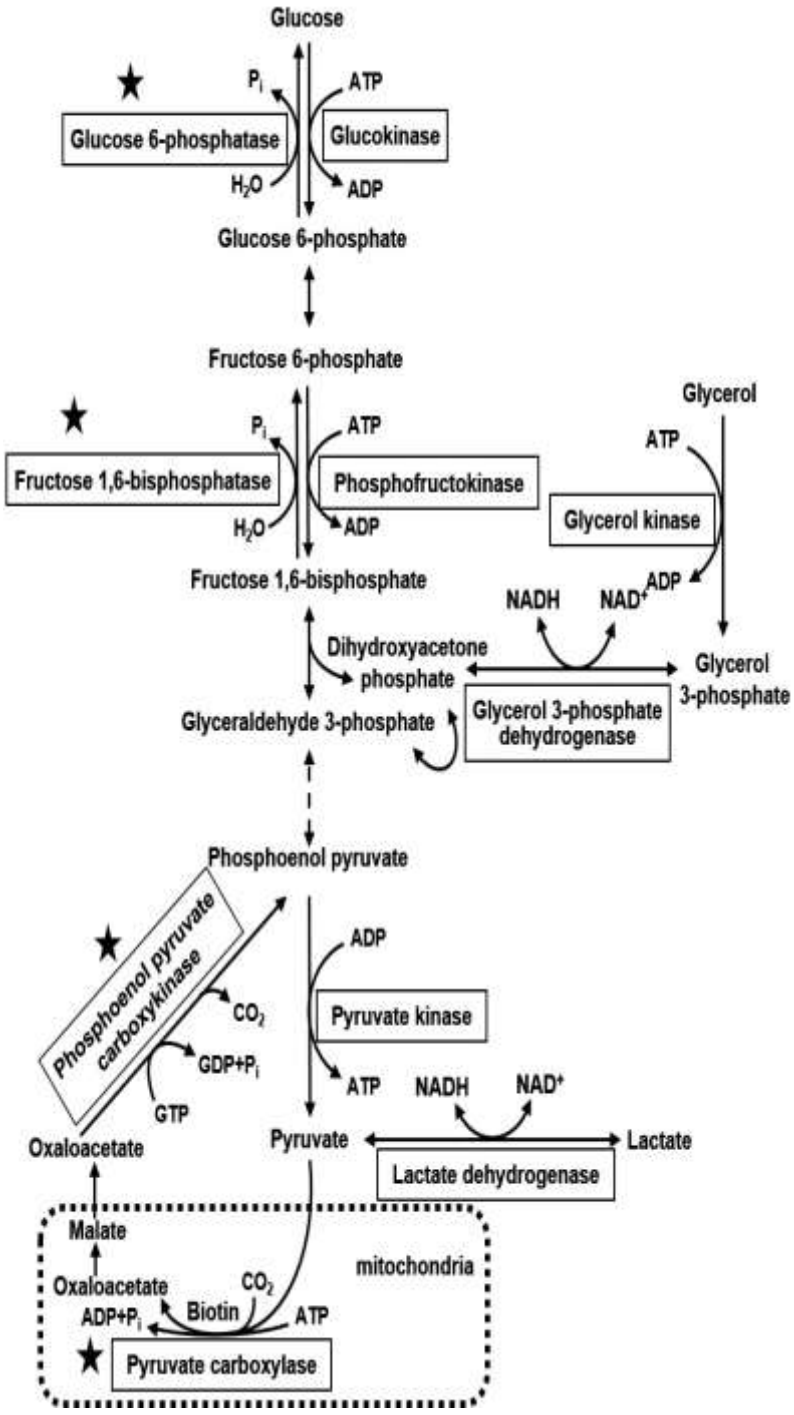
- Aminoacids resulting from protein degradation. Alanine goes from muscles to the liver to be used for glucose synthesis, and glucose goes to the muscles to be oxidized to pyruvate that is transaminated to alanine, thus forming a glucose-alanine cycle.
- Lactic acid produced by anaerobic glucose oxidation in muscle and red blood cells. This goes to the liver for glucose synthesis. Glucose passes to the muscles and red blood cells to be oxidized to lactate, thus forming a glucose-lactate cycle (Cori cycle).
- Glycerol resulting from lipolysis in adipose tissue.
- Propionyl CoA resulting from oxidation of odd-number and branched fatty acids and from some aminoacids. Most fatty acids and acetyl CoA are not glucogenic substrates.

Gluconeogenesis pathway is the reverse of glycolysis. The three one- way reactions of glycolysis are reversed by four reactions of gluconeogenesis (starred in the figure).

Glucose synthesis from lactate and glycerol is shown. Glucogenic aminoacids give pyruvate or intermediates of citrate cycle (including succinyl CoA from propionyl CoA), which form oxalo-acetate.

Energy is consumed in pyruvate carboxylase, phospho-enol pyruvate carboxykinase, and phosphoglycerate kinase (not shown) reactions. This means 6 ATP per glucose molecule formed from pyruvate. Also, 2 NADH are needed for the glyceraldehyde 3-phosphate dehydrogenase reaction (not shown).

Gluconeogenesis needs biotin for pyruvate carboxylation, niacin as a source of NAD^+ and NADH for lactate dehydrogenase, glyceraldehyde 3-phosphate de-hydrogenase, malate dehydro-genase, and glycerol 3-phosphate dehydrogenase and pyridoxine as the source of pyridoxal phosphate for transamination reactions.



The enzymes of gluconeogenesis are induced by glucagon and cortisol. Fructose 1,6-bisphosphatase is allosterically inhibited by fructose 2,6-bisphosphate in a reciprocal manner to phosphofructokinase-1. Pyruvate carboxylase is allosterically activated by acetyl CoA produced by fatty acid oxidation.

Alcohol consumption leads to increased NADH/NAD^+ resulting from ethanol metabolism by alcohol dehydrogenase. This leads to conversion of pyruvate to lactate, oxaloacetate to malate, and dihydroxyacetone phosphate to glycerol 3-phsphate. This interferes with gluconeogenesis and can lead to hypoglycemia.

Study Questions

Choose one best answer for every question of the following:

9- Which of the following may not be considered glucogenic?
(A) Glycerol.
(B) Lactic acid.
(C) Proteins.
(D) Palmitic acid.

10- In what condition does gluconeogenesis proceed?
(A) Fasting state to provide glucose for the brain and red blood cells.
(B) Muscular exercise to deal with lactate produced by muscle cells.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

11- What would result from a genetic absence of fructose 1,6-bisphosphatase?
(A) Accumulation of fructose phosphates in the liver.
(B) Failure to metabolize glucose 6-phosphate via the glycolytic pathway.
(C) Inability to produce glucose from aminoacids.
(D) Decreased production of acetyl CoA.

12- How many one-way reactions are needed for production of glucose from glycerol?
(A) 1
(B) 2
(C) 3
(D) 4

13- NADH is consumed by which reaction of gluconeogenesis?
(A) Lactate dehydrogenase.
(B) Glycerol 3-phosphate dehydrogenase.
(C) Malate dehydrogenase.
(D) Glyceraldehyde 3-phosphate dehydrogenase.

14- Energy for gluconeogenesis is obtained from
(A) creatine phosphate.
(B) oxidation of fatty acids.
(C) pyruvate dehydrogenase reaction.
(D) cAMP.

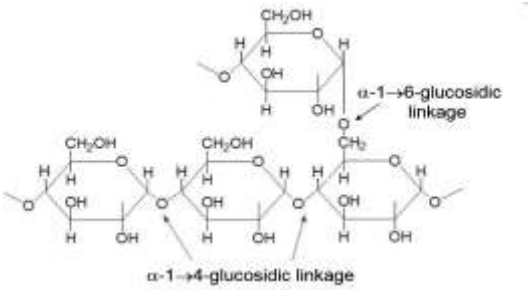
15- What is the energy cost for synthesis of one glucose molecule from pyruvate?
(A) 3 ATP + 1 NADH
(B) 4 ATP + 1 NADH
(C) 6 ATP + 2 NADH
(D) 9 ATP + 2 NADH

16- Lactate dehydrogenase reaction is a reaction of
(A) aerobic glycolysis.
(B) gluconeogenesis.
(C) pyruvate oxidation.
(D) lactate reduction.

Glycogen metabolism

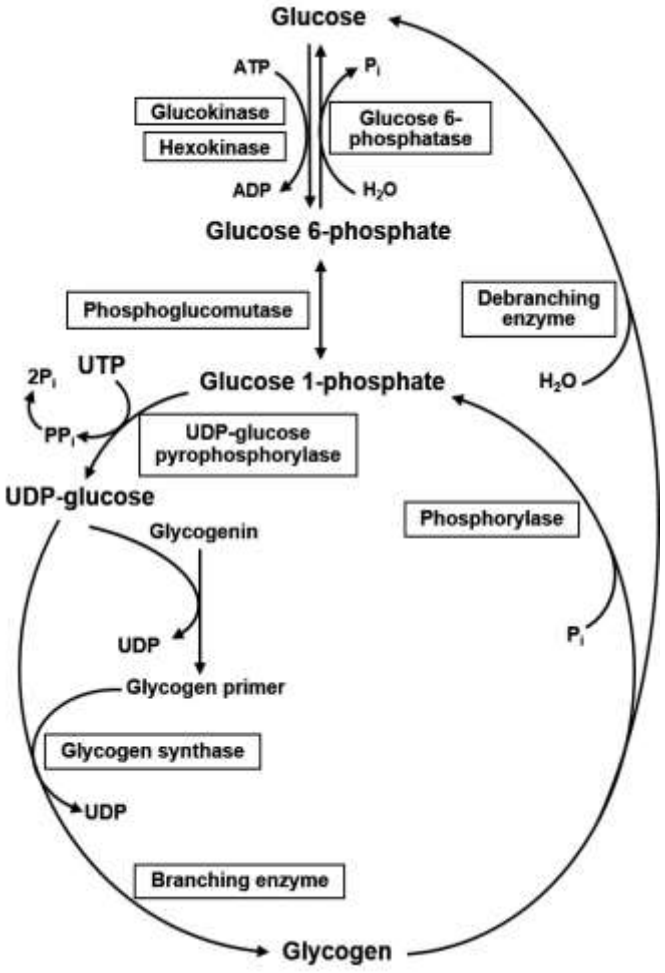
Glycogen is a carbohydrate store present mainly in the cytosol of liver and muscle cells. It constitutes up to 10% of the weight of the liver and 2% of the muscle weight. Since the total mass of muscle is much greater than that of the liver, total glycogen stored in the muscle is about twice that of the liver. Stores of glycogen in the liver are the main buffer of blood glucose levels, while muscle glycogen is a fuel store for the muscle cell itself.

Glycogen is a highly branched glucose polymer. Glucose units are connected in a chain by α-1→4-glucosidic linkage. At branching points, there is an α-1→6-glucosidic linkage. This branching is important for solubility and increasing the number of terminal glucose residues.



Glycogenesis

Glycogenesis is the synthesis of glycogen from glucose. The key enzyme is glycogen synthase which elongates a chain by adding glucose units from UDP-glucose (uridine diphosphate-glucose) by α-1→4-glucosidic linkage. The energy cost for each glucose added is two ATP (1 ATP and 1 UTP). A branching enzyme transfers a part of the straight chain (6-7 glucose residues) and links it at the branching point by an α-1→6-glucosidic linkage. Glycogen primer is formed by the protein glycogenin, which self catalyzes the addition of six glucose units to its tyrosine.



Glycogenolysis

Glycogenolysis is the breakdown of glycogen. The key enzyme is glycogen phosphorylase, which breaks the terminal α-1→4-glucosidic linkage, producing glucose 1-phosphate. The latter is isomerized to glucose 6-phosphate, which is translocated by glucose 6-phosphate translocase enzyme to the endoplasmic reticulum where it produces glucose by the action of glucose 6-phosphatase.

Muscle cells lack glucose 6-phosphatase; therefore, muscle glycogen is not converted to glucose but utilized by the muscle cell itself as a cell fuel.

Phosphorylase stops four glucose residues from a branching point. Debranching enzyme, with its glucan transferase activity transfers three residues to another chain, and by glucosidase activity hydrolyzes the α-1→6-glucosidic linkage, releasing free glucose. This free glucose in the muscle cell is rapidly phosphorylated by the high-affinity hexokinase.

Control of glycogen metabolism

After meals, glucose is stored as glycogen mainly in the liver and muscle cells. In post-absorptive period, blood glucose is obtained from the breakdown of liver glycogen. Muscle glycogen is used during muscular activity.

The two key enzymes: glycogen synthase and glycogen phosphorylase are reciprocally controlled by phosphorylation-dephosphorylation. Enzyme phosphorylation is under the influence of the rise of cAMP induced by the action of glucagon in liver cells and epinephrine (β-adrenergic) in liver and muscle cells, through G-proteins on adenylate cyclase. Cyclic AMP activates protein kinase A, which phosphorylates glycogen synthase, inactivating it, and phosphorylase kinase. The latter is activated, thus phosphorylates and activates glycogen phosphorylase. This cascade of activation amplifies the hormonal signal and insures efficient glycogenolysis, with inhibition of glycogenesis. This insures a rapid supply of glucose to the blood to guard against fasting hypoglycemia, and a rapid supply of muscle glucose 6-phosphate for glycolysis in emergencies. Phosphorylation effects are reversed by the drop of cAMP by the action of phosphodiesterase, and the dephosphorylation of the enzymes by protein phosphatases activated by insulin.

Allosteric control plays a role in controlling glycogen metabolism, even before hormonal signals reach the cell. Calcium ions that increase in the sarcoplasm upon muscle cell activation, or by the effect of α-adrenergic stimulation in liver cells activate phosphorylase kinase. Muscle phosphorylase is activated by AMP, which increases in cases of cell need for energy. Glucose and ATP inhibit liver phosphorylase, while glucose 6-phosphate activates glycogen synthase.

Glycogen storage diseases

Type Ia (von Gierke disease), deficiency of glucose 6-phosphatase: There is increased glycogen with normal structure, hepato-renomegaly, hypoglycemia, lactic acidosis, hyper-uricemia, and hyperlipemia. Type Ib (glucose 6-phosphate translocase deficiency) also shows neutropenia and recurrent infections. Treatment is regular glucose or starch feeding.

Type II (Pompe disease), deficiency of lysosomal α-1$\rightarrow$4-glucosidase: There is massive accumulation of glycogen in all tissues. The disease is usually fatal before the age of two.

Type III (Cori disease), deficiency of debranching enzyme: There is increased glycogen with short outer branches in the liver and muscles. This is usually milder than type I.

Type IV (Andersen disease), deficiency of branching enzyme: There is accumulation of abnormal glycogen that has limited solubility, with liver cirrhosis and death usually before the age of two.

Type V (McArdle disease), deficiency of muscle phosphorylase: There is accumulation of glycogen in the muscle with low tolerance to anaerobic exercise.

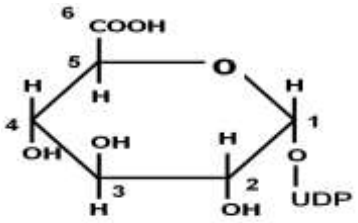
Type VI (Hers disease), deficiency of liver phosphorylase: There is accumulation of liver glycogen, but milder than type I. There is no accumulation of glucose 6-phosphate that stimulates glycogenesis.

Type VII, deficiency of muscle phosphofructokinase: There is inhibition of glycolysis and accumulation of glycogen in the muscle, with decreased tolerance to anaerobic exercise.

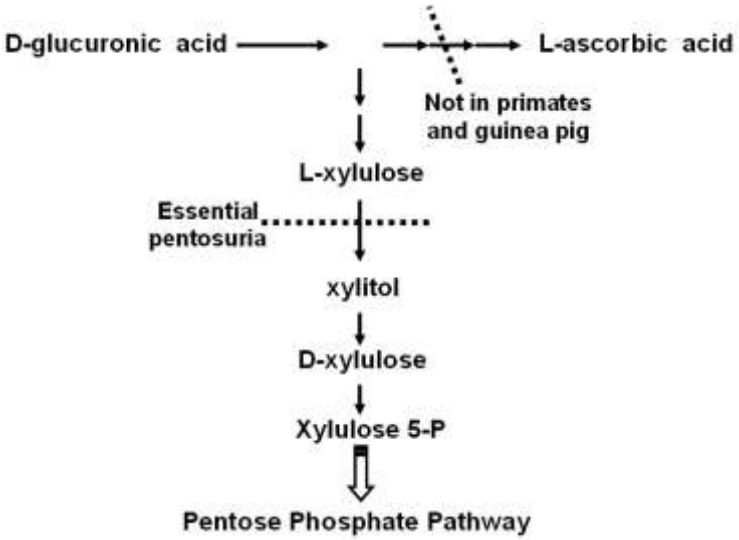
Type VIII, deficiency of liver phosphorylase kinase: There is accumulation of glycogen with mild liver enlargement and hypoglycemia.

Uronic Acid Pathway

UDP-glucose is oxidized at carbon 6 of glucose giving UDP-glucuronic acid. This is the active form of glucuronic acid used for synthesis of glycosaminoglycans, e.g., heparin, and for conjugation reactions, e.g., bilirubin conjugation.



UDP-glucuronic acid



Glucuronic acid is the precursor of ascorbic acid in animals other than primates and guinea pigs. In humans, glucuronic acid is metabolized to pentoses. A genetic block in the conversion of L-xylulose to D-xylulose results in essential pentosuria.

Study Questions

Choose one best answer for every question of the following:

17- Formation of glucose from aminoacids is called
(A) glycogenesis.
(B) glycogenolysis.
(C) gluconeogenesis.
(D) glycolysis.

18- Which of the following liver enzymes is active in a fasting person?
(A) Phosphofructokinase.
(B) Glucokinase.
(C) Glycogen phosphorylase.
(D) Glycogen synthase.

19- Glucagon activates which of the following enzymes?
(A) Phosphofructokinase.
(B) Muscle glycogen phosphorylase.
(C) Liver glycogen synthase.
(D) Phosphoenolpyruvate carboxykinase.

20- The first reaction in glycogenesis is catalyzed by
(A) glucokinase or hexokinase.
(B) phosphoglucomutase.
(C) UDP-glucose pyrophosphorylase.
(D) glycogen synthase.

21- UDP-glucose is formed by reaction of
(A) UDP and glucose.
(B) UDP, glucose and ATP.
(C) UTP and glucose 1-phsphate.
(D) UTP and glucose 6-phsphate.

22- Free glucose is released during glycogenolysis from
(A) glucose residues in 1→4 linkage.
(B) glucose residues in 1→6 linkage.
(C) the terminal glucose residues.
(D) glucose residues linked by their carbons 1,4 and 6.

23- Glycogenolysis in muscle does not contribute to blood glucose due to absence of
(A) hexokinase.
(B) glucose 6-phosphatase.
(C) phosphoglucomutase.
(D) phosphohexose isomerase.

24- Oxidation of glycogen anaerobically yields how many ATP per glucose 6-phosphate?
(A) 1
(B) 2
(C) 3
(D) 4

25- Which of the following is active in the dephosphorylated form?
(A) Glycogen synthase.
(B) Glycogen phosphorylase.
(C) Phosphorylase kinase.
(D) Fructose 2,6-bisphosphatase.

26- Which of the following is active in its phosphorylated form?
(A) Glycogen phosphorylase.
(B) Glycogen synthase.
(C) Pyruvate dehydrogenase.
(D) Phosphofructokinase-2.

27- Which of the following is a common intermediate of glycolysis, gluconeogenesis, glycogenesis and glycogenolysis?
(A) Glucose 1-phosphate.
(B) Glucose 6-phosphate.
(C) Fructose 6-phosphate.
(D) Fructose 1,6-bisphosphate.

28- Muscle glycogenolysis is activated during contraction by
(A) AMP and calcium ions.
(B) glucose 6-phosphate.
(C) phosphodiesterase.
(D) insulin.

29- Glycogen synthase is activated with high level of
(A) cAMP.
(B) calcium ions.
(C) glucose 6-phosphate.
(D) AMP.

30- Glycogen is present in the cell as
(A) a component of endoplasmic reticulum.
(B) a constituent of the mitochondrial matrix.
(C) free cytosolic solution.
(D) cytosolic granules that also contain enzymes of glycogenesis and glycogenolysis.

31- Glycogen storage diseases are due to a genetic defect in glycogen
(A) synthesis.
(B) degradation.
(C) either (A) or (B).
(D) neither (A) nor (B).

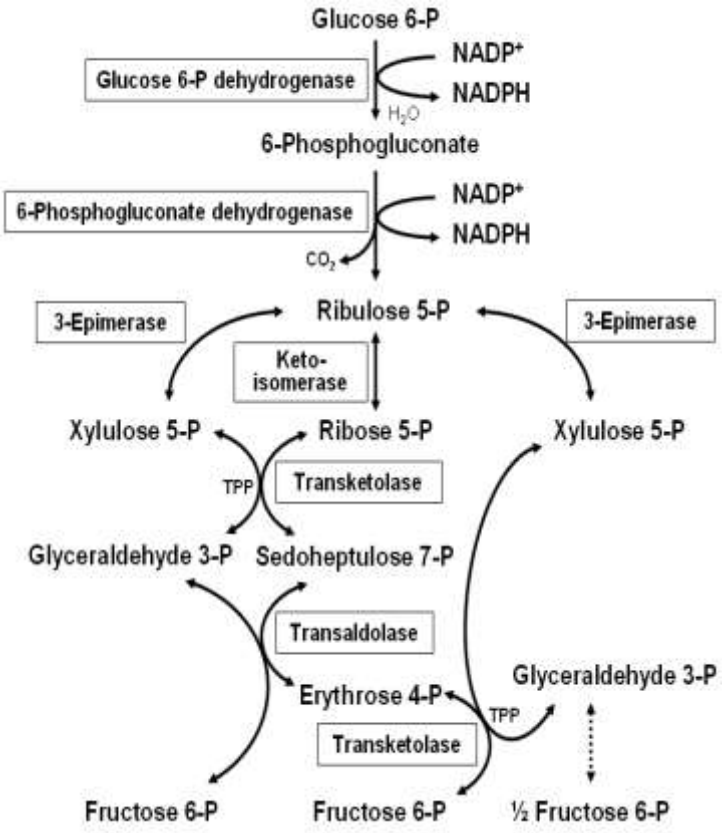
32- Uronic acid pathway is important for
(A) formation of glucose from glucuronic acid.
(B) synthesis of ascorbic acid in growing children.
(C) energy production through an alternative oxidative pathway.
(D) synthesis of glycosaminoglycans and excretion of bilirubin.

33- Ascorbic acid is synthesized from glucuronic acid in
(A) animals other than primates and guinea pigs.
(B) man and other primates.
(C) children only.
(D) cases of nutritional deficiency of vitamin C.

HMP pathway (Pentose phosphate pathway)

Hexose mono-phosphate (HMP) pathway, which is also called pentose phosphate pathway (PPP), is an oxidative pathway for glucose. Unlike glycolysis, it does not aim at energy production. It aims at the production of pentoses and NADPH. Pentoses are needed for synthesis of nucleotides, nucleic acids, coenzymes, and glycoproteins. Unlike energy producing pathways, which reduce the coenzyme NAD^+, HMP pathway uses $NADP^+$ (nicotinamide adenine dinucleotide phosphate) and reduces it to NADPH. The latter is needed for lipogenesis (fat synthesis) and cholesterol and steroid hormone synthesis (steroidogenesis), and as an antioxidant by red blood cells. NADPH is also needed for the aldose reductase reaction, cytochrome P450 monooxygenase, and NADPH oxidase of phagocytic cells. HMP pathway is active in the liver, adipose tissue, mammary glands, adrenal glands, and erythrocytes.

In this pathway, glucose is oxidized at carbon 1. Two dehydrogenases produce NADPH and convert glucose 6-phosphate to ribulose 5-phosphate (oxidative part of the pathway). In the non-oxidative part of the pathway different pentose phosphates are produced by isomerization. Three pentose phosphates are converted to two and half hexose phosphates, by the action of transketolase and transaldolase enzymes. Transketolase needs thiamin pyrophosphate (TPP) coenzyme. This non-oxidative part of the pathway is reversible, meaning that pentose phosphates can be synthesized from either side of the pathway.



Control of HMP pathway

Control of HMP pathway reactions is mainly by the availability (concentration) of different reactants. Therefore, the oxidative part, the non-oxidative part, or both may be active according to tissue needs. If only NADPH is needed, e.g., during lipogenesis with no nucleic acid synthesis, both the oxidative and non-oxidative parts of the pathway are active, together with the glycolytic pathway. During cell division, with no consumption of NADPH, pentoses are provided by an active non-oxidative part of the pathway going in reverse direction.

HMP pathway is considered an anabolic pathway and is activated by insulin. The enzyme glucose 6-phosphate dehydrogenase is induced by feeding.

Glucose 6-phsphate dehydrogenase (G6PD) deficiency

Glucose 6-phsphate dehydrogenase (G6PD) deficiency is an X-linked genetic disease with varying grades of severity. Since NADPH is required for maintaining adequate intracellular reduced glutathione, the antioxidant capacity of the cell is compromised in this disease.

$$G\text{-}S\text{-}S\text{-}G + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2G\text{-}SH + NADP^+$$

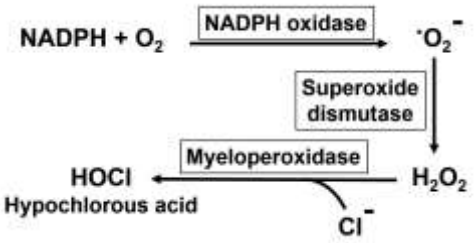
This is the most common enzyme deficiency that leads to hemolytic anemia.

Other functions of NADPH

- It provides the reducing equivalents in anabolic reductive pathways, e.g., lipogenesis (fat synthesis) and cholesterol and steroid hormone synthesis (steroidogenesis).
- It provides the reducing equivalents for the aldose reductase reaction. The consumption of NADPH in this reaction in case of hyperglycemia, with decreased activity of the HMP pathway, may cause the decreased antioxidant power in diabetic patients.
- Cytochrome P450 hydroxylases (mono-oxygenases) hydroxylate many endogenous substances as well as exogenous substances (xenobiotics). This makes these substances more water-soluble. Conjugation with glucuronate or sulfate further increases their solubility and facilitates their excretion.

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + NADP^+ + H_2O$$

- NADPH oxidase reaction is important in phagocytic cell pathway for the production of oxidizing species that help killing microorganisms. This pathway is characterized by the increased cellular oxygen consumption (respiratory burst).



Study Questions

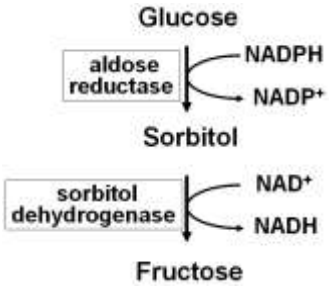
Choose one best answer for every question of the following:

34- Glucose 6-phosphate dehydrogenase requires which of the following?
(A) NAD^+.
(B) NADH.
(C) FAD.
(D) $NADP^+$.

35- Which of the following reactions produces carbon dioxide?
(A) Glucose 6-phsphate dehydrogenase.
(B) 6-Phosphogluconate dehydrogenase.
(C) Glucose 6-phosphatase.
(D) Phosphoglucomutase.

36- Cells with severe deficiency of glucose 6-phosphate dehydrogenase can get pentoses for nucleic acid synthesis from
(A) non-oxidative part of the HMP pathway.
(B) uronic acid pathway.
(C) both (A) and (B).
(D) neither (A nor (B).

Sorbitol metabolism (polyol pathway)



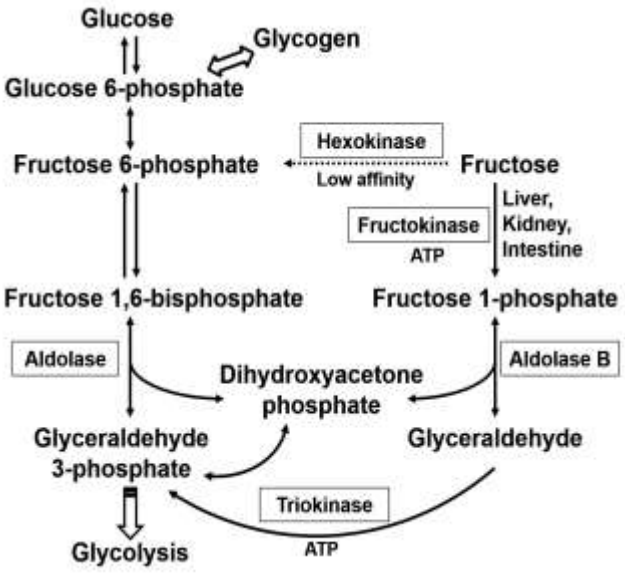
Glucose (an aldohexose) is converted to fructose (keto-hexose) through the intermediate sugar alcohol, sorbitol (a polyol). This pathway takes place in the seminal vesicles to provide fructose in the semen.

The enzyme aldose reductase is also present in the kidney, eye, and nerve cells. In these tissues, glucose is reduced to sorbitol when blood glucose is high in diabetic patients. Sorbitol dehydrogenase is lacking in these tissues. Intracellular accumulation of sorbitol produces an osmotic effect, which may contribute to the development of diabetic complications involving these tissues. Fluctuation of blood glucose in a diabetic patient can lead to imprecision of eyeglass measurements. The consumption of NADPH is believed to contribute to the lowered antioxidant capacity of diabetic patients. Aldose reductase inhibitors, including those of natural sources, are being considered for prevention of diabetic complications.

Sorbitol, when used as a sweetener, is not absorbed from the intestine. In excess, it may cause osmotic diarrhea. By bacterial action, it can cause bloating, colic and discomfort. Its intake is restricted in the low FODMAPs diet for irritable bowel syndrome (Lecture 25).

Fructose metabolism

Fructose is obtained from the diet at variable amounts. It results from the digestion of dietary sucrose. Fructose itself is present in fruits, bee honey, and the high fructose corn syrup. The latter is prepared by hydrolysis of starch, and subjecting the resulting glucose solution to an isomerization process. It is used as a sweetener of food and drinks.



Fructose is metabolized mainly by fructokinase and aldolase B in the liver, kidney and intestine. Fructose is converted to glucose, glycogen, lactate and triglycerides.

The uptake of fructose is not insulin dependent. Fructokinase is not insulin-dependent. Fructose metabolism escapes the rate-limiting step of glycolysis, the phosphofructokinase reaction. Therefore, the clearance of fructose is faster than that of glucose, especially in diabetic patients. This also makes fructose highly lipogenic, and a high fructose intake may lead to fatty liver and hypertriglyceridemia.

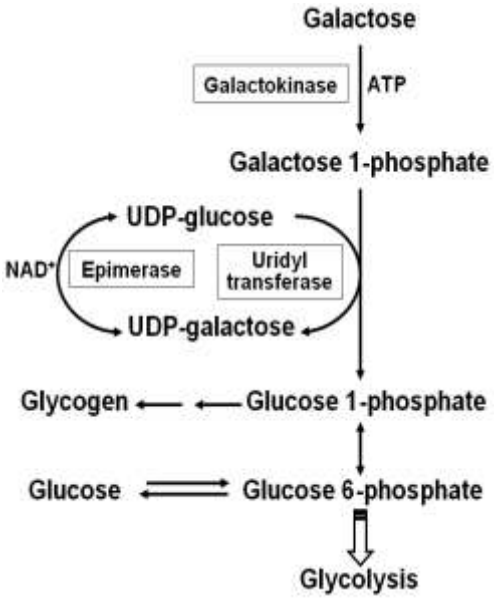
Deficiency of fructokinase leads to essential fructosuria, a benign condition. Deficiency of aldolase B leads to hereditary fructose intolerance. This condition is characterized by fructose-induced hypoglycemia. This hypoglycemia is attributed to inhibition of glycogen phosphorylase by the accumulated fructose 1-phosphate.

Fructose is absorbed from the intestine mainly by GLUT5. It is not well absorbed like glucose. Therefore, excess fructose can cause osmotic diarrhea and bowel irritation. Its intake is restricted in the low FODMAPs diet recommended for irritable bowel syndrome (Lecture 25).

Galactose metabolism

Dietary source of galactose is the milk lactose. Lactose is digested by intestinal lactase to glucose and galactose, which are actively absorbed by the same mechanism. This means that galactose comprises half the carbohydrate intake of a baby.

Galactose is metabolized by galactokinase, producing galactose 1-phosphate, which is eventually converted to glucose 1-phosphate by the action of uridyl transferase and 4-epimerase. The pathway continues as glycolysis for energy production, or glycogenesis for storage of glycogen.



Deficiency of uridyl transferase causes a serious condition, galactosemia. The accumulation of galactose and galactitol (from galactose reduction) in the eye lens leads to cataract. With the depletion of inorganic phosphate needed for ATP regeneration, there is liver failure and mental retardation. Galactokinase deficiency leads to non-classical galactosemia, with less severe symptoms, though the eye effect is still there.

UDP-galactose is the active form of galactose needed for the synthesis of lactose, glycoproteins, and glycolipids. With the restriction of lactose in diet, e.g., in cases of milk intolerance, or in galactosemia, glucose is the source of UDP-galactose by 4-epimerization of UDP-glucose.

Study Questions

Choose one best answer for every question of the following:

37- Fructose is metabolized by
(A) only spermatozoa.
(B) mainly the liver, kidney, and intestine.
(C) hexokinase-containing cells.
(D) cells dependent on insulin for fructose uptake.

38- Fructose was recommended for diabetic patients because
(A) fructose is less sweet than glucose.
(B) fructose is harder to metabolize than glucose.
(C) fructose cellular uptake and metabolism are not insulin-dependent.
(D) fructose does not raise blood glucose.

39- Fructose can be converted to glucose in man via which of the following?
(A) Isomerization by hexose isomerase.
(B) Action of aldose reductase.
(C) Formation of triose phosphates.
(D) Formation of sorbitol.

Study Questions

Choose one best answer for every question of the following:

40- Hereditary fructose intolerance is managed by
(A) avoidance of fructose and sucrose.
(B) intravenous glucose in hypoglycemic attacks.
(C) both (A) and (B).
(D) neither (A) nor (B).

41- Lactose is synthesized by reaction of which of the following?
(A) Glucose and galactose.
(B) UDP-glucose and galactose.
(C) UDP-galactose and glucose.
(D) UDP-galactose and UDP-glucose.

42- Dulcitol (galactitol) is formed by
(A) galactokinase.
(B) aldose reductase.
(C) 4-epimerase.
(D) galactosidase.

43- Patients with galactosemia are managed by
(A) lactose-free feeding formula.
(B) replacing lactose with galactose.
(C) enzyme therapy.
(D) gene therapy.

44- An inhibitor of glucose 6-phosphate dehydrogenase in the liver might result in
(A) accumulation of glucose 6-phosphate.
(B) impaired fatty acid synthesis.
(C) inability to catabolize glucose.
(D) inability to synthesize ribose 5-phosphate.

45- HMP pathway is active in which of the following conditions?
(A) Glucose 6-phosphate dehydrogenase deficiency.
(B) Glucose 6-phosphatase deficiency.
(C) Prolonged fasting.
(D) Low-carbohydrate diet.

46- Which of the following is a metabolite of glycolysis, gluconeogenesis and HMP pathway?
(A) Glucose 1-phosphate.
(B) Glyceraldehyde 3-phosphate.
(C) Ribulose 5-phosphate.
(D) 1,3-Bisphosphoglycerate.

47- Which of the following may <u>not</u> be converted to blood glucose?
(A) Fructose.
(B) Galactose.
(C) Glucogenic aminoacids.
(D) Muscle glycogen.

48- Glucose may be produced from
(A) glycogen in the muscle.
(B) alanine in adipose tissue.
(C) maltose in the intestine.
(D) lactose in mammary gland.

49- Uronic acid pathway is important for
(A) excretion of bilirubin.
(B) formation of UDP-galactose in cases of galactosemia.
(C) production of hypochlorous acid in phagocytes.
(D) alternative oxidation of galactose.

50- Which part of the HMP pathway may be active in actively dividing cells?
(A) Oxidative.
(B) Non-oxidative.
(C) Oxidative and non-oxidative.
(D) Oxidative and non-oxidative in the reverse direction.

51- Type Ia glycogen storage disease (von Gierke disease) is characterized by
(A) abnormal deficiency of glucose 6-phosphatase in the muscle.
(B) hepatomegaly, with accumulation of abnormal glycogen.
(C) hyperglycemia.
(D) hyperlipidemia and ketosis.

52- Which of the following enzymes is used in both gluconeogenesis and glycogenolysis?
(A) Glucose 6-phsphatase.
(B) Phosphoglucomutase.
(C) Phosphohexose isomerase.
(D) Hexokinase.

53- ATP is needed in the first reaction for synthesis of glucose from
(A) alanine.
(B) lactate.
(C) glycerol.
(D) glutamate.

54- Phosphatase enzymes require
(A) ATP.
(B) water.
(C) NAD^+.
(D) NADPH.

55- Glucose 6-phosphate allosterically inhibits
(A) hexokinase.
(B) glucokinase.
(C) glycogen synthase.
(D) 6-phosphogluconate dehydrogenase.

56- Apical GLUT2 in intestinal mucosal cells may help glucose absorption from the intestinal lumen
(A) following high-carbohydrate meals.
(B) with low-carbohydrate feeding.
(C) in diabetic persons.
(D) with insulin-mediated up-regulation.

57- GLUT1 is up-regulated by
(A) insulin.
(B) low blood glucose.
(C) food intake.
(D) high-carbohydrate diet.

58- Cyclic AMP activates the production of
(A) glucose from glycogen in the muscle.
(B) glucose from glycogen in the liver.
(C) glycogen from glucose in the liver.
(D) glycogen from glucose in the muscle.

59- Tricarboxylic acid cycle follows glycolysis in which cells?
(A) Red blood cells.
(B) Nerve cells.
(C) Cells undergoing anaerobic metabolism.
(D) Liver cells in fasting conditions.

60- SGLT transport glucose and sodium into which cells?
(A) Red blood cells and white blood cells.
(B) Red blood cells and nerve cells.
(C) Intestinal mucosal cells and renal tubular cells.
(D) Liver cells and beta-cells of the pancreas.

Fatty Acid Metabolism

Digestion and absorption of dietary lipids

The most common form of dietary lipids is triacylglycerols. Dietary lipids are digested in the small intestine. Bile salts are required to emulsify the lipids, dividing them into small droplets that can be acted upon by digestive enzymes. Pancreatic lipase is the main enzyme that digests triacylglycerols in the intestine. Orlistat, the anti-obesity drug, is a lipase inhibitor that produces steatorrhea as a side effect.

Bile salts are also required for the absorption of digested lipids. In the intestinal mucosal cells, long chain fatty acids are re-esterified forming triacylglycerols. These triacylglycerols together with cholesterol and cholesterol esters are packed with phospholipids and apolipoprotein B-48 in lipoprotein particles, the chylomicrons, which pass to the lymph then to the general circulation.

Due to the presence of chylomicrons with their high fat content in the circulation after meals, the plasma becomes turbid. This turbidity is rapidly cleared by the enzyme lipoprotein lipase present on the endothelium of blood capillaries. The enzyme lipoprotein lipase hydrolyzes the triacylglycerols, releasing free fatty acids for the uptake by the cells. Glycerol passes to the liver to be phosphorylated by glycerol kinase, forming glycerol 3-phosphate, which is oxidized giving the glycolytic intermediate: dihydroxyacetone phosphate.

Salivary (lingual lipase) acts in acidic pH and mainly on the short- and medium-chain triacyclglycerols in the stomach. It is important in infants, who take the short-chain milk fat and have their pancreatic lipase not yet well-developed. It is also important in cystic fibrosis patients who lack the pancreatic exocrine secretion and have an acidic duodenal medium. Short- and medium-chain fatty acids can be absorbed directly to the blood.

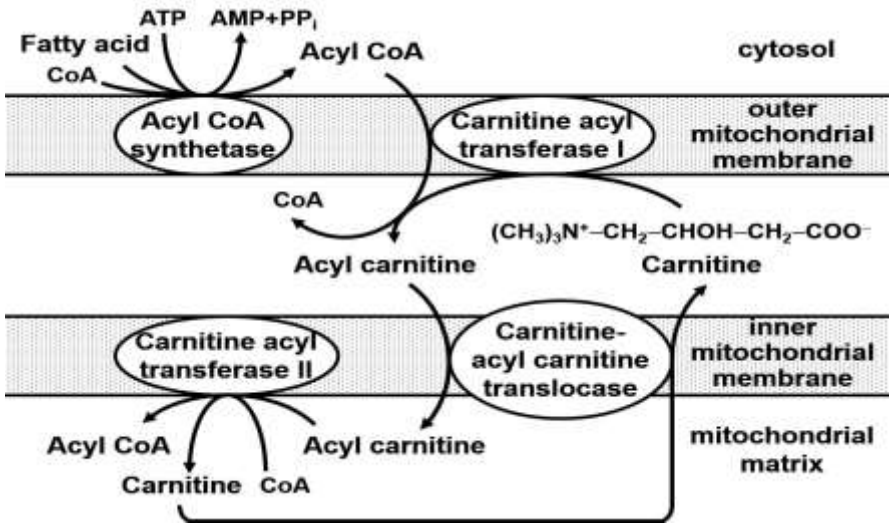
Fat mobilization

In the post-absorptive state, fatty acids enter the circulation from the adipose tissue. Lipases hydrolyze the stored triacylglycerols to free fatty acids and glycerol. The hormone-sensitive lipase of fat cells is activated by cAMP-dependent phosphorylation. Glucagon, epinephrine, and cortisol activate this enzyme, while insulin inhibits it. Fatty acids are carried on blood albumin to different tissues. Glycerol passes to the liver to be converted to dihydroxy-acetone phosphate for gluconeogenesis.

Fatty acid oxidation

The uptake of fatty acids by the cells is not insulin-dependent. Therefore, fatty acids are a suitable fasting cell fuel. Fatty acids are oxidized as cell fuel mainly by muscle, heart, liver, and fat cells. Beta-oxidation (β-oxidation) pathway in the mitochondria degrades fatty acids to acetyl CoA, which is oxidized by the tricarboxylic acid (citric acid) cycle. The oxidation of fatty acids as a fuel is strictly aerobic and produces high amounts of energy. Neither erythrocytes nor brain can use fatty acids. Erythrocytes lack mitochondria, and fatty acids do not cross the blood-brain barrier efficiently. Liver cells oxidize more fatty acids than they need for energy. Excess acetyl CoA cannot be oxidized in the mitochondria, thus they form ketone bodies (ketogenesis) to be exported as a water-soluble fuel, which can be used by extrahepatic cells during fasting. Acetyl CoA also activates pyruvate carboxylase, thus activating gluco-neogenesis. Glucose synthesis proceeds, getting the required energy from fatty acid oxidation.

Role of carnitine



Long-chain fatty acids are activated in the cytosol to fatty acyl CoA and translocated into the mitochondria with the help of carnitine. Carnitine is synthesized from lysine and methionine, or taken in the diet (meat is a rich source). Carnitine deficiency can occur in newborns, particularly in pre-term infants, and also in patients undergoing hemodialysis. Inability to transport fatty acids into the mitochondria for oxidation may manifest in systemic symptomology or may be limited to only muscles. Symptoms can range from mild occasional muscle cramping to severe weakness or even death. Treatment is by oral carnitine administration.

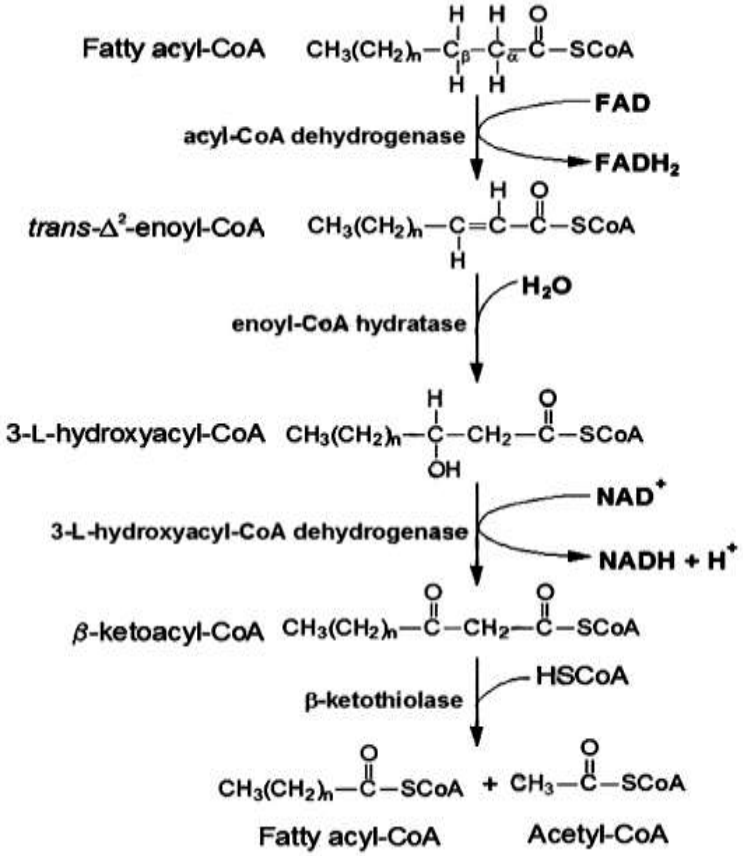
Deficiency of carnitine acyl-transferase (carnitine palmitoyl-transferase) leads to failure of fatty acid oxidation. If it involves the liver cells, it leads to hypoketotic hypoglycemia. Severe forms may lead to hepatomegaly, seizures and cardiomyopathy, which lead to infantile death. The most common form is the adult myopathic carnitine acyl-transferase II deficiency. This disease is characterized by muscle aches and weakness, and myoglobinuria with brown urine. Episodes are provoked by prolonged exercise especially after fasting, cold, or associated stress. Symptoms may be exacerbated by high-fat, low-carbohydrate diet. Triglycerides accumulate in the muscle cells. Treatment is glucose administration with cessation of muscle activity. A somewhat similar syndrome can be produced by muscle carnitine deficiency due to a defect in the transport system for carnitine into the muscle cells. Carnitine acyl-transferases may also be inhibited by sulfonylurea drugs such as tolbutamide and glyburide.

Eating unripe fruit of the Jamaican ackee tree, with its toxin: hypoglycin A, causes shutdown of carnitine transport system and fatty acid oxidation in all cells, with severe hypoglycemia.

Carnitine acyl-transferase I is inhibited by malonyl CoA formed for fatty acid synthesis. Thus, fatty acid oxidation is inhibited during periods of fatty acid synthesis. Insulin indirectly inhibits β-oxidation by activating acetyl CoA carboxylase and increasing malonyl CoA concentration in the cytoplasm (lipogenesis). Glucagon reverses this process.

Short- and medium-chain fatty acids are activated in the mitochondrial matrix. They are carnitine-independent.

Beta-oxidation pathway



β-Oxidation pathway is a repetition of four steps. Each four-step cycle releases one acetyl CoA (2 acetyl CoA in the last cycle) and reduces FAD and NAD^+ to $FADH_2$ and NADH, which are oxidized by the electron transport chain, providing 5 ATP. Each acetyl CoA yields 12 ATP on oxidation. Two ATP used in activation are subtracted to calculate the energy from a fatty acid.

Medium-chain acyl CoA dehydrogenase (MCAD) deficiency

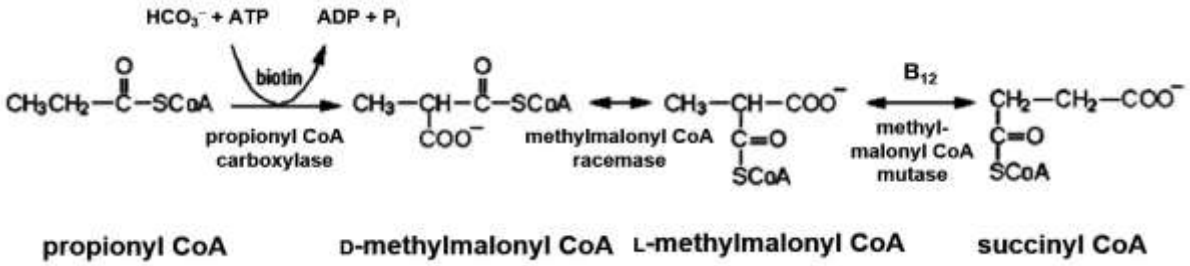
In this genetic deficiency disease, β-oxidation stops when the acyl CoA reaches 6-10 carbons in length. In the first years of life, this deficiency appears following a fasting period. Episode may be provoked by overnight fast in an infant. Symptoms include vomiting, lethargy and frequently coma. There is hypoglycemia with low or absent ketone bodies. The disease is characterized by urinary excretion of medium-chain dicarboxylic acids that result from omega-oxidation of the accumulating acyl CoA. Prolonged fasting should be avoided to prevent clinical problems. Feeding should be frequent, with high-carbohydrate, low-fat diet. Treatment of an episode is by intravenous glucose.

Oxidation of unsaturated fatty acids

The oxidation of unsaturated fatty acids, e.g., oleic acid (*cis*Δ^9 C18:1) is essentially the same process as for saturated ones, except when a double bond is encountered. The *cis*Δ^3 is isomerized to *trans*Δ^2 bond by a specific enoyl-CoA isomerase and oxidation continues. $FADH_2$ is not produced in this cycle. In the case of linoleate (*cis*Δ^9,Δ^{12} C18:2), there is formation of 2,4-dienoyl CoA during oxidation. This molecule is the substrate for an additional enzyme, the NADPH-requiring 2,4-dienoyl CoA reductase.

Propionic acid pathway

Fatty acids with an odd number of carbon atoms are oxidized by β-oxidation. In the final cycle, one acetyl CoA and one propionyl CoA are formed. Propionyl CoA results also from oxidation of branched-chain fatty acids, e.g., phytanic acid and from the metabolism of some aminoacids. Propionyl CoA is converted to succinyl CoA, a citric acid cycle intermediate, in the two-step propionic acid pathway.



Succinyl CoA can form malate, which passes to the cytosol for gluconeogenesis. Odd-carbon (and branched-chain) fatty acids represent an exception to the rule that fatty acids cannot be converted to glucose in humans. The propionic acid pathway includes two important enzymes, both in the mitochondria: propionyl CoA carboxylase, which requires biotin, and methylmalonyl CoA mutase, which requires vitamin B_{12} coenzyme. Vitamin B_{12} deficiency can cause a megaloblastic anemia of the same type seen in folate deficiency. Excretion of methylmalonic acid indicates a vitamin B_{12} rather than folate deficiency.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following should be present for dietary lipids to be digested and absorbed?
(A) Hydrochloric acid.
(B) Cholesterol.
(C) Bile salts.
(D) Chymotrypsin.

2- Fatty acids are oxidized as cell fuel by which of the following cells?
(A) Brain cells.
(B) Red blood cells.
(C) Liver and muscle cells.
(D) Any cells that have no mitochondria.

3- To differentiate between carnitine acyltransferase II deficiency and defective uptake of carnitine by muscle cells, we may measure
(A) acyl CoA.
(B) acylcarnitine.
(C) free fatty acids.
(D) triglycerides.

4- Episodes due to defective carnitine transport system are treated by
(A) glucose and muscle rest.
(B) long-chain fatty acids.
(C) insulin.
(D) carnitine.

5- Caffeine (a xanthine drug) stimulates lipolysis in adipose tissue through
(A) release of glucagon from pancreas.
(B) inhibition of insulin release.
(C) inhibition of cAMP phosphodiesterase.
(D) activation of protein phosphatase.

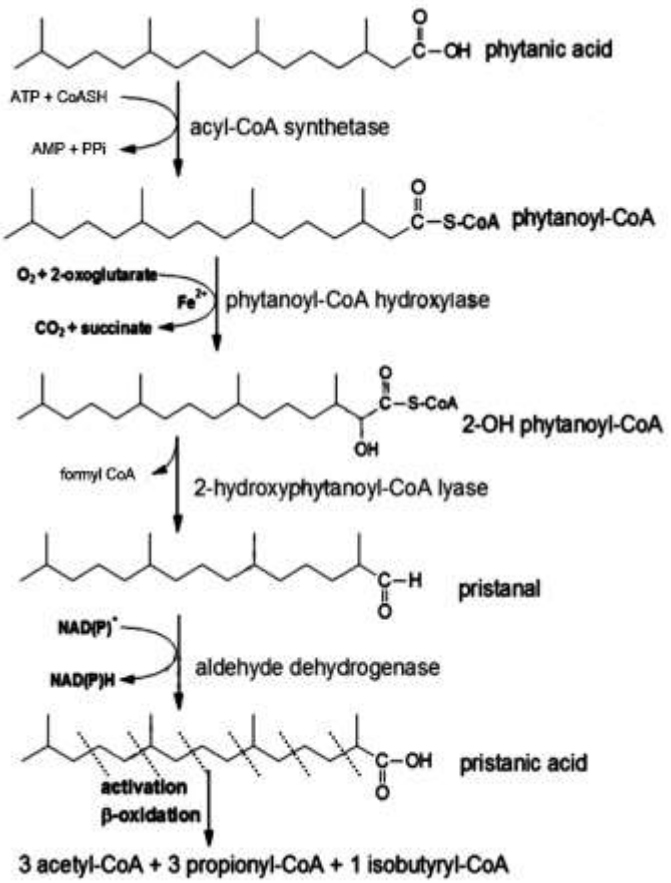
Peroxisomal β-oxidation reactions

The peroxisomes play an important role in overall fatty acid metabolism. Very-long-chain fatty acids (VLCFAs: C17–C26) are preferentially oxidized in the peroxisomes with cerotic acid (C26:0) being solely oxidized in these organelles. The peroxisomes also metabolize long-chain dicarboxylic acids that are produced by ω-oxidation of long-chain monocarboxylic acids, phytanic acid via the α-oxidation pathway, certain polyunsaturated fatty acids (PUFAs) such as tetracosahexaenoic acid (24:6), which by β-oxidation yields the important PUFA docosa-hexaenoic acid (DHA), and certain prostaglandins and leukotrienes.

The enzymatic processes of peroxisomal β-oxidation are very similar to those of mitochondrial β-oxidation with one major difference. During mitochondrial oxidation the first oxidation step, catalyzed by various acyl-CoA dehydrogenases, results in the reduced electron carrier $FADH_2$ that delivers its' electrons to coenzyme Q of the electron transport chain (via electron transfer flavoprotein). In the peroxisome, the first oxidation step is catalyzed by acyl-CoA oxidase, which reduces O_2 to hydrogen peroxide (H_2O_2). Thus, the reaction is not coupled to energy production, but instead yields a significant reactive oxygen species (ROS). Peroxisomes contain the enzyme catalase that degrades hydrogen peroxide.

Alpha-oxidation

Phytanic acid, derived from chlorophyll, is a fatty acid present in the tissues of ruminants and in dairy products. Because it is methylated at the β-carbon, it cannot undergo mitochondrial β-oxidation. It is oxidized within the peroxisomes. The first step involves an oxidation of the α-carbon, and the process is termed α-oxidation. With the removal of the first carbon, the new β-carbon becomes suitable for β-oxidation.

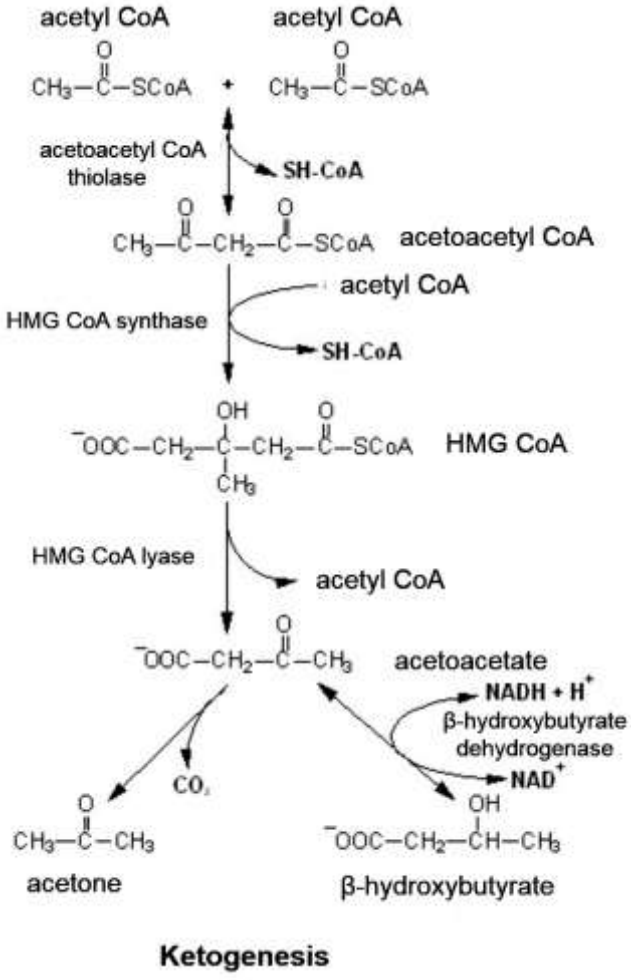


Refsum disease is a rare inherited defect in the peroxisomal α-oxidizing enzyme: phytanoyl-CoA hydro-xylase. Patients accumulate large quantities of phytanic acid in their tissues and serum. This leads to severe symptoms, including cerebellar ataxia, retinitis pigmentosa, nerve deafness and peripheral neuropathy. The restriction of dairy products and ruminant meat from the diet can ameliorate these symptoms.

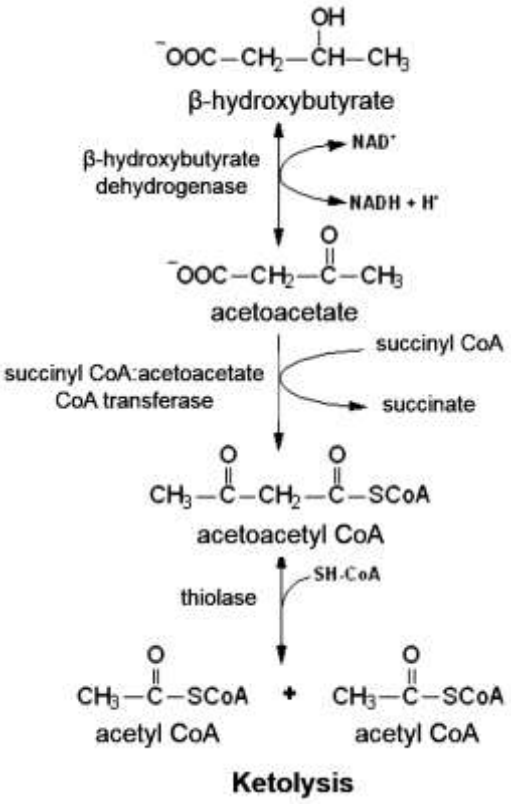
Zellweger's (cerebrohepatorenal) syndrome is caused by a rare inherited absence of peroxisomes in all tissues. There is accumulation of phytanic acid and very long chain polyenoic acids.

Ketone bodies

Ketogenesis takes place in the liver mitochondria during fasting. In the fasting state, with mobilization of fatty acids from the adipose tissue, the liver produces more acetyl CoA from β-oxidation than is used in the citric acid cycle. This is converted to the ketone bodies: acetoacetate and 3-hydroxybutyrate (β-hydroxybutyrate). Ketone bodies are water-soluble fuel that can be used by extra-hepatic cells (ketolysis). Therefore, ketone bodies are an important fasting cell fuel. Acetone results from spontaneous decomposition of acetoacetate and appears in expired air. It gives the characteristic smell of ketosis (ketoacidosis) that is observed in uncontrolled diabetes mellitus.



Ketogenesis



Ketolysis

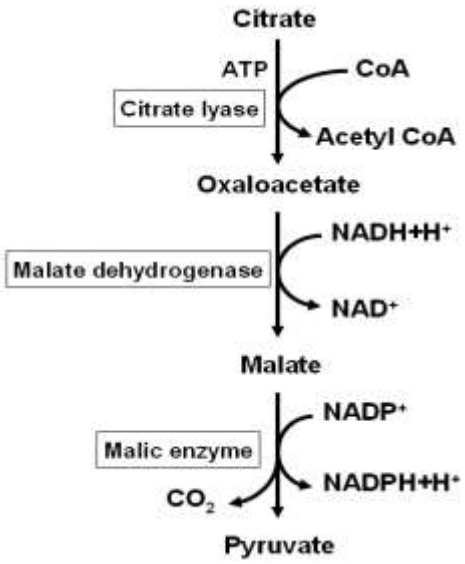
Ketolysis takes place in the mitochondria of extra-hepatic tissues, mainly muscle and heart. The key enzyme is thiophorase (syccinyl CoA:acetoacetate CoA transferase), which is absent in liver cells. After one week of fasting, ketones reach a concentration in blood high enough for the brain to begin metabolizing them. When ketones are metabolized to acetyl CoA, pyruvate dehydrogenase is inhibited. Glycolysis, and subsequently glucose uptake in the brain, decreases. This spares tissue proteins, the main gluconeogenic substrates.

Ketoacidosis results from increased ketogenesis, more than ketolysis, like in diabetes (the main cause) and prolonged fasting. An infection or trauma (causing an increase in cortisol or epinephrine) may precipitate an episode of ketoacidosis. Alcoholics can also develop ketoacidosis. In alcoholic ketoacidosis, 3-hydroxybutyrate is the major ketone body produced because there is usually a high $NADH/NAD^+$ ratio in the liver. The urinary nitroprusside test detects only acetoacetate and may dramatically underestimate the extent of ketosis in an alcoholic.

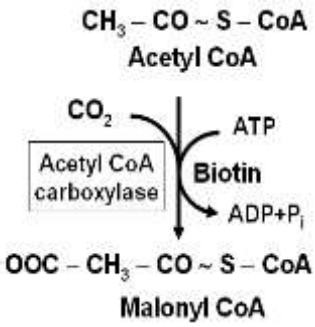
Fatty acid synthesis (lipogenesis)

Fatty acids are synthesized from the excess acetyl CoA resulting from carbohydrate oxidation. The synthesis of fatty acids occurs mainly in the liver, adipose tissue and mammary glands. Lipogenesis is a cytosolic pathway. The reactions of the fatty acid synthesis are the reverse of the mitochondrial β-oxidation reactions. An acetate moiety is added, from malonyl CoA, in each cycle to the growing fatty acid. Reducing equivalents needed are provided by NADPH, not NADH or $FADH_2$. Lipogenic enzymes are induced by feeding. These enzymes include citrate lyase, malic enzyme, glucose 6-phsphate dehydrogenase, acetyl CoA carboxylase, and fatty acid synthase.

Acetyl CoA combines with oxaloacetate in the mitochondria to form citrate, which is transported to the cytoplasm. In the cytoplasm, ATP- citrate lyase acts on citrate and coenzyme A to form oxaloacetate and acetyl CoA.



Oxaloacetate captures the reducing equivalents from NADH (formed by the glycolytic pathway) and is reduced to malate, the substrate of malic enzyme that produces NADPH for lipogenesis. Malic enzyme reaction represents an additional source of cytoplasmic NADPH, added to that from the HMP pathway. Tissues that show active lipogenesis also show an active HMP pathway. Cytosolic isocitrate dehydrogenase is another minor source of NADPH.



Acetyl CoA carboxylase is the key, rate-limiting, enzyme of lipogenesis. It activates acetyl CoA to act as the source of the two-carbon moiety for fatty acid synthase by converting it to malonyl CoA. However, the added CO_2 is not incorporated into the fatty acid being synthesized. This key enzyme is allosterically activated by citrate, which causes its polymerization, and inhibited by long chain fatty acyl CoA. It is induced by feeding and activated by insulin (dephosphorylated). Malonyl CoA inhibits carnitine palmitoyl transferase I, thus inhibiting fatty acid oxidation.

Fatty acid synthase is a large multienzyme complex that contains the enzymatic activities for all the steps of fatty acid synthesis and an acyl carrier protein (ACP) that requires the vitamin pantothenic acid. The two-carbon primer used by the enzyme is from acetyl CoA, and the two carbons added in every cycle are from malonyl CoA. NADPH is required in the two reduction reactions. The final product is palmitate (C16:0). In the mammary gland, the product may be C8-C12 fatty acids.

Fatty acyl CoA may be elongated and desaturated (to a limited extent in humans) using enzymes of the smooth endoplasmic reticulum (fatty acid elongase and desaturase). These enzymes cannot introduce double bonds past position 9 in the fatty acid.

Storage of fatty acids

Fatty acids are stored as triacylglycerols (triglycerides). This occurs primarily in the liver and adipose tissue. Fatty acids are activated to fatty acyl CoA, then reacted with glycerol 3-phosphate. The latter is derived from glucose through dihydroxyacetone phosphate, since fat cells do not have glycerol kinase. Liver cells however have glycerol kinase, which enables them to recycle glycerol, whether in fed states to synthesize triglycerides, or during fasting to synthesize glucose. The liver exports triglycerides packaged as very low-density lipoproteins (VLDL). A small amount of triglycerides may be stored in the liver. Accumulation of significant triglycerides in tissues other than the adipose tissue usually indicates a pathologic state.

Study Questions

Choose one best answer for every question of the following:

6- Ketolysis normally takes place in which cells?
(A) Heart cells.
(B) Brain cells.
(C) Liver cells.
(D) Red blood cells.

7- Ketogenesis is a
(A) Physiological process during fasting.
(B) Normal phenomenon following food intake.
(C) Pathological alteration of metabolism in diabetic patients.
(D) Useless pathway in normal persons.

8- Ketoacidosis may be observed in which of the following conditions?
(A) Well-fed state.
(B) After a fatty meal.
(C) Prolonged fasting.
(D) Controlled diabetes mellitus.

9- Which of the following hormones inhibits lipolysis in adipose tissue?
(A) Insulin.
(B) Glucagon.
(C) Epinephrine.
(D) Thyroxine.

10- Ketone bodies are formed from
(A) beta-ketoacyl CoA.
(B) acetyl CoA.
(C) cytosolic HMG CoA.
(D) ketosugars.

11- Beta oxidation pathway is controlled by
(A) phosphorylation-dephosphorylation of acyl CoA dehydrogenase.
(B) allosteric inhibition of enoyl CoA hydratase by acetyl CoA.
(C) availability of acyl CoA in the mitochondria.
(D) induction by a high insulin/glucagon ratio.

12- Compared to the energy yield of palmitic acid, the energy yield of stearic acid oxidation is
(A) 12 ATP lower.
(B) 12 ATP higher.
(C) 17 ATP lower.
(D) 17 ATP higher.

13- Water-soluble fuel produced by the liver during fasting includes
(A) glucose and fatty acids.
(B) glucose and ketone bodies.
(C) ketone bodies and fatty acids.
(D) fatty acids and glycerol.

14- Fatty acid oxidation is inhibited by
(A) insulin.
(B) glucagon.
(C) fatty acyl CoA.
(D) acetyl CoA.

Aminoacid Metabolism

Sources of aminoacids:

- Digested diet proteins.
- Broken down tissue proteins.
- Synthesis of non-essential aminoacids.

Fate of aminoacids:

- Synthesis of body proteins and peptides.
- Synthesis of nitrogenous compounds.
- Synthesis of non-essential aminoacids.
- Sharing in metabolic reactions such as transmethylation and sulfation.
- After removal of nitrogen, an aminoacid becomes an oxygenated hydrocarbon, which is converted to carbohydrate or fat or used as a fuel for energy production.

Digestion and absorption of dietary protein

Digestion of dietary proteins takes place in the stomach and the intestine. Proteases of the gastrointestinal tract differ from each other in their specificity to certain aminoacids and their optimum pH. A protease of the stomach, pepsin, has an acidic optimum pH, while the proteases acting in the intestine have alkaline optimum pH.

In the stomach, hydrochloric acid denatures dietary proteins. The unfolding of proteins makes them more accessible by the digestive enzymes. Pepsinogen is activated to pepsin by hydrochloric acid and pepsin itself, a process of auto-activation. The stomach also secretes rennin, which is important in infants for the coagulation of milk casein.

Pancreatic proteases include trypsin, chymotrypsin, elastase and carboxypeptidase. The first three enzymes are endopeptidases while the last one is an exopeptidase that releases one aminoacid at a time from the carboxyl end. They all work in the intestinal lumen at an alkaline pH. They are secreted as zymogens. Trypsinogen is activated by enterokinase, an enzyme secreted by the intestinal mucosal cells. Trypsin in turn auto-activates trypsinogen as well as the rest of pancreatic proteases.

Intestinal enzymes complete the digestion of proteins. Aminopeptidase is an exopeptidase that splits off one aminoacid at a time from the amino terminus of the peptide. Dipeptidases, some of which may be within the intestinal epithelium ensure the complete digestion of dipeptides to free aminoacids.

The final products of protein digestion are free aminoacids. Several aminoacid transport systems of overlapping specificity participate in active absorption of these aminoacids. Aminoacids absorbed from the intestine pass in the portal circulation to the liver.

Protein turnover

Tissue proteins are subject to turnover. Protein molecules are broken down and new molecules are formed at a rate of about 1-2% per day. This ensures the youth of molecules and provides a means of controlling pathways by controlling the concentrations of critical enzymes or modulators. Tissue proteins have a half-life that differs from one protein to another. This may be a few minutes or months. Breaking down of body proteins usually takes place in the lysosomes, but proteolysis may also take place in the cytosol, or even in the extracellular matrix or the plasma. Upon prolonged fasting, proteolysis of tissue protein provides aminoacids to be used for synthesis of glucose or ketone bodies.

Glucogenic and ketogenic aminoacids

In fed states, excess aminoacids contribute to acetyl CoA pool that is oxidized for energy or converted to fat stores. In fasting states, the carbon skeletons of aminoacids are converted to glucose or ketone bodies.

Glucogenic aminoacids are those that give pyruvate or an intermediate of citrate cycle, which can be converted to glucose by gluconeogenesis. Ketogenic aminoacids are those that give acetoacetate, acetoacetyl CoA or acetyl CoA. These intermediates cannot be converted to glucose, but they are components of the ketogenesis pathway. Purely ketogenic aminoacids are lysine and leucine. Mixed fate, glucogenic and ketogenic, aminoacids are isoleucine, phenylalanine, tyrosine, tryptophan and threonine. The remaining aminoacids are purely glucogenic.

Essential aminoacids

Essential aminoacids are those that cannot be synthesized in the body, or not synthesized at a sufficient rate. Therefore, their presence in the diet is essential. These are ten aminoacids: valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, lysine, histidine, and arginine. Arginine is essential only in special cases of increased demand like in growing children and pregnant women. By restriction of phenylalanine intake, tyrosine becomes essential as it is synthesized from phenylalanine.

Proteins that contain all the essential aminoacids are high biological value proteins. These are usually animal proteins like those from milk, eggs, and meat. Plant proteins lack one or more essential aminoacids. Vegetarians can obtain all the essential aminoacids by taking a combination of different plant proteins.

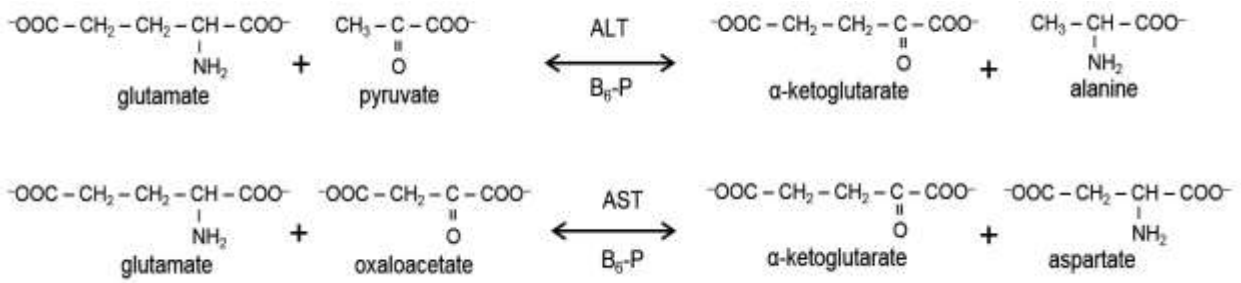
Transamination

Non-essential aminoacids may be synthesized by transamination. An aminoacid reacts with an α-ketoacid, generating a new aminoacid. Transaminases (amino-transferases) require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate is derived from vitamin B_6 (pyridoxine). These reactions are reversible, i.e., any of the two aminoacids may be synthesized from its corresponding α-ketoacid.

Clinical significance

Transaminases are intracellular enzymes. They increase in the plasma in cases of active disease accompanied by cell injury. Clinically important transaminases include:

- ALT (Alanine Transaminase), formerly called GPT (Glutamate:Pyruvate Transaminase).
- AST (Aspartate Transaminase), formerly GOT (Glutamate:Oxaloacetate Transaminase).



Nitrogen balance

Atmospheric nitrogen, which constitutes about 80% of air, is not metabolized. Dietary proteins provide the main source of nitrogen input. This is used for building up processes or replacing losses. Unlike carbohydrate and lipids, nitrogen is not stored. In a normal healthy adult: nitrogen input equals nitrogen output. In other words, there is equilibrium between nitrogen intake in the form of protein and nitrogen excretion in the form of nitrogenous compounds, mainly urea.

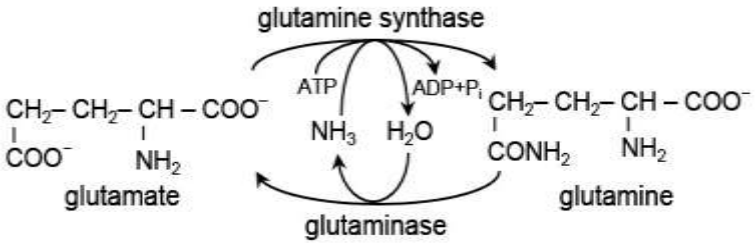
Negative nitrogen balance means that nitrogen intake is less than nitrogen excretion. This is observed in some conditions like prolonged fasting or protein malnutrition, e.g., kwashiorkor in children taking enough-calorie but low-protein diet. It is also seen in fevers, when there is increased catabolism and decreased food intake.

Positive nitrogen balance means a nitrogen intake more than nitrogen excretion. This is seen in growing children, pregnant women and in cases of convalescence after illness.

Nitrogen catabolism

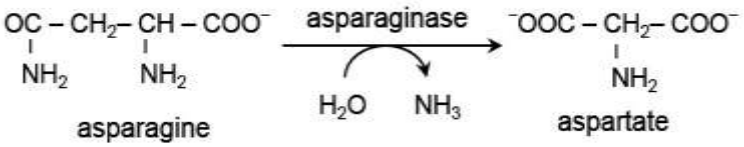
Catabolism of aminoacid nitrogen starts by removal of the amino group from the aminoacids in the form of ammonia (NH_3), a process known as deamination. Ammonia is toxic, especially to the nervous system. It is eliminated by being converted to urea by the liver cells, a pathway known as urea cycle. Urea is then excreted by the kidneys as the main nitrogenous excretory product in the urine.

Many tissues, including the brain, contain glutamine synthetase that enables them to get rid of ammonia in the form of glutamine. Glutamine has the highest plasma aminoacid concentration.

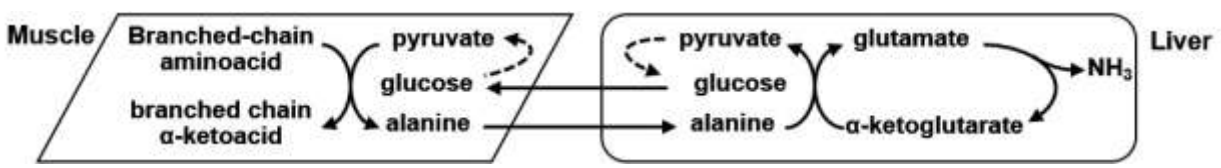


In the liver cells, glutaminase reaction releases ammonia. The kidney also has glutaminase enzyme that releases ammonia to be used for buffering the excreted acids.

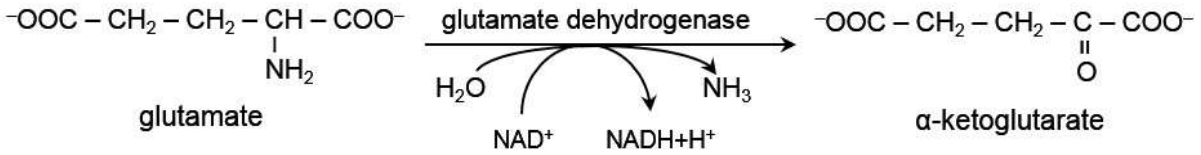
Asparaginase, an analogous enzyme, converts asparagine to aspartate. This enzyme may be used for cancer therapy since it can deplete plasma asparagine, which is necessary for tumor growth.



The nitrogen may be transported on alanine, e.g., from muscle cells, by transamination of pyruvate, then delivered to α-ketoglutarate in the liver cells. The resulting pyruvate in the liver cells can be used to synthesize glucose, which is transported to the muscle to be a source of pyruvate. This completes the glucose-alanine cycle mentioned with gluconeogenesis.



The nitrogen of many aminoacids ends in glutamate by transamination, or by conversion of the aminoacid to glutamate. Glutamate undergoes oxidative deamination in the liver cells, catalyzed by the enzyme L-glutamate dehydrogenase, releasing ammonia:



D- and L-aminoacid oxidases are flavoproteins that may also participate in oxidative deamination of some aminoacids and production of ammonia.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is a gastric protease?
(A) Pepsin.
(B) Trypsin.
(C) Pepsinogen.
(D) Chymotrypsin.

2- Which of the following is <u>not</u> a function of aminoacids?
(A) Synthesis of nucleic acids.
(B) Formation of tissue proteins.
(C) Synthesis of essential aminoacids.
(D) Production of important nitrogenous compounds.

3- What is meant by glucogenic aminoacids?
(A) Aminoacids that are synthesized from glucose.
(B) Aminoacids that can produce glucose by gluconeogenesis.
(C) Aminoacids present with glucose in food.
(D) Aminoacids that cannot be converted to glucose.

4- Protein turnover means which of the following?
(A) A molecule is folded on itself.
(B) A molecule is taken back by the cell after being excreted.
(C) Molecules are broken down and new molecules are synthesized.
(D) Molecules have a half-life of only seconds or minutes.

5- Which of the following causes an increase in plasma alanine transaminase?
(A) Active liver disease.
(B) Renal failure.
(C) Enhanced gluconeogenesis.
(D) Lowered functional capacity of the liver.

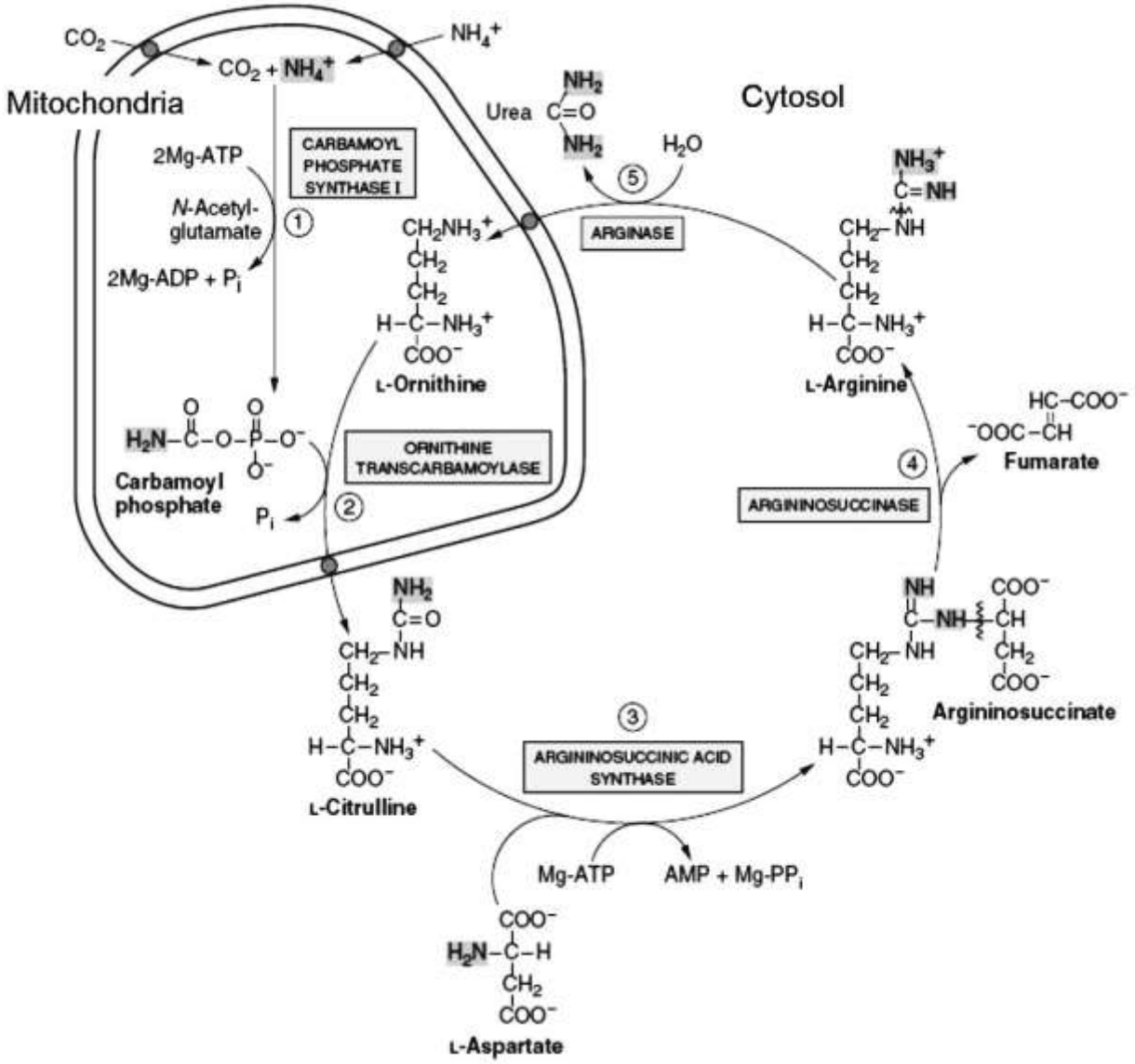
6- Which aminoacid is the main one that undergoes oxidative deamination?
(A) Alanine.
(B) Aspartic acid.
(C) Glutamine.
(D) Glutamic acid.

7- Which of the following is usually accompanied by positive nitrogen balance?
(C) Heavy proteinuria.
(D) Pregnancy.
(C) A high protein diet.
(D) Fever.

8- Ammonia is safely transported in the blood from various tissues to the liver as
(A) dissolved ammonia.
(B) ammonium ions.
(C) amide of glutamine.
(D) amide of asparagine.

Urea synthesis

Five reactions, catalyzed by five enzymes constitute the urea cycle. Reactions 1 and 2 take place in the mitochondria; the remaining three reactions take place in the cytosol.



Respiratory carbon dioxide (reaction 1) plus two nitrogen atoms are used for urea synthesis. The two nitrogen atoms are derived from ammonia (reaction 1) and aspartate (reaction 3). N-acetyl glutamate is an allosteric activator of the mitochondrial carbamoyl phosphate synthetase. Its synthesis is allosterically activated by arginine. Arginine is formed in the cycle, but still it is an essential aminoacid for children and pregnant women.

Fumarate (reaction 4) can be recycled: converted to malate, oxaloacetate, and then to aspartate by fumarase, malate dehydrogenase and aspartate transaminase successively. Ornithine can be synthesized from glutamic acid, and is regenerated in the cycle (reaction 5).

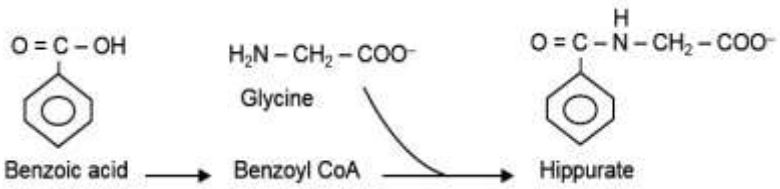
Note that the urea cycle is energy consuming: 4 ATP/mol urea. Getting rid of ammonia is worth spending energy. However, there are 2 NADH generated by glutamate dehydrogenase (that provides ammonia) and malate dehydrogenase (during fumarate recycling), whose oxidation compensates the energy consumed by the urea cycle.

Important clinical notes:

- Nitrogen constitutes about half (28/60) the urea weight. Considering that nitrogen constitutes about one-sixth the protein weight, then the daily excretion of urea equals one-third the daily protein intake.
- Reference range for blood or plasma urea is relatively wide (10-50 mg/dL). BUN is a term frequently used, meaning blood urea nitrogen.
- Blood urea concentration depends on the state of protein nutrition.
- Blood urea is elevated with renal impairment.
- Liver cell failure is characterized by hyper-ammonemia. A patient with liver cell failure is maintained on a low protein diet.
- Functional capacity of the liver is measured by blood ammonia, albumin, and bilirubin, and by prothrombin time, while elevation of plasma transaminases indicates an active liver disease. Elevated alkaline phosphatase may indicate biliary obstruction or bone disease, in addition to intestinal and placental sources. Since enzyme electrophoresis is impractical as a routine clinical lab test, the liver as a source is confirmed by other enzymes. Gamma-glutamyl transferase, the earliest liver enzyme, is useful for this purpose.
- Genetic defect of any of the enzymes of urea cycle leads to congenital hyperammonemia. The same is true for enzymes that synthesize ornithine from glutamate.

Genetic deficiency of the Urea Cycle

A genetic defect of urea synthesis leads to congenital (primary) hyperammonemia, accompanied by decreased blood urea and elevated blood glutamine. With neonatal onset, infants typically appear normal for the first 24 hours. Sometime during the 24-72 hour postnatal period, symptoms of lethargy, vomiting, and hyperventilation begin and, if not treated, progress to coma, respiratory failure, and death.

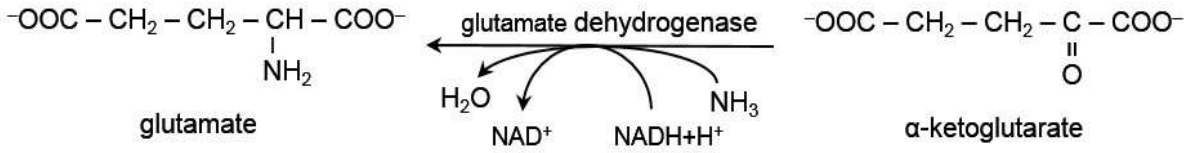


These conditions may be treated with a low protein diet and administration of sodium benzoate and phenyl butyrate or phenyl acetate to provide an alternative route for capturing and excreting excess nitrogen. Benzoic acid is conjugated with glycine and excreted as hippuric acid in urine. Phenyl butyrate is activated by beta-oxidation to phenyl acetate, which is conjugated with glutamine to be excreted in urine. Acidification of intestinal lumen may also help getting rid of ammonia as ammonium ions. Giving arginine and ornithine may stimulate the urea cycle. Arginine gives ornithine and also activates glutamate N-acetylation. Glucose is given for energy, sparing tissue proteins, and providing substrates for synthesis of non-essential aminoacids.

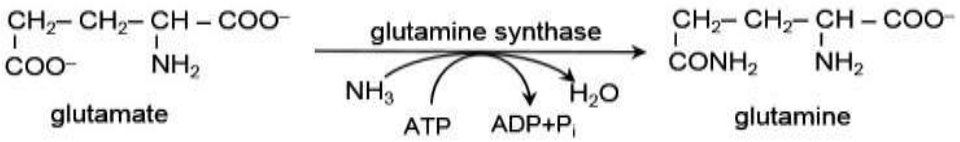
Ornithine transcarbamoylase (OTC) deficiency, a recessive X-linked disease, leads to accumulation of carbamoyl phosphate, which leaks from the mitochondria to the cytoplasm where it participates in uncontrolled pyrimidine synthesis. Therefore, this condition is characterized by high levels of orotic acid and uracil, an observation not seen with the deficiency of mitochondrial carbamoyl phosphate synthetase. Both conditions are characterized by low citrulline production.

Ammonia toxicity

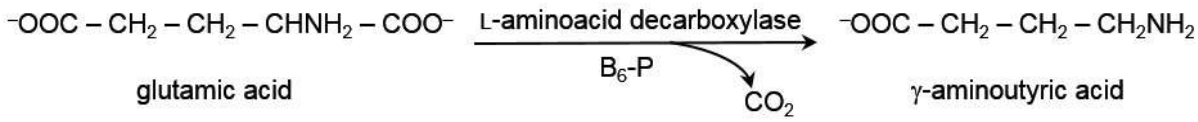
Ammonia is toxic, especially to the nervous system. Ammonia toxicity to the brain is probably due to reversal of the glutamate dehydrogenase reaction under the high concentration of ammonia. Depleting alpha-ketoglutarate by its conversion to glutamate leads to inhibition of the TCA cycle (the major energy provider).



Ammonia in the brain is loaded on glutamate to synthesize glutamine by glutamine synthase. This can lead to depletion of brain glutamate.



Glutamate is a brain neurotransmitter and it is the source of the neurotransmitter gamma-aminobutyric acid (GABA) in the brain. Increased ammonia can lead to decrease in these two neurotransmitters.



Elevated blood ammonia may also increase permeability of brain cells to potassium and chloride ions leading to disturbance of the brain electricity.

Sources of Ammonia:

- Deamination of aminoacids and other nitrogenous compounds is the major source of ammonia. In cases of hyperammonemia, a low protein diet is mandatory.
- Action of bacterial urease in the intestine on urea diffused from the blood is a minor source of ammonia in healthy people. In patients with impaired liver function, this becomes a big problem. Bacterial putrefaction in the large intestine also produces ammonia. Thus, antibiotics are a justifiable measure for treatment of hyperammonemia.
- Hemorrhage into the stomach, in cases of liver cirrhosis and ruptured esophageal varices, lays a protein load that increases the hyperammonemia of liver cell failure. It is to be remembered that one liter of blood escaping to the stomach may equal feeding with one kilogram of meat in those patients kept on a restricted protein diet. Measures such as gastric lavage and enema are important in these conditions. Acidification of the intestine, e.g., using lactulose may be beneficial for decreasing ammonia absorption.

Study Questions

Choose one best answer for every question of the following:

9- What is the daily urea excretion by a healthy adult that takes 90 g protein/day?
(A) 30 mg.
(B) 90 mg.
(C) 30 g.
(D) 90 g.

10- Why may patients with liver cell failure be given oral antibiotics?
(A) To combat hepatitis virus.
(B) To improve liver function.
(C) To kill intestinal bacteria.
(D) To inhibit gluconeogenesis.

11- A 20-year-old healthy girl develops hyperammonemia upon intake of a high-protein diet. What may be the cause?
(A) Liver cell failure.
(B) OTC deficiency carrier.
(C) Arginase deficiency.
(D) Vitamin B_6 deficiency.

12- A genetic defect of N-acetylglutamate production leads to a high level of
(A) urea.
(B) arginine.
(C) glutamine.
(D) citrulline.

13- Increased plasma alanine during fasting is due to
(A) increased breakdown of tissue proteins.
(B) increased plasma alanine transaminase.
(C) decreased utilization of alanine.
(D) decreased formation of urea.

14- To test for hepatitis, which plasma analyte would you measure?
(A) Albumin.
(B) ammonia.
(C) ALT.
(D) AST.

15- Which of the following my <u>not</u> be a direct fate of ammonia?
(A) Glutamine.
(B) Urea cycle.
(C) Purines pyrimidines, and uric acid.
(D) Ammonium ions in urine.

16- High blood urea is mostly seen with
(A) liver cell failure.
(B) renal failure.
(C) high-protein diet.
(D) low-protein diet.

17- Which of the following would you give to a patient with hyperammonemia?
(A) Glutamine and asparagine.
(B) A combination of non-essential aminoacids.
(C) Ammonium hydroxide.
(D) Alpha-ketoacid analogues of phenylalanine, methionine, valine, leucine and isoleucine.

18- Which of the following may be considered a physiological ammonia-fixing reaction?
(A) Glutamate dehydrogenase.
(B) Alanine transaminase.
(C) Glutamine synthase.
(D) Asparagine synthase.

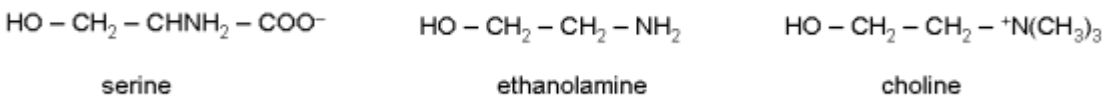
19- Glutamine is important for which of the following?
(A) Safe transport of ammonia as the amide nitrogen.
(B) The major source of ammonia for urinary excretion.
(C) Synthesis of asparagine, purines and pyrimidines.
(D) All the above.

20- Which of the following produces ammonia?
(A) Glutamine synthetase.
(B) Glutamate:oxaloacetate transaminase.
(C) Arginase.
(D) Urease.

Important nitrogenous compounds

Since diet protein provides the main nitrogen source, all nitrogenous compounds are derived from aminoacids. In addition to hormones and neurotransmitters discussed before (Lecture 9), many other nitrogenous compounds are biologically important. Below is a short list:

- Nicotinic acid (Niacin), a member of vitamin B complex, is synthesized from tryptophan.
- Creatine and creatinine are from three aminoacids: glycine, arginine, and methionine.
- Melanin pigment is from tyrosine.
- Purine and pyrimidine nucleotides depend on aminoacids for their synthesis.
- Uric acid is the catabolite of the purine bases.
- Heme is synthesized from glycine and succinyl CoA. It is catabolized to bilirubin.
- Bile acids: Conjugation of the steroidal bile acids with glycine or taurine (derived from cysteine) makes them more soluble, e.g., glycocholic and taurocholic acids.
- Carnitine for fatty acid oxidation is synthesized from lysine and methionine.
- Aminosugars, important for glycosaminoglycans and glycoprotein get their nitrogen from glutamine.
- Sphingosine, which is essential for synthesis of sphingomyelin and glycolipids, is synthesized from serine and palmitoyl CoA.
- Phosphatidyl serine contains the aminoacid serine in its structure. Phosphatidyl ethanolamine contains ethanolamine, which results from decarboxylation of serine. Phosphatidyl choline contains choline, which results by adding 3 methyl groups to ethanolamine



serine ethanolamine choline

Sulfur compounds

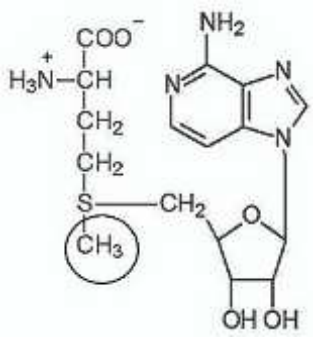
Dietary protein is the main source of sulfur for human metabolism. The incorporation of cysteine in the polypeptide chain provides the means for formation of the disulfide bond important for the peptide or protein structure, e.g., insulin and immunoglobulins. From cysteine, important sulfur compounds are formed. Below is a short list:

- Taurine is synthesized from cysteine by oxidation then decarboxylation. It is used for conjugation of bile acids.
- Active sulfate: 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is used for sulfation reactions, e.g., for synthesis of sulfolipids and glycosaminoglycans and for conjugation reactions.
- Thiosulfate is used for detoxication of cyanide by producing thiocyanate, a reaction catalyzed by rhodanese (sulfur transferase) enzyme.
- Glutathione (gamma-glutamyl-cysteinyl-glycine) is an important tripeptide that functions as an antioxidant, a coenzyme and a hydrogen carrier required for inactivation of insulin by glutathione:insulin transhydrogenase. It shares in detoxication by conjugating halogenated and nitrogenous compounds, organophosphorus compounds, drugs and heavy metals. It also acts as a glutamyl donor (substrate for γ-glutamyl transferase), thus it may be required for absorption of aminoacids in the renal tubules (γ-glutamyl cycle).

Dietary sulfur, in cysteine and methionine, is excreted in the urine mainly as sulfate, sulfated steroids and other sulfur-containing conjugates, and thio-compounds. The odor of stool is mainly due to hydrogen sulfide and indole compounds produced by bacterial putrefaction of sulfur-containing aminoacids and tryptophan respectively.

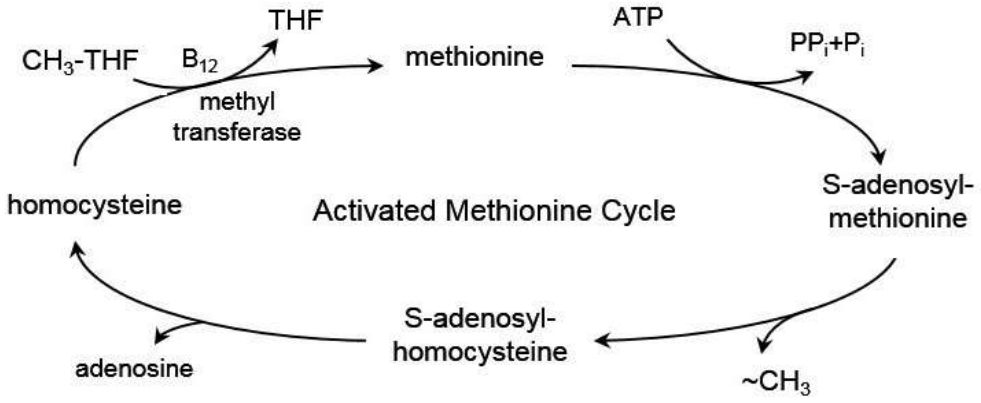
Active methionine cycle

Methionine, besides sharing in protein synthesis, is important for methylation reactions. Methionine reacts with ATP to generate S-adenosyl methionine (SAM), the major methyl donor. Methyl transferases catalyze the transfer of the methyl group (CH_3) from SAM to different methyl acceptors, e.g.,



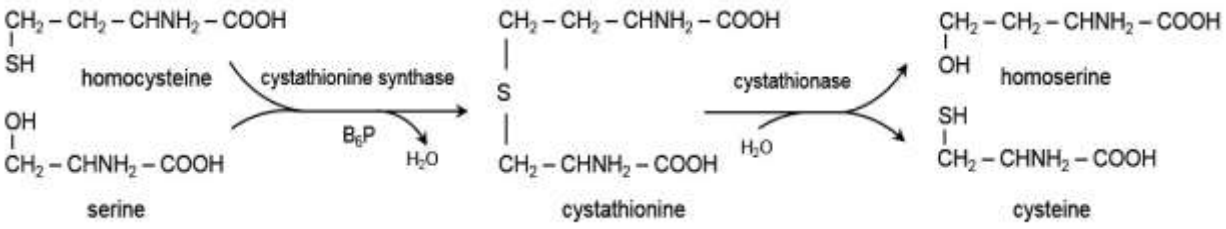
- Norepinephrine + (CH_3) → epinephrine.
- Guanidoacetate + (CH_3) → creatine.
- Ethanolamine + 3 (CH_3) → choline.
- Serotonin + acetyl + (CH_3) → melatonin.

Losing the methyl group, SAM becomes S-adenosyl homocysteine, which then splits to adenosine and homocysteine ($HS\text{-}CH_2\text{-}CH_2\text{-}CHNH_2\text{-}COO^-$). Homocysteine can accept a methyl group from N^5-methyl tetrahydrofolate (CH_3-THF) to regenerate methionine, a reaction catalyzed by methionine synthase (methyl transferase), which needs a vitamin B_{12} coenzyme.



Because of the role of methionine in methylation reactions and synthesis of phosphatidyl choline, methionine is considered a lipotropic factor. Lipotropic factors are those that help in prevention and treatment of fatty liver by helping the export of lipids from the liver in VLDL particles.

Homocysteine can condense with serine in the cystathionine pathway. By this pathway, the sulfur of methionine ends in cysteine, while the rest of cysteine molecule comes from serine. Methionine carbon skeleton goes in a glucogenic route.

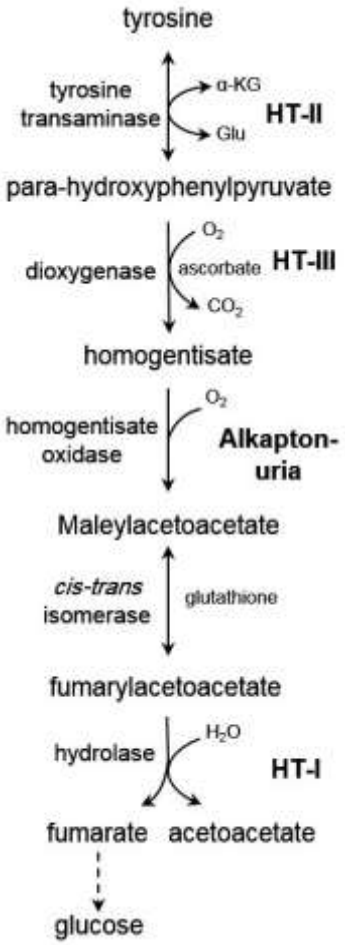


Inborn Errors of Aminoacid Metabolism

An alteration of a gene that encodes an enzyme is expected to result in deficiency of the reaction catalyzed by this enzyme. In reality, a defect of aminoacid metabolism may pass unnoticed or it may have a minor, a severe, or even a fatal consequence. We should remember that inborn errors of metabolism we see are only those that survived the intrauterine period, and pre-implantation before that. Because of the close relation of aminoacid metabolism to neuro-transmitters, it is not surprising that many of the inborn errors of aminoacid metabolism are associated with neurological manifestations and mental deficiency. Below are few of these abnormalities of aminoacid metabolism.

Alkaptonuria

This was the first inborn error of metabolism to be described and characterized. It involves an error of tyrosine catabolism. In alkaptonuria, there is a deficiency of homogentisate oxidase. This leads to accumulation of homogentisic acid, which passes in the urine (homogentisic aciduria). Homogentisic acid is oxidized by air and gives the black color characteristic of the disease. Apart from the black urine, ochronosis (black pigment in the cartilage) and arthritis later in life, alkaptonuria is not considered a serious disease.



Tyrosinemia

Hereditary tyrosinemia type I (HT-I) is caused by a deficiency of fumarylacetoacetate hydrolase. This leads to accumulation of toxic catabolites such as succinylacetone, resulting in liver and kidney failure, bone softening, and neurological crises. Affected children, if not treated, do not survive past the age of 10.

HT-II results from deficiency of tyrosine transaminase. This leads to photophobia, palmoplantar hyperkeratosis, and some degree of intellectual disability. HT-III results from insufficiency of hydroxyphenylpyruvate dioxygenase. Its characteristic features include intellectual disability, seizures, and intermittent ataxia. Transient tyrosinemia of newborns is most likely caused by vitamin C deficiency or immature liver enzymes.

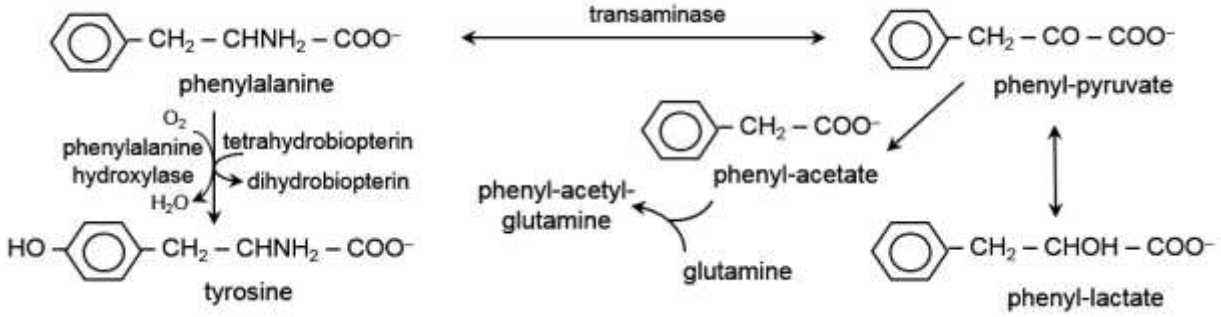
Treatment of HT is by diet control. Since 1992, patients with HT-I have been treated with nitisinone, which inhibits hydroxyphenylpyruvate dioxygenase. This pharmacological block is incomplete, with still a higher incidence of hepatocellular carcinoma.

Albinism

This is another abnormality of tyrosine metabolism. Various types of this disease are characterized by decreased production of melanin, whether in skin and hair or the eye. Melanin pigment is produced in melanocytes from tyrosine. The initial reaction for melanin synthesis is the hydroxylation of tyrosine to dopa, catalyzed by tyrosinase enzyme. This is a copper-containing enzyme, and it does not require tetrahydrobiopterin, unlike tyrosine hydroxylase of the catecholamine synthetic pathway. Therefore, the production of neurotransmitters is not affected in albinism. Albinism is an autosomal recessive disease, which appears in homozygous or double-heterozygous patients.

Phenylketonuria

This is a disorder of phenylalanine metabolism. Phenylalanine is normally hydroxylated to tyrosine by phenylalanine hydroxylase. Deficiency of this enzyme leads to accumulation of phenylalanine (phenylalaninemia) and its transamination producing phenyl-pyruvate, which passes in the urine giving the disease its name: phenylketonuria. Phenyl-pyruvate reduction product: phenyl-lactate, and decarboxylation product: phenyl-acetate pass in the urine too.



Phenylalaninemia interferes with the normal brain uptake of large neutral aminoacids such as tyrosine, tryptophan, and leucine. Disease manifestations include hypo-pigmentation and mental deficiency, which is the most serious consequence. Patients should be diagnosed as early as possible and treated before the age of one month; otherwise, a permanent brain damage takes place. Treatment is a low-phenylalanine diet for the whole childhood period. Tyrosine becomes an essential aminoacid in this condition. The artificial sweetener aspartame (aspartyl-phenylalanine methyl ester) should be avoided. Pregnant women with phenyl-alaninemia should have a controlled diet to prevent adverse effects on the fetus.

Phenylketonuria may be caused be a deficiency of tetrahydrobiopterin, the cofactor needed for phenylalanine hydroxylase. Deficiency of this cofactor synthesis or reduction affects not only the phenylalanine hydroxylase reaction, but also tyrosine hydroxylase and tryptophan hydroxylase. This leads to deficiency of the production of catecholamines and serotonin and melatonin as well. Therefore, the patient is to be supplied with L-dopa and 5-hydroxy-tryptophan.

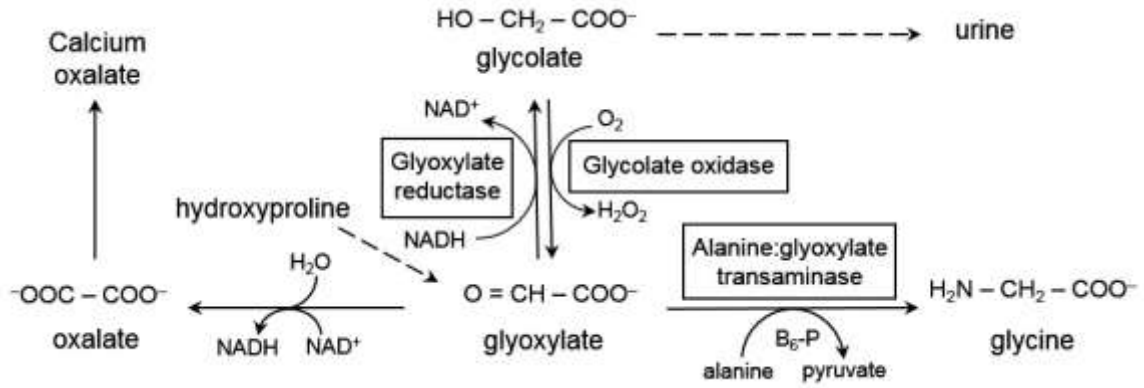
Maple syrup urine disease

This disease results from impaired metabolism of branched chain aminoacids: valine, leucine and isoleucine. The metabolism of these aminoacids normally starts by transamination yielding the corresponding branched chain α-ketoacids. The latter undergo oxidative de-carboxylation by α-ketoacid dehydrogenase complex, similar to pyruvate dehydrogenase reaction, needing the five coenzymes: TPP, lipoic acid, coenzyme A, FAD and NAD^+. Deficiency of branched chain α-ketoacid dehydrogenase leads to accumulation of branched chain aminoacids and their corresponding α-ketoacids, which pass in the urine giving it the smell of maple syrup or burnt sugar.

There are different variants of the disease varying in severity. It may lead to neurological manifestations and may be fatal at an early age. If the mutation affects the first enzyme subunit decreasing its affinity to TPP, the patient may benefit from large doses of thiamine. A concomitant lactic acidosis suggests that the mutation affects the third enzyme subunit, dihydrolipoyl dehydrogenase, common to pyruvate dehydrogenase and α-ketoglutarate dehydrogenase.

Primary hyperoxaluria

The equilibrium of the alanine:glyoxylate transamination reaction is in favor of glycine formation. Primary hyperoxaluria (type I) is a rare autosomal recessive genetic disease associated with deficiency of alanine:glyoxylate transaminase in the liver cells. This results in hyperoxaluria, not related to oxalate intake in diet, and glycolic aciduria.



The defect is in the liver, but the organ that suffers is the kidney. Calcium oxalate urinary stones and nephrocalcinosis are the main manifestations. The child usually dies of renal failure. The disease may not appear until adulthood. Calcium oxalate also deposits in different tissues (oxalosis). Treatment is low oxalate in food, plenty of fluids, vitamin B_6, and dialysis. Liver or combined liver and kidney transplantation may be life-saving.

Type II primary hyperoxaluria is a rarer disease associated with deficiency of the enzyme glyoxylate reductase in the liver. Other causes of primary hyperoxaluria remain to be discovered.

Hyperglycinemia

The glycine cleavage complex of liver mitochondria splits glycine to CO_2 and NH_3 and forms N^5,N^{10}-methylene tetrahydrofolate. In nonketotic hyperglycinemia, a rare inborn error of glycine degradation, glycine accumulates in all body tissues including the central nervous system.

Glycinuria

Glycinuria results from a defect in renal tubular reabsorption of glycine.

Hartnup disease

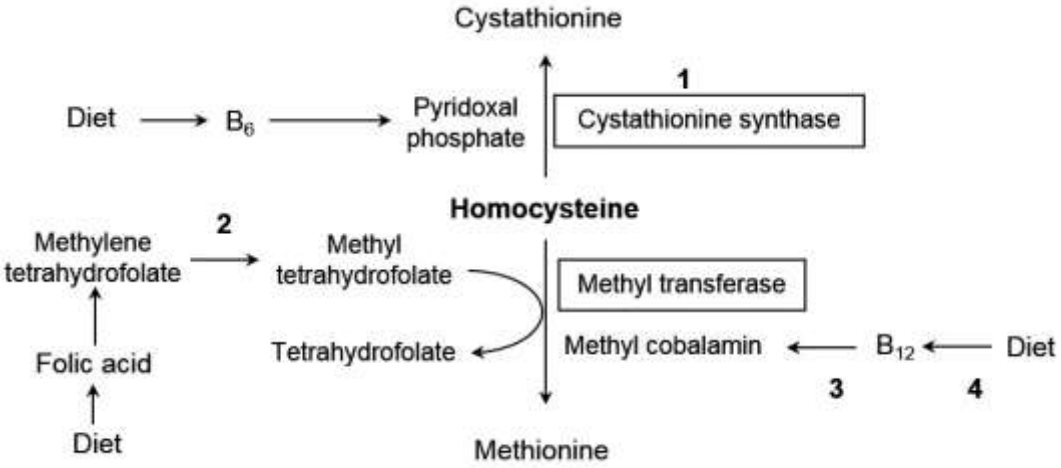
Hartnup disease is due to a defect of tryptophan absorption in the intestine and kidney. It is characterized by pellagra-like skin rash and neurological manifestations.

Cystinuria (Cystine-lysinuria)

This condition is due to defective renal transport of cystine, lysine, arginine, and ornithine (cystine is two cysteine molecules linked by S-S bond). Cystine deposits as urinary stones. Otherwise, the disease may pass unnoticed.

Hyperhomocysteinemia and homocystinuria

These result from deficient utilization of homocysteine. Accumulated homocysteine passes in the urine and is oxidized to homocystine (by formation of S-S bond). Normally, homocysteine undergoes methylation to regenerate methionine or condensation with serine to form cystathionine. As explained before, coenzymes derived from the vitamins B_6 and B_{12} and folic acid are required for this purpose. The figure below shows an outline for these pathways.



In this figure, possible defects are indicated by numbers:

1. Deficiency of cystathionine synthase.
2. Defective reduction of methylene tetrahydrofolate to methyl tetrahydrofolate.
3. Defective formation of the coenzyme from vitamin B_{12}.
4. Defect in absorption of vitamin B_{12} (pernicious anemia).

Increased homocysteine in the blood is an independent risk factor for the development of atherosclerosis and coronary heart disease. This is probably through formation of homocysteine thiolactone, which thiolates LDL particles. These altered particles are endocytosed by macrophages, which turn to foam cells and proceed in atherosclerosis process. The detrimental effects of homocysteine may be due to its binding to lysyl oxidase, responsible for proper maturation of the extracellular matrix proteins collagen and elastin. Production of defective collagen and elastin has a negative impact on arteries, bone and skin. Consequences of hyperhomocysteinemia include thromboses, cardiovascular disease, stroke, dislocation of the lens, osteoporosis and frequently mental retardation. Presence of megaloblastic anemia indicates a defect in the methyl transferase reaction. Type 1 patients may respond to vitamin B_6 therapy. Vitamin B_{12} is essential for treatment of type 4. Treatment includes a diet low in methionine.

Cystinosis

Cystinosis is a rare autosomal recessive genetic disease characterized by accumulation of cystine in lysosomes due to deficient production of its transporter protein. Cystine crystals in the cornea may be seen by slit lamp examination. Often child death results from kidney failure.

Study Questions

Choose one best answer for every question of the following:

21- What is the main end product of protein nitrogen catabolism?
(A) Uric acid.
(B) Free aminoacids.
(C) Urea.
(D) Creatinine.

22- Liver cell failure is characterized by rise of which of the following plasma analytes?
(A) Urea.
(B) Albumin.
(C) Ammonia.
(D) Creatine kinase.

23- Which of the following aminoacids is important for formation of choline from ethanolamine?
(A) Cysteine.
(B) Methionine.
(C) Threonine.
(D) Arginine.

24- Production of taurocholic acid requires which aminoacid?
A) Cysteine.
B) Serine.
(C) Methionine.
(D) Glycine.

25- The compound 5-hydroxyindoleacetate found in the urine results from metabolism of which aminoacid?
(A) Tryptophan.
(B) Tyrosine.
(C) Methionine.
(D) Isoleucine.

26- Methionine synthase needs which of the following?
(A) Thiamine and lipoic acid.
(B) Cobalamin and folic acid.
(C) Coenzyme A.
(D) Pyridoxine.

27- Phenylketonuria cases may show which of the following?
(A) Black urine.
(B) Low phenylalanine concentration in the blood.
(C) Dark hair and skin.
(D) Mental retardation.

28- Hyperhomocysteinemia may be treated with which of the following vitamins?
(A) Thiamine.
(B) Lipoic acid.
(C) Pyridoxine.
(D) Biotin.

29- Parents of a phenylketonuria patient may show high blood levels of
(A) phenylalanine.
(B) ketone bodies.
(C) tyrosine.
(D) tetrahydrobiopterin.

30- What is the non-essential aminoacid that becomes essential with treatment of phenylketonuria patients?
(A) Phenylalanine.
(B) Alanine.
(C) Tyrosine.
(D) Glutamine.

31- Phenylalanine hydroxylase is a mono-oxygenase (mixed-function oxygenase) that
(A) is a copper-containing metalloenzyme.
(B) oxidizes phenylalanine and reduces tetrahydrobiopterin.
(C) incorporates the two atoms of the oxygen molecule in one substrate molecule.
(D) needs a NADPH-dependent reductase to restore the active cofactor.

32- Treatment of hyperhomocysteinemia includes a diet low in
(A) cysteine.
(B) homocysteine.
(C) methionine.
(D) glycine.

33- What is to be supplied to a patient with dihydrobiopterin reductase deficiency?
(A) Tyrosine and tryptophan.
(B) Tyrosine and dopa.
(C) Dopa and 5-hydroxytryptophan.
(D) Dopamine and norepinephrine.

34- Maple syrup urine disease is characterized by
(A) deficiency of branched-chain aminoacid transaminase.
(B) low levels of branched-chain aminoacids and their ketoacid derivatives.
(C) burnt-sugar smell of urine.
(D) urine turning black in air.

35- In Hartnup disease, pellagra-like skin rash and neurological manifestations are due to
(A) increased tryptophan level in the blood.
(B) deficiency of niacin production.
(C) deficiency of vitamin B_6.
(D) increased production of 5-hydroxyindole acetate.

36- Which of the following cases of hyperhomocysteinemia may respond to treatment with vitamin B_6?
(A) pernicious anemia.
(B) cystathionine synthase deficiency.
(C) deficient reduction of methylene-tetrahydrofolate to methyl-tetrahydrofolate.
(D) deficient synthesis of methylcobalamin.

37- Albinism results from deficient
(A) hydroxylation of phenylalanine.
(B) hydroxylation of tyrosine.
(C) reduction of dihydrobiopterin.
(D) methylation of dopa.

38- A patient with nephrocalcinosis resulting from primary hyperoxaluria may be saved by
(A) high doses of glycine.
(B) fluid restriction.
(C) kidney transplantation.
(D) liver and kidney transplantation.

39- A case of maple syrup urine disease with lactic acidosis is probably due to defective dealing with
(A) thiamin pyrophosphate.
(B) coenzyme A.
(C) lipoic acid.
(D) lactic acid.

40- A case of dislocation of the lens, bone and vascular lesions, and no megaloblastic anemia is most probably due to defect of which of the following?
(A) Homocysteine methyl transferase.
(B) Cystathionine synthase.
(C) Dihydrofolate reductase.
(D) Intrinsic factor.

Match each clinical condition to the aminoacid involved:
(A) Tyrosine.
(B) Tryptophan.
(C) Methionine.
(D) Glycine.

41- Hartnup disease.
42- Pellagra.
43- Oxalate stones.
44- Pheochromocytoma.
45- Homocystinuria.
46- Carcinoid syndrome.
47- Alkaptonuria.

Vitamins and Coenzymes

Vitamins are organic compounds that are essential in diet, in minute quantities, though not incorporated in tissue structure, nor oxidized for energy production. Their deficiency causes specific disease. They are generally classified as water-soluble and fat-soluble vitamins. Water-soluble vitamins include vitamin C and members of vitamin B complex. Fat-soluble vitamins include vitamins A, D, E, and K.

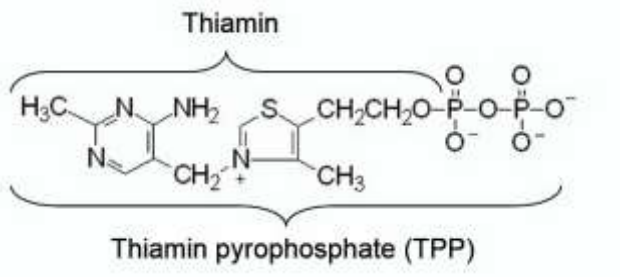
Fat-soluble vitamins need bile salts for absorption, and are transmitted in plasma lipoproteins. Their absorption is compromised with fat malabsorption states, e.g., biliary obstruction, pancreatitis, and abeta-lipoproteinemia. They are stored in tissue fat, and their over-intake can produce toxicity symptoms, except for vitamin E, which has no sure toxicity.

Water-soluble vitamins are readily absorbed, and readily excreted in urine. They are not stored, except for vitamin B_{12}, and produce no toxicity. They generally form cofactors for enzymes of intermediary metabolism.

The fat-soluble vitamins and vitamin C are discussed in this text in their related lectures. Below is a summary of the importance of vitamin B in nutrition and metabolism.

Thiamin

Thiamin (thiamine, vitamin B_1), as its name indicates, contains sulfur and nitrogen. It is the first vitamin to be identified, giving vitamins their name though not all are amines. It is the precursor of thiamin pyrophosphate (TPP). The latter is a cofactor for oxidative decarboxylation of α-ketoacids and the transketolase reaction of the HMP pathway.

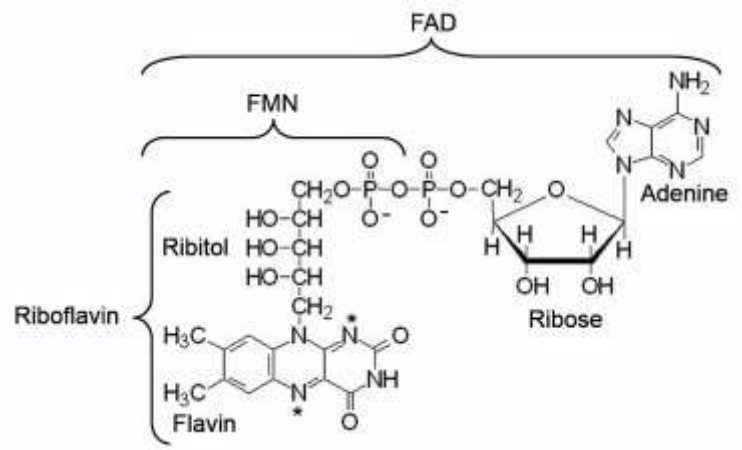


The dietary requirement for thiamin is proportional to the caloric intake of the diet and is about 1.0–1.5 mg/day for normal adults. An increase in thiamin intake will be required if the carbohydrate content of the diet is excessive. Thiamin is obtained from plant sources including whole grains, bran, green vegetables, nuts, and legumes, and yeast. Animal sources include organ meat (liver, kidney, etc.), eggs, and milk.

A deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy because of its role in pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase reactions. Thiamin deficiency can be diagnosed in the lab by increase in transketolase activity of red blood cells upon adding thiamin pyrophosphate.

The classical deficiency disease is beriberi, associated with living on white (polished) rice (carbohydrate rich and thiamin deficient). An additional thiamin deficiency related disease is known as Wernicke-Korsakoff syndrome. This disease is most commonly found in chronic alcoholics due to their poor dietetic lifestyles. It is characterized by acute encephalopathy followed by chronic impairment of short-term memory. Other neurological symptoms include ataxia, mental confusion and loss of eye coordination (nystagmus). Other clinical symptoms are related to cardiovascular and musculature defects, e.g., heart failure.

Riboflavin

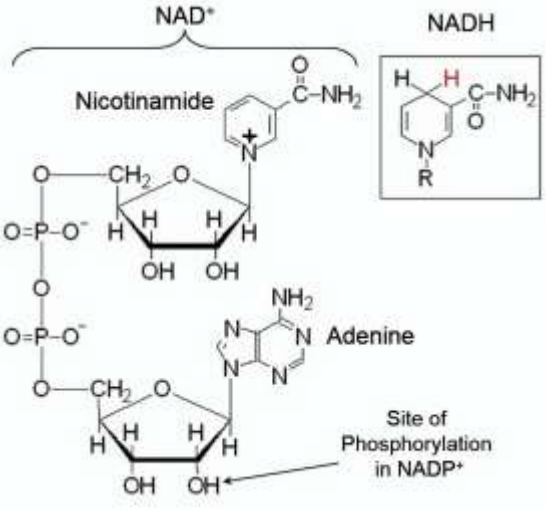


Riboflavin (vitamin B_2) is formed of ribitol and flavin. It is yellow in color and it is sensitive to light. It is the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metallo-flavoproteins. Both classes of enzymes are involved in a wide range of redox reactions, e.g. succinate dehydrogenase and xanthine oxidase. During the course of the enzymatic reactions involving the flavoproteins the reduced forms of FMN and FAD are formed, $FMNH_2$ and $FADH_2$, respectively. The hydrogens are carried on the starred nitrogen atoms in the figure.

The normal daily requirement for riboflavin is 1.2–1.7 mg/day for normal adults. The vitamin is present in eggs, milk, meat, and cereals.

Isolated deficiency of riboflavin is not usually seen. Deficiency is often seen in chronic alcoholics, and in infants treated with phototherapy for hyperbilirubinemia. Associated symptoms include itching and burning eyes, angular stomatitis and cheilosis (cracks and sores in the mouth and lips), glossitis (inflammation of the tongue leading to purplish discoloration), seborrhea (dandruff, flaking skin on scalp and face), trembling, sluggishness, and photophobia.

Niacin

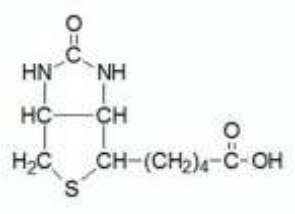


Niacin (nicotinic acid, vitamin B_3) and nicotinamide serve as the dietary source of the vitamin. They contain the pyridine ring. They are the precursor of coenzyme I and II: nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both coenzymes function as cofactors for numerous dehydrogenases involved in energy-producing pathways (NAD^+) as well as anabolic pathways (NADPH). They act as one electron and one hydrogen atom carriers. Nicotinic acid is used therapeutically as a plasma lipid lowering agent.

The recommended daily requirement for niacin is 13-19 mg. It is widely spread in plant and animal sources, like vitamin B_1. Niacin is not a true vitamin in the strictest definition since it can be derived from the amino acid tryptophan. However, only 1 mg of niacin can be synthesized from 60 mg of tryptophan. Vitamin B_6 is required in this process.

Niacin deficiency results from a multi-vitamin and protein deficient diet. Living on corn flour bread used to be a cause of niacin deficiency since maize (corn) is deficient in niacin and tryptophan. Niacin deficiency can result from defective absorption, e.g., Hartnup disease, or shifting of tryptophan to serotonin synthesis in carcinoid syndrome. Niacin deficiency leads to the manifestations of pellagra: diarrhea, dermatitis, and dementia.

Biotin

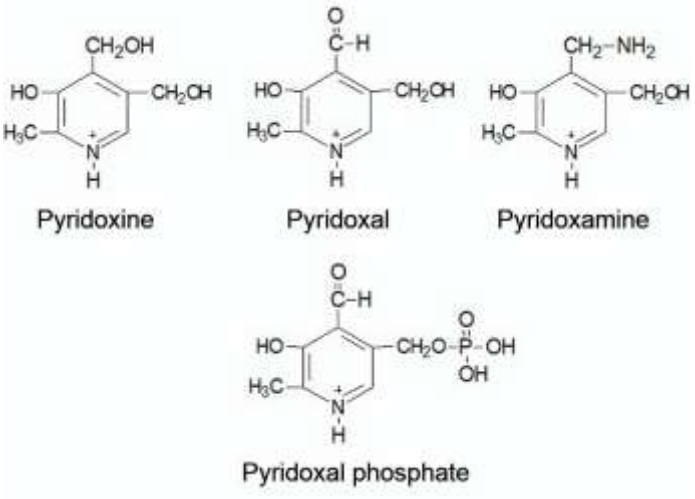


Biotin (vitamin H, vitamin B_7) is the cofactor required for carboxylase enzymes (co-carboxylase), e.g., acetyl-CoA carboxylase (for lipogenesis), pyruvate carboxylase (providing oxaloacetate for citrate cycle and gluconeogenesis), and propionyl CoA carboxylase.

Biotin is found in numerous foods and is also synthesized by intestinal bacteria, and as such, deficiencies of the vitamin are rare. Deficiencies are generally seen only after long antibiotic therapies, which deplete the intestinal flora or following habitual intake of raw eggs. An egg white protein, avidin, has a high affinity to biotin, which prevents intestinal absorption of biotin. Deficiency of biotinidase, which is required to remove covalently linked biotin from diet proteins, can also lead to biotin deficiency.

Biotin deficiency results in fasting hypoglycemia due to defective gluconeogenesis. Ketosis follows the failure of oxidation of acetyl CoA from fatty acids in citric acid cycle. Accumulation of pyruvate leads to lactic acidosis. Deficient carboxylation reactions also lead to deficient fatty acid synthesis and deficient metabolism of propionate.

Vitamin B_6



Pyridoxine, pyridoxal, and pyridoxamine are collectively known as vitamin B_6. All three compounds are efficiently converted to the biologically active form of vitamin B_6, pyridoxal phosphate (PLP). This conversion is catalyzed by pyridoxal kinase, a zinc metalloenzyme.

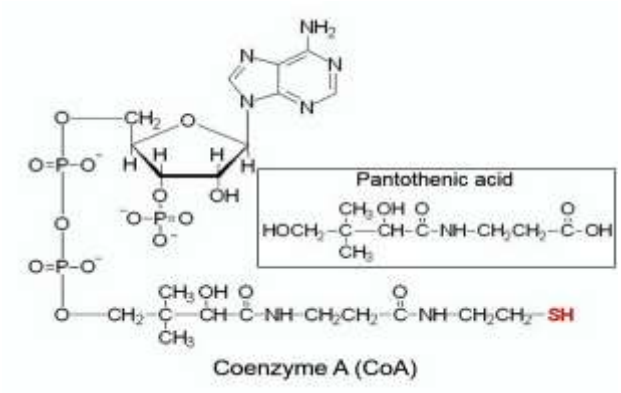
Pyridoxal phosphate functions as a cofactor for transamination reactions (co-transaminase), serine dehydratase, which converts serine to pyruvate, and aminoacid decarboxylases, which are responsible for the production of a number of hormones and neurotransmitters. It is also required for production of niacin from tryptophan, for the key reaction of heme synthesis, delta-aminolevulinic acid synthase, and for synthesis of sphingosine from serine and palmitoyl CoA. It is also a cofactor for glycogen phosphorylase, as it is necessary for stabilization of the enzyme.

The requirement for vitamin B_6 in the diet is proportional to protein consumption, about 1.4-2.0 mg/day for a normal adult. During pregnancy and lactation, the requirement for vitamin B_6 increases by approximately 0.6 mg/day. Sources of the vitamin are widespread in food like other B complex vitamins.

Deficiencies of vitamin B_6 are rare and are usually related to an overall deficiency of all the B-complex vitamins. Isoniazid (anti-tuberculosis drug) and penicillamine (used to treat rheumatoid arthritis and Wilson's disease) complex with pyridoxal and PLP resulting in a deficiency of this vitamin. Deficiencies of pyridoxal kinase result in reduced synthesis of PLP. Neurological manifestations of the cofactor deficiency (irritability, peripheral neuropathy and convulsions) are related to a reduction in the synthesis of GABA and sphingolipids. Other manifestations may be related to defective niacin production (pellagra) and heme synthesis (sideroblastic anemia).

Pantothenic Acid

Pantothenic acid (vitamin B_5) is required for synthesis of coenzyme A (CoA) and is a component of the acyl carrier protein (ACP) domain of fatty acid synthase. It is, therefore, required for the metabolism of carbohydrate, fats and proteins. At least 70 enzymes have been identified as requiring CoA or ACP derivatives for their function.



Pantothenic acid has a widespread distribution in whole grain cereals, legumes and meat. No recommended daily allowance has been established for this vitamin. Deficiency of pantothenic acid is extremely rare and symptoms specific to pantothenate deficiency are difficult to assess.

Study Questions

Choose one best answer for every question of the following:

1- Pellagra may be attributed to deficiency of which of the following?
(A) Vitamin A.
(B) Iron and copper.
(C) High biological value protein.
(D) Thiamin.

2- Which of the following are needed for synthesis of cofactors of dehydrogenase enzymes?
(A) Folic acid and cobalamin.
(B) Niacin and riboflavin.
(C) Pyridoxine and pyridoxamine.
(D) Retinol and retinoic acid.

3- Which of the following enzyme activity is an indicator of thiamin nutritional status?
(A) Erythrocyte transketolase.
(B) Liver lactate dehydrogenase.
(C) Muscle creatine kinase.
(D) Fat cell lipase.

4- Hydrolysis of coenzyme I (NAD^+) gives
(A) nicotinic acid, adenine, and phosphate.
(B) nicotinic acid, adenine, ribose, and phosphate.
(C) nicotinamide, adenine, two ribose molecules and two phosphates.
(D) nicotinamide, adenine, two ribose molecules and three phosphates.

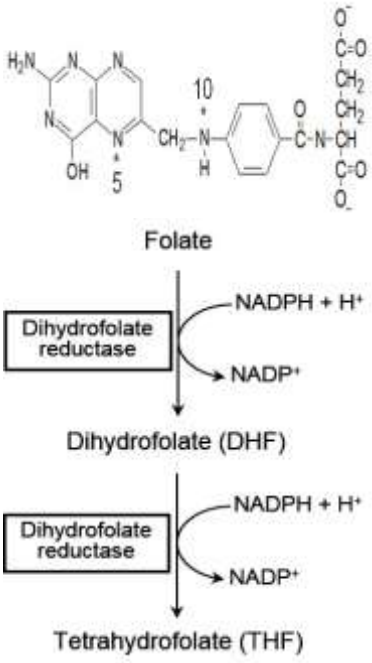
5- NADPH is a coenzyme for
(A) citrate synthase.
(B) fatty acid synthase.
(C) pyruvate dehydrogenase.
(D) fatty acyl CoA dehydrogenase.

6- The zinc metalloenzyme: pyridoxal kinase converts vitamin B_6 to which coenzyme?
(A) Cotransaminase.
(B) Coenzyme A.
(C) Coenzyme I.
(D) Coenzyme II.

7- Pyridoxal phosphate acts as a coenzyme by
(A) forming Schiff bases with aminoacids.
(B) carrying two electrons in oxidation-reduction reactions.
(C) changing at the end of the reaction to balance the change in substrate.
(D) providing energy for the reaction.

Folic Acid

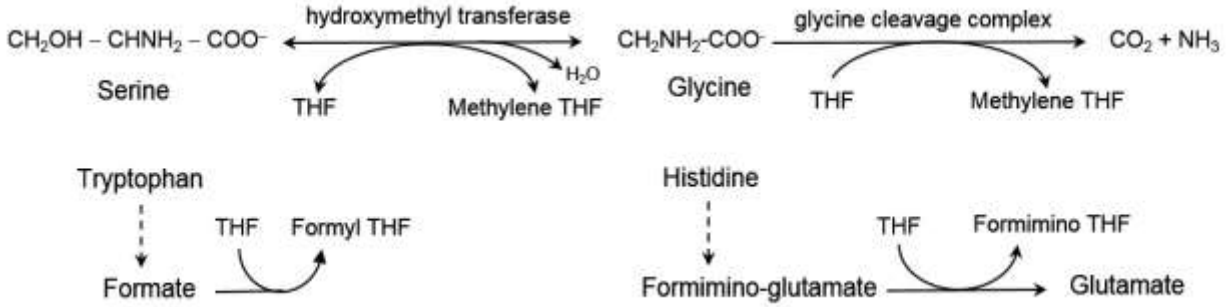
Folic acid (folacin, vitamin B_9) is a conjugated molecule consisting of a pteridine ring structure linked to para-amino-benzoic acid (PABA), conjugated with glutamic acid. Folic acid is obtained primarily from leafy vegetables (hence its name) and yeasts as well as animal liver. Animals cannot synthesize folic acid, thus, folate is required in the diet.



When stored in the liver or ingested, folic acid exists in a polyglutamate form. Intestinal mucosal cells conjugase enzyme removes some of the glutamate residues. Folate becomes less negatively charged and therefore more capable of passing through the basal laminal membrane of the epithelial cells of the intestine and into the bloodstream. Folic acid is reduced within cells (principally the liver where it is stored) to tetrahydrofolate (THF) through the action of dihydrofolate reductase, a NADPH-requiring enzyme.

The function of THF is to carry and transfer one-carbon units during biosynthetic reactions. The one-carbon units are methyl (-CH_3), methylene (-CH_2-), methenyl (-CH=), formyl (-CH=O) or formimino (-CH=NH) groups. The N^5 position is the site of attachment of methyl groups, N^{10} is for formyl and formimino groups and both N^5 and N^{10} are bridged by the methylene or methenyl groups. Inter conversion of these groups is possible except for the methyl group, which once formed by reduction of methylene cannot be oxidized back.

The source of the one carbon unit is primarily serine, in addition to other aminoacids: glycine, histidine, and tryptophan.



Serine hydroxymethyl transferase is a bidirectional enzyme and can be used for serine synthesis from glycine and methylene tetrahydrofolate.

The most important function of the one carbon group carried on tetrahydrofolate is the synthesis of purine nucleotides (carbons 2 and 8 of the purine ring) by formyl and methenyl THF, and the methylation of deoxyuridylate to thymidylate for DNA synthesis by methylene THF (nucleotide metabolism, lecture 28).

Methyl tetrahydrofolate is important for the methylation of homocysteine by methionine synthase (methyl transferase), liberating tetrahydrofolate and regenerating methionine used in transmethylation reactions (aminoacid metabolism, lecture 14).

Folic acid requirement is about 300-400 µg/day. Folate deficiencies are rare due to the adequate presence of folate in food. Poor dietary habits as those of chronic alcoholics can lead to folate deficiency. The predominant causes of folate deficiency in non-alcoholics are impaired absorption or metabolism or an increased demand for the vitamin. The predominant condition requiring an increase in the daily intake of folate is pregnancy. The need for folate nearly doubles by the third trimester of pregnancy. Therefore, it is a good routine practice to give pregnant women folic acid supplement. Certain drugs such as anticonvulsants and oral contraceptives can impair the absorption of folate. Anticonvulsants also increase the rate of folate metabolism.

Folic acid deficiency leads to impaired synthesis of purine nucleotides and thymidylate needed for DNA synthesis. This results in megaloblastic anemia, especially with increased requirement like during pregnancy. Folate deficiency also leads to defective methionine-dependent transmethylation needed for formation of choline of myelin phospholipids. Fetal anomalies, e.g., neural tube defect (spina bifida) may develop due to folate deficiency during pregnancy.

Methylation of homocysteine is the only reaction for methyl THF, which cannot be oxidized back to methylene THF. Since vitamin B_{12} is essential for this reaction, deficiency of B_{12} leads to trapping of THF in the methylated form, with deficiency of tetrahydrofolate needed for purine nucleotide and thymidylate synthesis, leading to megaloblastic anemia.

DNA synthesis can be therapeutically inhibited by folic acid analogues such as methotrexate, which inhibits the enzyme dihydrofolate reductase responsible for the activation of folic acid. In bacteria, folic acid is not a vitamin. It is synthesized by the bacterial cell. Sulfonamides are structural analogues of para-aminobenzoic acid (PABA), a component of folic acid molecule. Therefore, sulfonamides inhibit the synthesis of folic acid and are used for bacterial chemotherapy. The bacterial chemotherapeutic trimethoprim inhibits dihydrofolate reductase. A combination of the two chemotherapeutics has a bactericidal action.

Folinic acid (leukovorin, citrovorum factor, 5-formyl tetrahydrofolate) is used as an adjuvant in chemotherapy. It ameliorates the action of methotrexate, and potentiates the action of 5-fluorouracil.

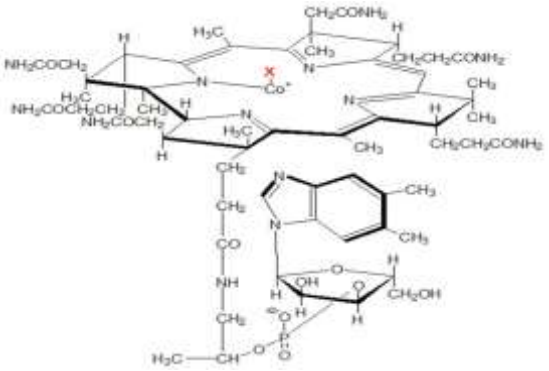
Study Questions

Choose one best answer for every question of the following:

8- Which of the following is the best timing of folic acid supplementation in pregnancy?
(A) Starting before pregnancy.
(B) Starting from the end of second trimester.
(C) When plasma folate concentration falls down.
(D) When erythrocyte folate concentration is low.

9- How can methylene THF give a methyl group to deoxyuridylate to form thymidylate?
(A) Reducing methylene to methyl and oxidizing tetrahydrofolate to dihydrofolate.
(B) Reducing one methylene to methyl and oxidizing another to methenyl group.
(C) Reducing methylene to methyl and oxidizing the deoxyribose moiety of deoxyuridylate.
(D) Participation of S-adenosyl methionine as a methyl donor.

Cobalamin



Cobalamin (vitamin B_{12}) has a complex structure. It contains a tetrapyrrole ring with a cobalt ion in the center. Attached to the cobalt ion is a hydroxyl group (hydroxycobalamin), a methyl group (methylcobalamin), or 5'-deoxyadenosine (5'-deoxyadenosylcobalamin). Cyanocobalamin is a form that is used therapeutically. The vitamin has a characteristic red color.

Vitamin B_{12} is synthesized exclusively by microorganisms and is found in the liver of animals bound to protein as methylcobalamin or 5'-deoxyadenosylcobalamin. The vitamin is hydrolyzed from protein by gastric acid or trypsin digestion following consumption of animal meat. The vitamin is then bound by intrinsic factor, a protein secreted by parietal cells of the stomach, and carried to the ileum where it is absorbed. Following absorption, the vitamin is transported, bound to blood transcobalamin II, to the liver.

There are only two clinically significant reactions in the body that require vitamin B_{12} as a cofactor. The first is methylmalonyl CoA mutase important in metabolism of propionyl CoA, which results from catabolism of odd chain fatty acids, and the aminoacids: valine, isoleucine, threonine, and methionine. It converts methylmalonyl CoA to succinyl-CoA. The 5'-deoxy-adenosine derivative of cobalamin is required for this reaction. The second reaction is the conversion of homocysteine to methionine, catalyzed by methionine synthase (methyl transferase). In this reaction, a methyl group is transferred from N^5-methyltetrahydrofolate to hydroxycobalamin, generating methylcobalamin as an intermediate.

The recommended daily allowance of vitamin B_{12} is 2.4 µg. The vitamin is present in food of animal origin, especially liver, and also meat and eggs. The liver can store up to six-year worth of vitamin B_{12}, hence deficiencies of this vitamin are rare. Pernicious anemia develops because of a lack of intrinsic factor in the stomach leading to malabsorption of the vitamin. It can follow gastrectomy. The vitamin deficiency can also occur in long-term vegans.

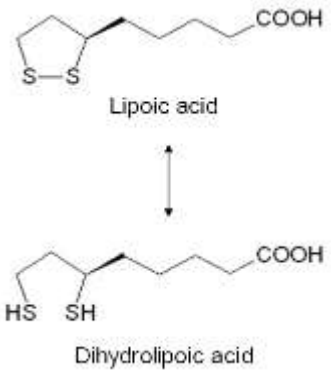
Patients with pernicious anemia used to be treated with lifelong intramuscular vitamin B_{12} replacement. However, oral vitamin B_{12} at a high dose (1000 µg daily) was found comparable to the injectable vitamin in efficacy, but easier and cheaper. Absorption in this case is by mass action (passive diffusion).

When vitamin B_{12} is deficient essentially all of the folate becomes trapped as the N^5-methyl THF derivative as a result of the loss of functional methionine synthase. This trapping prevents the synthesis of other THF derivatives required for the purine and thymidine nucleotide biosynthesis pathways, which leads to megaloblastic anemia.

Neurological complications also are associated with vitamin B_{12} deficiency and result from a progressive demyelination of nerve cells. The demyelination may be due to lack of choline formation as a result of S-adenosyl methionine deficiency, or increase in methylmalonyl CoA, which competes with malonyl CoA in fatty acid biosynthesis. Methylmalonyl CoA can inhibit fatty acid synthesis or produce branched-chain fatty acids that may severely alter the architecture of the normal membrane structure of nerve cells. Treating megaloblastic anemia with folic acid alone can mask, and lead to a missed diagnosis of, vitamin B_{12} deficiency.

Deficiencies of vitamin B_{12} can also lead to elevations in circulating homocysteine, known to lead to cardiovascular dysfunction and a negative impact on arteries, bone and skin.

Lipoic Acid



Alpha-lipoic acid (LA) is a dithiol compound, enzymatically synthesized in the mitochondria from the medium-chain fatty acid octanoic acid. Because LA can be synthesized in the body it is not technically considered a vitamin but because of its vital role in overall cellular metabolism it is considered as an important, but not necessary, dietary supplement.

Enzymes containing lipoamide are typically mitochondrial multi-enzyme complexes that catalyze the oxidative decarboxylation of α-keto acids and glycine cleavage. Lipoic acid serves a critical role in mitochondrial energy metabolism. It is an essential cofactor for the E2 component of α-ketoacid dehydrogenase complexes, exclusively located in the mitochondria. These include the pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KGDH), and branched-chain α-keto-acid dehydrogenase (BCKDH) complexes.

LA supplements may not be used as a metabolic cofactor but instead, elicit a unique set of biochemical activities with potential therapeutic value against a host of pathophysiological insults. LA has been described as a potent biological antioxidant, a detoxification agent, e.g., for arsenic, and a diabetes medicine. It has been used to improve age-associated cardio-vascular, cognitive, and neuromuscular deficits, and has been implicated as a modulator of various inflammatory signaling pathways.

LA may have therapeutic usefulness in lowering blood glucose levels and modulation of insulin resistance. Lipoic acid has been shown to stimulate glucose uptake by affecting components of the insulin signaling pathway. In addition, LA stimulates glucose uptake upon translocation and regulation of the intrinsic activity of the glucose transporter, GLUT4.

Vitamin B_{17} - amygdalin - laetrile

There is no vitamin B_{17}. It is a misnomer of amygdalin, a cyanogenic glycoside in the kernels of bitter almonds, apricots, peaches, and plums, and apples seeds, or its derivative "laetrile". It is claimed to selectively kill cancer cells by the released hydrogen cyanide, a claim with no solid proof. Cyanide poisoning may result upon overconsumption.

Study Questions

Choose one best answer for every question of the following:

10- Megaloblastic anemia seen with vitamin B_{12} deficiency is probably due to which of the following?
(A) Participation of vitamin B_{12} in normal erythropoiesis.
(B) Functional deficiency of folic acid.
(C) Deficiency of homocysteine.
(D) Concomitant deficiency of iron absorption.

11- Absorption of vitamin B_{12} may <u>not</u> require
(A) bile salts.
(B) intrinsic factor.
(C) intestinal receptors.
(D) healthy gastric mucosa.

Minerals

The term "minerals" is intended to describe ions not actual minerals. There are both quantity elements required by the body and trace elements. The quantity elements are sodium, potassium, calcium, magnesium, phosphorous, chlorine, and sulfur. The essential trace elements are iron, copper, manganese, zinc, cobalt, nickel, selenium, iodine, and molybdenum. The terminology of "trace" relates to the fact that these minerals are effective and necessary in only minute concentration. Additional trace elements (although not considered essential) are boron, chromium, fluoride, and silicon. The functions of the "minerals" are numerous and either quite broad or highly specific.

Sodium, potassium, and chlorine

Sodium is the major extracellular cation. Na^+/K^+-ATPases function primarily in maintaining transmembrane potential and the transmission of nerve impulses. Potassium is the major intracellular cation. Chlorine (as chloride ion) is important to maintain the electric balance of the tissues. It is used in the production of hydrochloric acid in the stomach.

Calcium, phosphorus and magnesium

As discussed in lecture 7, calcium is required for bone mineralization, cardiac function, muscle contraction, and secretory processes. In addition, calcium is necessary for blood coagulation. Phosphorous is an important buffer in the blood in the form of phosphate ion: $H_2PO_4^-/HPO_4^{2-}$. Phosphate is also required for bone mineralization, and is necessary for energy utilization and formation of phosphate-containing compounds. Magnesium is required for bone mineralization as well as for the proper functioning of ATP. In this latter function, essentially all of ATP in the cell has magnesium bound to the phosphates. This magnesium-ATP complex allows ATP to more readily release the terminal phosphate to provide energy for cellular metabolism.

Sulfur

Sulfur is taken primarily in proteins. It has a primary function in amino acid metabolism but is also necessary for glycosaminoglycans, sulfolipids, and conjugation reactions.

Manganese

Manganese is involved in reactions of protein and fat metabolism, promotes a healthy nervous system, necessary for digestive function, bone growth, and immune function. In addition, manganese is necessary for manganese-superoxide dismutase (mitochondrial SOD).

Zinc

Zinc is found as a co-factor in over 300 different enzymes and thus is involved in a wide variety of biochemical processes. Zinc interacts with the hormone insulin to ensure proper function and thus this trace mineral has an important role in regulation of blood glucose level via insulin action. Zinc also promotes wound healing, regulates immune function, serves as a co-factor for numerous antioxidant enzymes, and is necessary for protein synthesis and the processing of collagen.

Iodine

Iodine is required for the synthesis of the thyroid hormones, as discussed in lecture 9.

Molybdenum

Molybdenum is primarily involved as a co-factor in several oxidases such as xanthine oxidase, aldehyde oxidase, and sulfite oxidase.

Selenium

Selenium is incorporated, in the form of selenocysteine, in glutathione peroxidases. Therefore, it is classified as an antioxidant. Other selenoproteins are also identified including iodothyronine deiodinases, involved in activation and inactivation of thyroid hormones, and thioredoxin reductases.

Selenium is needed for the proper functioning of the immune system and sperm motility and may reduce the risk of miscarriage. Deficiency has been linked to adverse mood states. An elevated selenium intake may be associated with reduced cancer risk.

Selenium is found in the environment in the soil. It most often occurs in combination with proteins, thus products with high protein content typically have a higher selenium content. These products include meat, fish, offal, and cereals. Extremely high levels are found in Brazil nuts and mushrooms. Onion and garlic too are a good source of selenium.

Deficiency of selenium mainly results from insufficient supply in the diet. It was confirmed in geographical regions where soils are characterized by low contents of this element. Selenium deficiency leads primarily to degeneration of many organs and tissues, resulting from decreased expression of selenoproteins. Moderate deficiencies may have a negative impacts on human health, for example: increasing the risk of infertility in men, prostate cancer, nephropathy, or the risk of the occurrence of neurological diseases. In addition, selenium deficiency causes a dilated cardiomyopathy (Keshan disease) and endemic osteoarthropathy (Kashin-Beck disease).

Dietary supplements contain inorganic selenium, usually selenite, or organic forms, e.g., selenomethionine. The recommended dietary allowance is 55 µg/day with a tolerable upper intake limit of 400 µg/day. Symptoms of selenium toxicity include diarrhea, nausea, vomiting, joint pain, nail discoloration, brittleness, and loss, hair loss, fatigue, irritability, foul breath odor (often described as “garlic breath”), and fever.

Copper

Copper is required for several oxidoreductases including: cytochrome oxidase (for energy production), tyrosinase (for melanin synthesis), dopamine β-hydroxylase (for noradrenaline synthesis), Cu-Zn superoxide dismutase (antioxidant), ceruloplasmin ferroxidase activity (for iron metabolism), and lysyl oxidase (for cross-linking of collagen and elastin).

Copper is absorbed from the stomach and intestine, transported on albumin to the liver, which exports it to the plasma mainly on ceruloplasmin. Copper deficiency may be under-diagnosed, giving structural, neurological, hematological, immunological and hair and skin manifestations. Deficiency may be due to deviation from a balanced diet. Copper absorption from the stomach and intestine may decrease in presence of high concentrations of other trace elements. Therefore, it is advised to give copper along with iron supplements.

Menkes disease is a rare X-linked fatal hereditary disorder, in which copper is sequestered in intestinal cells leading to copper deficiency. Wilson’s disease is a rare autosomal (chromosome 13) hereditary disorder resulting from different mutations impairing the function of the Wilson copper ATPase. These genetic mutations produce copper toxicosis due to excess copper accumulation, predominantly in the liver and brain (hepato-lenticular degeneration) and, to a lesser extent, in kidneys, eyes, and other organs. There is failure to excrete copper in the bile and to incorporate it in ceruloplasmin. This disease is treatable. Penicillamine chelates copper and promotes its excretion. Zinc induces metallothionein, which binds copper in intestinal mucosal cells until they slough off and are eliminated in the feces, and it competes with copper for absorption in the intestine by DMT1 (Divalent Metal transporter 1).

Iron

Iron is considered a dietary trace element. The total body iron is about 3-5 g, mostly (about 75%) in hemoglobin. Iron is present in other heme-proteins like myoglobin, cytochromes, and peroxidases. Iron is also found in non-heme proteins like iron-sulfur proteins of the respiratory chain, the iron transport protein transferrin, and the iron storage protein ferritin.

Body iron is highly conserved. Iron losses are minor, only 1-2 mg/day in menstrual blood and shed epithelial cells. Therefore, iron is required as a trace element of the diet, 10-20 mg/day, of which only about 10% is absorbed.

The main dietary source is meat, especially organ meat (liver, spleen), which contains heme iron. Other sources include egg yolk, dates, molasses, nuts, and vegetables.

Iron is absorbed in the duodenum. To be absorbed it should be in the reduced, ferrous (Fe^{2+}) state. Stomach hydrochloric acid and ascorbic acid help in dissolving and reducing the ferric (Fe^{3+}) iron. A ferrireductase enzyme is present on the surface of duodenal cells. Ferrous ions are then absorbed via the divalent metal transporter DMT1. Heme is absorbed intact via the heme carrier protein HCP1. Inside the enterocytes, iron is released from heme by heme oxygenase enzyme. Ferrous ions are oxidized to ferric and bound to ferritin. Iron is transported across the basolateral membrane of enterocytes into the circulation through the action of the transport protein ferroportin. Once in the circulation, ferric form of iron is bound to transferrin and passes through the portal circulation.

Body iron is regulated at the level of absorption. Gastric hydrochloric acid and vitamin C help iron absorption. Some molecules in food form insoluble complexes with iron and prevent its absorption, e.g., tannic acid (in tea), phytate (inositol hexaphosphate), oxalate, phosphate, and carbonate. Heme is taken intact by enterocytes and is not affected by these agents. Iron is either stored in enterocytes as ferritin or passed to the circulation. A hepatic regulatory protein: hepcidin has been discovered, which controls the expression of iron transporters.

Transferrin transports iron in the plasma. It can bind two moles of ferric iron. It is helped by the ferroxidase activity of the plasma ceruloplasmin that keeps iron in the ferric form. Iron released from heme by heme oxygenase in the reticuloendothelial system is also oxidized and carried on transferrin. Transferrin is taken by body cells, e.g., bone marrow cells via receptor-mediated endocytosis. Iron is released in the acidic endosomes, and transferrin receptors are recycled. To be incorporated in heme, iron should be reduced to the ferrous state.

It is to be noted here that ferrous iron generates free radicals by reacting with peroxides (Fenton reaction), hence its potential toxicity. The combined action of transferrin and ceruloplasmin guard against iron toxicity. These two proteins can be considered as antioxidants.

Ferritin is the labile store of iron. Apoferritin is a large polymer of 24 polypeptide subunits. It is able to bind up to 2,000 iron atoms in the form of ferric-phosphate. The majority of intracellular stored iron is found in the liver, skeletal muscle, and reticuloendothelial cells. Plasma ferritin is important for investigation of anemia. A high plasma ferritin excludes iron deficiency as a cause of anemia.

Hemosiderin is a histological amorphous iron deposit. If the storage capacity of ferritin is exceeded, iron will deposit adjacent to the ferritin-iron complexes in the cell. Hemosiderin is composed of ferritin, denatured ferritin, and other materials; its molecular structure is poorly defined. The iron present in hemosiderin is not readily available to the cell, thus hemosiderin cannot supply iron to the cell when it is needed. Hemosiderin is found most frequently in macrophages and is most abundant following hemorrhagic events.

Iron deficiency anemia is the most common type of anemia. Iron deficiency results from increased loss in menstrual flow or gastrointestinal bleeding. It may result from poor intake with increased demand like in children or pregnant women. Serum iron and plasma ferritin are low, while plasma iron binding capacity is high, in contrast to hemolytic anemia. The cause of iron deficiency should be adequately treated. Oral iron supplementation should be in a soluble, easily absorbed form like ferrous sulfate or ferrous gluconate. Iron injection is to be used if oral iron is not tolerated, with intestinal inflammation, or to compensate for a large deficit of iron store. It is to be noted that correction of anemia by iron supplementation needs time and is not achieved promptly.

Iron overload (hemochromatosis) is seen in patients undergoing repeated blood transfusion, e.g., thalassemia patients. Primary hemochromatosis is an autosomal recessive hereditary disorder characterized by increased intestinal absorption of iron due to loss of normal regulation. There is saturation of iron-binding proteins and deposition of hemosiderin in the tissues. The primary affected tissues are the liver, pancreas and skin. Iron deposition in the liver leads to cirrhosis and in the pancreas causes diabetes. The excess iron deposition leads to bronze pigmentation of the organs and skin. The condition is termed bronze diabetes.

Study Questions

Choose one best answer for every question of the following:

1- Selenium is needed for which of the following?
(A) Glutathione peroxidase.
(B) Glucuronyl transferase.
(C) Tyrosinase.
(D) Lactate dehydrogenase.

2- Selenocysteine is formed by
(A) reaction of selenium with cysteine.
(B) reaction of selenium with a protein.
(C) modification of a serine-tRNA.
(D) hydrolysis of selenium-containing food components.

3- Zinc-dependent enzymes include
(A) superoxide dismutase.
(B) RNA and DNA polymerases.
(C) carbonic anhydrase and alkaline phosphatase.
(D) all the above.

4- How would you manage a 30-year-old man presenting with iron deficiency anemia?
(A) Oral iron supplementation.
(B) Iron injection.
(C) Blood transfusion.
(D) Test for blood in stool.

5- Which of the following findings indicates iron deficiency anemia rather than hemolytic anemia?
(A) Low blood hemoglobin.
(B) High serum iron and ferritin.
(C) High plasma iron binding capacity.
(D) High urinary urobilinogen.

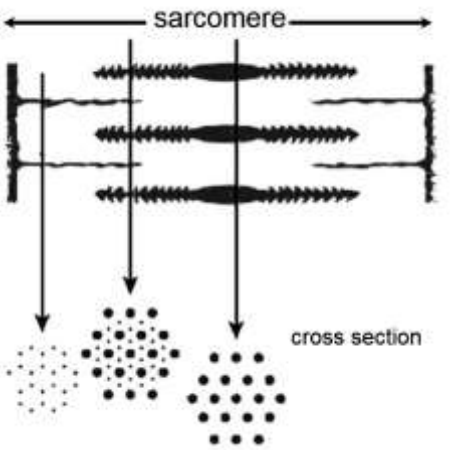
6- High intracellular iron triggers which of the following effects?
(A) increased ferritin.
(B) decreased ferritin.
(C) decreased hemosiderin.
(D) increased transferrin receptor.

7- Iron overload may result from:
(A) Thalassemia.
(B) Lead poisoning.
(C) Oxidant stress.
(D) Vitamin C deficiency.

Muscle Metabolism

Muscle proteins

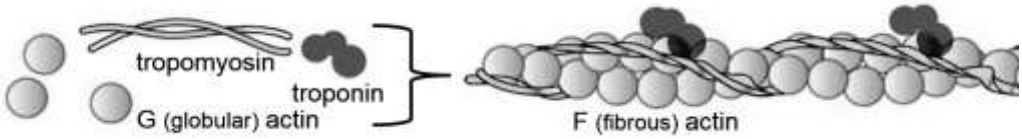
About 40 percent of the body weight of a healthy human adult is muscle. The mass of a muscle is made up of 75% water and more than 20% protein. Proteins play an important role in movement at both the organ, e.g., skeletal muscle, heart, and gut and cellular levels.



The two major proteins are actin and myosin. These major proteins are arranged in the sarcomere, the basic functional unit of the myofibril, in a way that allows for shortening and elongation of the sarcomere by sliding of thick and thin filaments against each other. The myofibrils are regularly arranged inside the muscle fiber to give the horizontal banding that characterizes the striated, skeletal and cardiac, muscles.

The thick filament contains chiefly myosin, a hexameric protein with a molecular mass of about 460 kDa, formed of one pair of heavy chains each of approximately 200 kDA molecular mass, and two pairs of light chains each with a molecular mass of approximately 20 kDa. Myosin has a fibrous tail consisting of two intertwined helices. Each helix has a globular head portion; these project as a flexible cross bridge that interacts with actin.

The thin filament contains the proteins actin, tropomyosin, and troponin. Troponin T (TnT) interacts with tropomyosin. Inhibitory troponin (TnI) inhibits the interaction of myosin and actin. Troponin C (TnC) is a calmodulin-like protein that can bind four calcium ions.



Muscle contraction is controlled by calcium ions. Sarcoplasmic calcium concentration in relaxing fibers is less than 10^{-7} M (plasma calcium $\approx$ 2.5 M). Depolarization leads to increased sarcoplasmic calcium, coming from the sarcoplasmic reticulum or more from the extracellular fluid in the heart cell. Upon binding calcium by TnC, a conformational change in the troponin-tropomyosin assembly leads to exposure of actin for myosin interaction. In the relaxed state, myosin attaches ATP and is energized. Upon myosin-actin interaction, ADP and P_i are release with movement of the myosin cross bridge to pull actin towards the center of the sarcomere. Myosin binds another ATP and the cycle is repeated until calcium concentration drops and the troponin-tropomyosin assembly inhibits myosin and actin interaction.

Relaxation issues by binding ATP to myosin and pumping calcium back into the sarcoplasmic reticulum and to the outside of the cell. This means that muscle relaxation too is ATP-dependent.

High cytosolic calcium and depletion of ATP occurs with repeated muscle stimulation or after death. This leads to hypercontraction (tetany) or postmortem rigidity (rigor mortis). Tetany is followed by fatigue due to utilization of mitochondrial energy production for directly pumping calcium ions into the mitochondria.

A number of additional proteins play various roles in the structure and function of muscle. Actinin in either end of the sarcomere (Z disc) anchors actin and seems to act to keep the myofibrils within a myofiber in register, causing the transverse banding. Myomesin in the center of the sarcomere (M band) is important for fixing myosin. Other proteins include titin (the largest protein known), nebulin, desmin, dystrophin, and calcineurin.

Dystrophin is of special interest. It forms a part of the dystrophin-glycoprotein complex, important for interactions between the cytoskeleton, membrane, and the extracellular matrix. Mutations in the gene encoding this protein, on the X-chromosome, cause Duchenne muscular dystrophy, the milder Becker muscular dystrophy, and X-linked dilated cardiomyopathy.

Smooth muscles

Smooth muscles have the sarcomeres not aligned to generate the striated appearance. They do not have the troponin system, and their myosin light chains differ from those of striated muscle myosin. Actin-myosin interaction is prevented by myosin light chains. Phosphorylation of the light chains allows the interaction that stimulates the attachment-detachment contraction cycle of the smooth muscle. This phosphorylation is catalyzed by Ca^{2+}-calmodulin-activated myosin light chain kinase.

Cyclic AMP-activated protein kinase (PKA) can phosphorylate the myosin light chain kinase (not the light chains themselves). The phosphorylated enzyme has a lower affinity for Ca^{2+}-calmodulin, and thus is less sensitive to activation. Accordingly, an increase in cAMP dampens the smooth muscle contraction. This can explain the relaxing effect of β_2-adrenergic stimulation on smooth muscle. Another path that explains this relaxing effect is the phosphorylation of a membrane potassium channel by PKA, which results in closure of the plasma membrane Ca^{2+} channels.

Nitric oxide (NO, endothelium-derived relaxing factor, EDRF) relaxes the smooth muscles of blood vessels by binding to the heme moiety of soluble guanylyl cyclase, thus activating the enzyme. Elevation of intracellular levels of cGMP stimulates the activities of certain cGMP-dependent protein kinases, which phosphorylate specific muscle proteins, causing decreased cytosolic calcium and increased myosin light chain phosphatase activity. Vasodilators such as acetylcholine initially interact with endothelial cell receptors, coupled to the phosphoinositide cycle, leading to the intracellular release of Ca^{2+} through the action of inositol trisphosphate. Calcium activates endothelial NO synthase (also the neuronal isoform). Nitric oxide diffuses into adjacent smooth muscles causing vasodilation. Nitrates and nitrites used as vasodilators are also a source of nitric oxide. Sildenafil citrate (Viagra®) inhibits a cGMP phosphodiesterase, which leads to smooth muscle relaxation.

Another protein that appears to play a Ca^{2+}-dependent role in the regulation of smooth muscle contraction is caldesmon (87 kDa). At low concentrations of Ca^{2+}, it binds to tropomyosin and actin, preventing actin-myosin interaction. At higher concentrations of Ca^{2+}, Ca^{2+}-calmodulin binds caldesmon, releasing it from actin, thus allowing the actin-myosin interaction. Caldesmon is also subject to phosphorylation–dephosphorylation; when phosphorylated, it cannot bind actin, again freeing the latter to interact with myosin.

Sources of ATP:

- Aerobic fuel oxidation in prolonged exercise, using mainly fatty acids and also glucose and ketone bodies.
- Anaerobic glycolysis in short intense exercise, using glycogen and glucose.
- Creatine phosphate as an immediate energy store:

  Creatine-P + ADP ⟵ (creatine kinase) ⟶ Creatine + ATP
- ADP by adenylate kinase reaction:

  ADP + ADP ⟵ (adenylate kinase) ⟶ ATP + AMP

Muscle cell fuel in different physiological states:

- Resting muscles in the well fed state uptake glucose and aminoacids, helped by insulin, and use them to restore their glycogen and proteins respectively. The excess can be oxidized for energy.
- In the fasting state, with no insulin, the muscles take fatty acids from the circulation. Ketone bodies can also be used. Muscle proteolysis provides aminoacids for gluconeogenesis and as a fuel. Skeletal muscle is the principal site of metabolism of branched-chain amino acids. Skeletal muscle protein is the major non-fat source of stored energy. Large losses of muscle mass, particularly in adults, result from prolonged caloric undernutrition.
- For intense exercise of short duration, the muscles depend on anaerobic glycolysis for energy. They use the glycogen store. Glucose from the blood is also used, helped by the recruitment of glucose transporters by the rise of AMP level.
- For prolonged exercise, aerobic energy production depends on fatty acids, and also ketone bodies during fasting.

The amount of ATP in skeletal muscle is only sufficient to provide energy for contraction for a few seconds; therefor, ATP must be constantly renewed. Two major types of muscle fibers are found in humans: white (anaerobic, fast twitch) and red (aerobic, slow twitch). Red fibers contain mitochondria and myoglobin, an oxygen reservoir that gives the red color. White fibers are particularly used in sprints while red fibers are used in prolonged aerobic exercise.

The major sources of energy in the 100-m sprint are creatine phosphate (first 4–5 sec) and then anaerobic glycolysis, using muscle glycogen as the fuel. The two main sites of metabolic control are at glycogen phosphorylase and at phosphofructokinase (PFK-1). The former is activated by Ca^{2+} (released from the sarcoplasmic reticulum during contraction), AMP (resulting from adenylate kinase reaction), and epinephrine. PFK-1 is activated by AMP, P_i, and NH_3. The bifunctional enzyme: phosphofructokinase-2/fructose 2,6-bisphosphatase regulation in the muscle cells is different from in the liver cells in that phosphorylation activates the kinase and inhibits the phosphatase. Thus, epinephrine activates glycolysis in muscle cells (glucagon does not due to lack of receptors). The flux through glycolysis can increase as much as 1000-fold during a sprint. Lactate produced by anaerobic metabolism in skeletal muscle passes to the liver, which uses it to synthesize glucose, which can then return to the muscle (Cori cycle).

In contrast, in the marathon, aerobic metabolism is the principal ATP source. The major fuels are blood glucose and free fatty acids, largely derived from lipolysis in adipose tissue, stimulated by epinephrine. Hepatic glycogen is degraded to maintain the level of blood glucose. Muscle glycogen is also a fuel source, but it is degraded much more gradually than in a sprint.

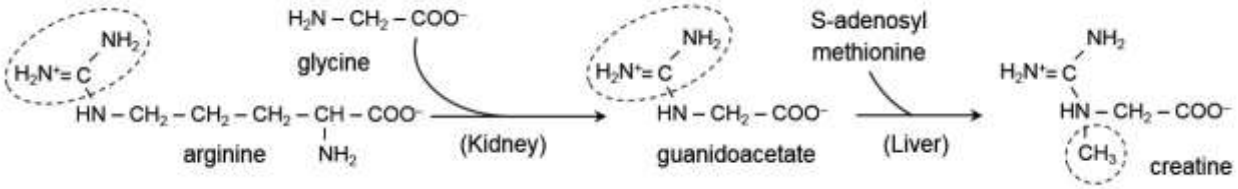
A number of procedures have been used by athletes to counteract muscle fatigue and enhance performance. These include carbohydrate loading, which builds up glycogen stores. Bicarbonate preloading may be used to neutralize acidity, especially in sprints . Ingestion of creatine has gained much popularity in the last few decades.

Heart cell fuel

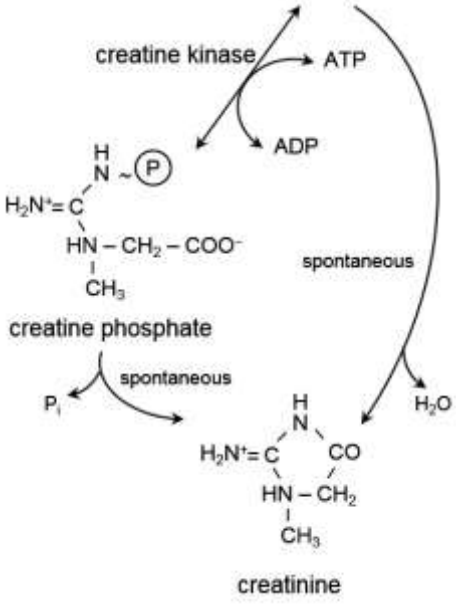
Cardiac metabolism is strictly aerobic. The preferred fuel for cardiac myocytes is fatty acids in fasting or glucose in fed state. Ketone bodies, elevated in the blood with fasting, are used by cardiac myocytes. With severe muscular exercise, lactic acid produced by the skeletal muscles is used as a fuel by the heart. Lactate dehydrogenase works in the reverse direction to that in skeletal muscle to direct lactic acid towards aerobic metabolism.

Creatine

Creatine is synthesized from three aminoacids: glycine, arginine and methionine in the kidney and liver. Methionine provides the methyl group in the hepatic, rate limiting reaction.



Creatine is used for energy storage as creatine phosphate, e.g., in skeletal muscle, heart and brain. Only traces of creatine pass in the urine.



Creatine is one of the most popular sports dietary supplements. It is not screened for or banned by the World Anti-Doping Agency (WADA) or the International Olympic Committee (IOC). Currently, the scientific literature supports creatine supplementation for increased performance in short-duration, maximal-intensity resistance training.

Creatinine

Creatinine is the catabolic cyclic product of creatine and creatine phosphate. Its concentration in the plasma and its excretion in 24 hour-urine are relatively constant, and proportionate to muscle mass. Its blood concentration is not affected by food intake, unlike urea. Plasma creatinine concentration increases with impaired kidney function. Reference range for plasma creatinine is 0.6-1.4 mg/dL, with higher level in men than in women due to difference in muscle mass. Creatinine clearance is used clinically instead of inulin clearance as a measure of glomerular filtration rate.

Defects of energy production

genetic abnormalities of enzymes of energy production lead to serious effects including myopathy and cardiomyopathy. Examples of mitochondrial defects include MERRF (myoclonic epilepsy with ragged red fibers) and MELAS (myopathy, encephalopathy, lactic acidosis, and stroke), caused by mutations in mitochondrial tRNA. Vitamin deficiency may also pause such health problems, e.g., thiamin deficiency accompanying alcoholism.

Cardiac Markers

What are cardiac markers?

These are plasma analytes whose concentration rises as a result of myocardial injury (infarction) since they are originally intracellular components of cardiac muscles. They are useful for early diagnosis and monitoring, hence better prognosis.

Commonly used markers:

- Enzymes (non-functional plasma enzymes):
  - Creatine kinase (CK).
  - Creatine kinase MB isozyme.
  - Aspartate transaminase (AST), aka glutamate:oxaloacetate transaminase (GOT).
  - Lactate dehydrogenase.
  - Glycogen phosphorylase BB.
- Other proteins:
  - Troponin.
  - Myoglobin.
- Other markers:
  - B-type natriuretic peptide.
  - C-reactive protein.
  - Myeloperoxidase.
  - Ischemia-modified albumin.

Cardiac markers are used in the diagnosis and risk stratification of patients with chest pain and suspected acute coronary syndrome (ACS). Cardiac markers are often discussed in the context of myocardial infarction (MI), but other conditions can lead to an elevation in cardiac marker level. Most of the early markers identified were enzymes, and as a result, the term "cardiac enzymes" is sometimes used. However, not all of the markers currently used are enzymes.

Depending on the marker, it can take 2-24 hours for the level to increase in the blood. Additionally, determining the levels of cardiac markers in the laboratory - like many other lab measurements - takes substantial time. The clinical presentation and results from an ECG may be more appropriate in the acute situation. Treatment should not be delayed to wait for cardiac marker results, especially since the sensitivity is low in the first 6 hours after symptom onset.

Different testing strategies for the use of cardiac markers employ single or serial evaluations, with the use of the 99 percentile cutoff values and 10% CV (coefficient of variation). The value of a marker is usually seen as a multiple of the cutoff value.

Up to 80% of patients with acute MI will have an elevated troponin level within 2-3 hours of emergency department (ED) arrival, versus 6-9 hours or more with CK-MB and other cardiac markers. Nevertheless, CK-MB and other markers continue to be used in some hospitals to rule out MI and to monitor for additional cardiac muscle injury over time.

The troponins are regulatory proteins found in skeletal and cardiac muscle. Three subunits have been identified: troponin I (TnI), troponin T (TnT), and troponin C (TnC). The genes that encode for the skeletal and cardiac isoforms of TnC are identical; thus, no structural difference exists between them. However, the skeletal and cardiac subforms for TnI and TnT are distinct, and immunoassays have been designed to differentiate between them. In addition to its use in the diagnosis of MI, an elevated troponin level can identify patients at high risk for adverse cardiac events.

Prior to the introduction of cardiac troponins, the biochemical marker of choice was the CK-MB isoenzyme. The criterion most commonly used for the diagnosis of acute MI was 2 serial elevations above the diagnostic cutoff level or a single result more than twice the upper limit of normal. Although CK-MB is more concentrated in the myocardium, it also exists in skeletal muscle and is elevated in a number of clinical settings, including trauma, heavy exertion, and myopathy. CK-MB first appears 4-6 hours after symptom onset, peaks at 24 hours, and returns to normal in 48-72 hours. Its value in the early and late (>72 hours) diagnosis of acute MI is limited. However, its release kinetics can assist in diagnosing reinfarction if levels rise after initially declining following acute MI.

CK-MB/total CK relative index was introduced to improve the specificity of CK-MB elevation for myocardial infarction. A ratio >5 is indicative of a cardiac source. Ratios between 3 and 5 represent a gray zone; no definitive diagnosis can be established without serial determinations to detect a rise. CK-MB elevation (leak) should be diagnostic for MI even with low total CK.

A false increase of CK-MB may be found in the absence of myocardial injury. The enzyme CK is made of 2 subunits which normally form either CK-MM (CK3, mainly found in the skeletal and cardiac muscles), CK-MB (CK2, mainly found in the heart representing 25-46% of CK activity and to a minor degree in skeletal muscle representing <5% of CK activity), or CK-BB (CK1, mainly found in the brain, prostate, gut, lung, bladder, uterus, placenta, and thyroid). Among the analytical methods that can be used to measure CK-MB, some methods consist of using an anti-M subunit antibody in order to specifically inhibit M subunits enzymatic activity, then measuring the remaining CK enzymatic activity. Assuming that CK-BB is normally absent from the serum, this measurement is supposed to be that of half CK-MB. It is therefore clear that if CK-BB is present in a patient' serum, we can get falsely elevated levels of measured CK-MB. The same is true in presence of macro-CKs, i.e., CK associated with immunoglobulin (macro-CK1) or mitochondrial/autoaggregated CK (macro-CK2). In some cases, the measured CK-MB can even be higher than total CK activity. In this case we measure myoglobin and troponin levels, and/or use another analytical method to measure CK-MB, e.g., electrophoresis. In some cases, it can also be useful to measure CK-BB, or even macro-CKs because they have their own diagnostic utilities, in particular, being tumor markers. CK-MB/total-CK>1 was found associated with colorectal, lung, liver, prostate, and hematological cancers.

The CK-MB isoenzyme exists as 2 isoforms separated by electrophoresis: CK-MB1 and CK-MB2. CK-MB2 is the tissue form, which is initially released from the myocardium after MI. It is converted peripherally in serum to the CK-MB1 rapidly by losing a lysine residue from the M subunit. Normally, CK-MB1 isoform predominates; thus, the CK-MB2/CK-MB1 ratio is typically less than 1. A result should be considered positive if the ratio is elevated to 1.5. The major disadvantage of this assay is that it is relatively labor-intensive for the laboratory.

Myoglobin typically rises 2-4 hours after onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours. However, it has low sensitivity and specificity.

Aspartate transaminase (AST) was the first marker used, however it lacks specificity. Lactate dehydrogenase (LDH) reaches a peak approximately at 72 hours. A high LDH-1 to LDH-2 isozyme ratio suggests MI (LDH is five isozymes). The heart LDH (1&2) has an α-hydroxybutyrate dehydrogenase (HBDH) activity, unlike the liver and muscle isozyme, LDH5.

Other markers under investigation include glycogen phosphorylase BB, which has a high sensitivity and specificity, CK-MM3/CK-MM1, B-type natriuretic peptide, C-reactive protein, myeloperoxidase, and ischemia modified albumin.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following plasma analytes is best for detecting renal impairment?
(A) Urea. (C) Creatine.
(B) Creatinine. (D) Carnitine.

2- What is the best-choice cardiac marker for a recent chest pain?
(A) Cardiac troponin. (C) Creatine kinase.
(B) Myoglobin. (D) Creatine kinase-2.

3- Lactate dehydrogenase is present in which cells?
(A) Muscle and heart cells. (C) Liver cells.
(B) Red blood cells. (D) All cells.

4- In terms of specificity, which of the following is best for diagnosis of myocardial infarction?
(A) Creatine kinase. (C) Troponin C.
(B) Creatine kinase-MB. (D) Aspartate transaminase.

5- Which of the following is an absolute requirement for cardiac myocytes at all times?
(A) Glucose. (C) Ketone bodies.
(B) Fatty acids. (D) Oxygen.

6- Heart cell fuel is oxidized to
(A) lactic acid. (C) carbon dioxide and water.
(B) pyruvic acid. (D) NAD^+ and NADH.

7- Lactate dehydrogenase enzyme in the heart muscle cells is important for
(A) conversion of pyruvate to lactate.
(B) conversion of lactate to pyruvate.
(C) maintenance of anaerobic glycolysis.
(D) maintenance of adequate supply of NAD^+.

8- Which of the following vitamins is needed for the first step in utilization of lactic acid?
(A) Thiamin. (C) Cobalamin.
(B) Niacin. (D) Pyridoxine.

9- How much ATP is produced from oxidation of one molecule of lactic acid in heart cells?
(A) 2 (C) 12
(B) 6 (D) 18

10- Muscle and heart cells take glucose from the circulation by
(A) simple diffusion. (C) active uptake.
(B) facilitated diffusion. (D) sodium-coupled uptake.

11- Glucose uptake by muscle and heart cells is
(A) insulin-dependent. (C) ATP-dependent.
(B) sodium-dependent. (D) none of the above.

12- Glucose transporters of the muscle and heart cell membrane are
(A) SGLT (C) GLUT3
(B) GLUT1 and GLUT2 (D) GLUT4

13- For energy production by glycolysis, the muscle cells oxidize
(A) glucose. (C) both (A) and (B).
(B) glycogen. (D) neither (A) nor (B).

14- Glucose transporters are recruited to the muscle cell membrane by the action of a
(A) product of adenylate kinase reaction.
(B) hormone that activates glycogenolysis.
(C) hyperglycemic hormone.
(D) cyclic nucleotide.

15- Glucose is phosphorylated in the muscle cells by
(A) glucokinase.
(B) glucose 6-phosphatase.
(C) a low-affinity enzyme.
(D) a high-affinity enzyme.

16- Glycolysis in muscle cells ends in
(A) pyruvate.
(B) lactate.
(C) either (A) or (B).
(D) neither (A) nor (B).

17- AMP stimulates energy production in muscle cells by
(A) down-regulation of glucose transporters.
(B) allosteric inhibition of phosphofructokinase.
(C) activation of glycogen phosphorylase without its phosphorylation.
(D) activation of cAMP-dependent protein kinase.

18- Red muscle fibers get their color from
(A) blood.
(B) myoglobin.
(C) ATP.
(D) glycogen.

19- Fatty acids are the muscle preferred fuel in
(A) the fed state.
(B) absence of insulin.
(C) anaerobic metabolism.
(D) heavy weight lifting.

20- Aminoacids exported from the muscle during prolonged fasting are mainly
(A) alanine and glutamine.
(B) phenylalanine and tyrosine.
(C) glycine, arginine, and methionine.
(D) branched-chain aminoacids (valine, leucine, and isoleucine).

21- The greatest body fuel store is which of the following?
(A) Blood glucose.
(B) Liver glycogen.
(C) Muscle glycogen.
(D) Adipose tissue triacylglycerols.

22- Muscle glycogen is not converted to blood glucose due to lack of which enzyme?
(A) Glucokinase.
(B) Debranching enzyme.
(C) Glucose 6-phosphatase.
(D) Glycogen phosphorylase.

23- McArdle disease is characterized by.
(A) genetic deficiency of glucose 6-phosphatase.
(B) decreased glycogen in the muscle.
(C) low tolerance to anaerobic exercise.
(D) lactic acidosis.

24- Genetic deficiency of muscle phosphofructokinase leads to
(A) inhibition of glycolysis.
(B) inhibition of glycogenesis.
(C) McArdle disease.
(D) inability to oxidize fatty acids.

25- Carnitine deficiency may lead to
(A) low tolerance to anaerobic exercise.
(B) depletion of triglycerides in muscle cells.
(C) hypoglycemia.
(D) ketoacidosis.

Blood Cell Metabolism

Red blood cells

Red Blood Cells have relatively simple functions. They are mostly involved in delivering oxygen to various tissues, in addition to helping in disposal of carbon dioxide and buffering generated acids. Simple functions need simple structure, a cell membrane surrounding hemoglobin solution, which constitutes 95% of intracellular protein.

Red blood cell membrane is about 50% protein and 50% lipid. Carbohydrate is in glycoprotein and glycolipids and is directed toward the outer surface. Carbohydrates form the blood group antigens (Lecture 5)

Spectrin is a peripheral membrane protein that interacts with the cytoskeleton to keep the biconcave shape, which increases surface to volume ratio. Defective spectrin causes hereditary spherocytosis and hemolysis.

Mature red blood cell has no nucleus and no organelles like mitochondria, ribosomes or lysosomes. About two million red blood cells enter the circulation per second. The new cells, or reticulocytes, still contain ribosomes and elements of the endoplasmic reticulum. Therefore, they can synthesize protein, a function that is lost within 24 hours by losing the cellular organelles. Reticulocytes constitute normally about 1% of red blood cell count. This ratio increases with hemolytic anemia.

Erythropoietin, secreted by the kidney in response to hypoxia, is the main hormone that stimulates erythropoiesis. Recombinant erythropoietin is used for maintenance of chronic renal failure patients.

Red blood cell metabolism

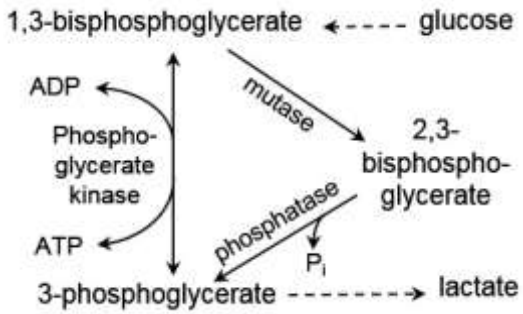
Red blood cells are not metabolically inert. ATP is needed for ion pump (Na^+-K^+-ATPase) important for keeping the biconcave shape. ATP is generated by anaerobic glycolysis.

Glucose is taken by facilitated diffusion, using a transporter that is not insulin-dependent (GLUT1). Once inside the cell, glucose is phosphorylated by hexokinase to glucose 6-phosphate. This keeps the concentration gradient of glucose across the cell membrane.

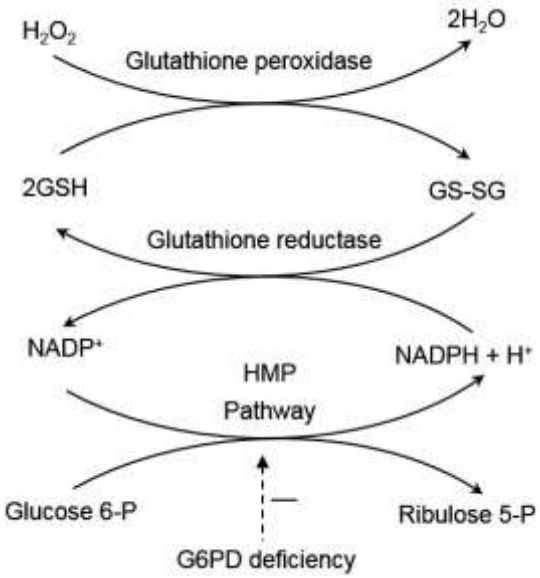
Lactate dehydrogenase, by oxidation of NADH, keeps glycolysis going on. Lactate therefore is the end product of glucose catabolism. Hemolyzed blood samples show elevated plasma lactate dehydrogenase activity.

Fluoride, which inhibits enolase enzyme of glycolysis, is added to blood samples waiting for glucose estimation. Deficiency of pyruvate kinase (red blood cell isoform) causes deficient energy production and is the second enzyme deficiency, after glucose 6-phosphate dehydrogenase, that causes hemolytic anemia. Other enzymes whose deficiency causes hemolysis include hexokinase and phosphohexose isomerase (Lecture 12).

The glycolytic pathway in red blood cells is characterized by the production of the byproduct, 2,3-bisphosphoglycerate (2,3-BPG), produced by R-L cycle. It is important for shifting the hemoglobin-oxygen saturation curve to the right, thus helping the delivery of oxygen to tissues. Its production increases with hypoxia. it plays a compensatory role in cases of pyruvate kinase deficiency.



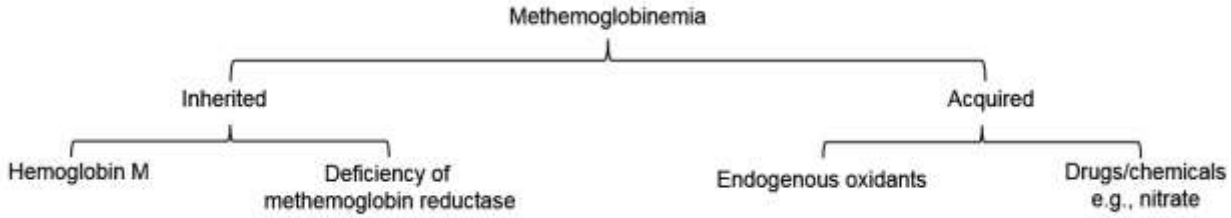
Antioxidants (Lecture 11) play an important role in protecting red blood cells against oxidant stress. Certain drugs or foods can lead to generation of reactive oxygen species, e.g., hydrogen peroxide. Hydroxyl radical can be generated by reaction of ferrous ions with hydrogen peroxide (Fenton reaction). A free-radical chain reaction can be harmful to the cells. Oxidation of the SH groups of hemoglobin leads to protein aggregation seen as Heinz bodies. Oxidation of membrane proteins and lipids can lead to hemolysis. The tripeptide glutathione acts as an antioxidant, in collaboration with NADPH generated in red blood cells by the two dehydrogenases of the HMP pathway (Lecture 12).



NADPH generation is compromised with glucose 6-phsphate dehydrogenase (G6PD) deficiency, an X-linked genetic disease that results from any of hundreds of mutations. G6PD deficiency is the leading enzymatic deficiency that causes hemolytic anemia. Hemolytic anemia varies in severity according to the type of mutation. Hemolysis occurs on increased oxidant stress like with infection or exposure to some drugs or foods such as fava beans, hence the name favism. Precipitating factors should be avoided in such a case, with the possible benefit of the use of antioxidants like tocopherols (vitamin E).

The activity of the enzyme transketolase of the HMP pathway in red blood cells can be a measure of thiamin (vitamin B_1) nutritional status. Transketolase activity is assayed with and without added thiamin pyrophosphate (TPP) to test for thiamin deficiency. TPP is the coenzyme required for transketolase and for oxidative decarboxylation of alpha-ketoacids (pyruvate, alpha-ketoglutarate, and branched-chain alpha-ketoacid dehydrogenases).

Oxidation of hemoglobin iron from Fe^{2+} to Fe^{3+} results in methemoglobinemia. Methemoglobin cannot carry oxygen, which leads to cyanosis. Normally, hemoglobin is protected against oxidation by the erythrocyte NADH-cytochrome b_5 methemoglobin reductase system. A genetic deficiency of this reductase system or a mutation leading to hemoglobin M formation can cause methemoglobinemia. Acquired causes include exposure to endogenous oxidants or some drugs or chemicals, e.g., nitrate. Methemoglobinemia is treated with reducing agents like vitamin C (ascorbic acid) or i.v. methylene blue.



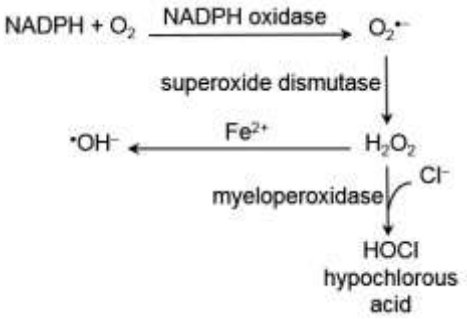
Blood platelets

Platelets are essential for blood clotting. Adhesion of platelets to exposed collagen, helped by von Willebrand factor, is followed by the release of their granule contents. The released thromboxane and ADP cause more platelet aggregation. Activation of the platelets exposes the inner surface of their plasma membrane, with its negative charges (of phosphatidyl serine and phosphatidyl inositol). These negative charges are important for the assembly of clotting factor activation complexes (Lecture 22).

Important metabolic pathways of white blood cells:

- Aerobic glycolysis and tricarboxylic acid cycle.
- HMP pathway.
- Oxidative phosphorylation (moderately active because mitochondria are relatively few).
- Lysosomal enzyme-mediated bacterial destruction.
- NADPH oxidase and myeloperoxidase pathway for bacterial killing (respiratory burst).
- Inducible nitric oxide synthase for bacterial killing.
- Protein synthesis machinery for the production of antibodies and cytokines.

Respiratory burst



Respiratory burst is the increased oxygen consumption by neutrophils and macrophages following phagocytosis. It is important for production of reactive oxygen species (ROS) that help killing the bacteria internalized by phagocytosis. NADPH reduces oxygen in a reaction catalyzed by NADPH oxidase, generating superoxide ($O_2^{\bullet -}$), a free radical that is converted to hydrogen peroxide (H_2O_2, a ROS) spontaneously or catalyzed by superoxide dismutase. Hydrogen peroxide reacts with chloride ions in a reaction catalyzed by myeloperoxidase generating hypochlorous acid (HOCl), a bactericidal. Hydrogen peroxide also generates hydroxyl radical ($^{\bullet}OH$, a ROS) by reacting with ferrous ion (Fenton reaction). In cases of the rare genetic deficiency of NADPH oxidase, this bactericidal mechanism is compromised leading to chronic granulomatous disease.

Nitric oxide

In macrophages, NADPH is required, along with oxygen, for production of nitric oxide (NO, a free radical) from arginine. The reaction is catalyzed by the inducible nitric oxide synthase (iNOS) upon engulfing invading bacteria. This free radical helps killing the bacteria, before being metabolized to nitrite and nitrate.

Fecal calprotectin

Calprotectin is a calcium and zinc-binding protein that constitutes about 60% of the neutrophil cytosolic proteins. It is resistant to proteolysis by pancreatic and intestinal enzymes. Fecal calprotectin is measured as a marker of inflammatory bowel disease.

Study Questions

Choose one best answer for every question of the following:

1- For production of energy, red blood cells catabolize
(A) glucose.
(B) fatty acids.
(C) ketone bodies.
(D) lactic acid.

2- Which of the following enzymes is important in the erythrocytes?
(A) Heme synthase.
(B) Citrate synthase.
(C) Lactate dehydrogenase.
(D) Glucose 6-phosphatase.

3- Which of the following is active in mature red blood cells?
(A) Hemoglobin synthesis.
(B) HMP pathway.
(C) Aerobic glycolysis.
(D) Respiratory chain.

4- HMP pathway in the red blood cells is important for which of the following?
(A) Antioxidant function.
(B) Lipogenesis.
(C) Production of pentoses.
(D) Aerobic energy production.

5- In a hemolyzed blood sample, which of the following is not elevated in the plasma?
(A) Hemoglobin.
(B) Lactate dehydrogenase.
(C) Sodium.
(D) Potassium.

6- Fluoride is added to blood samples primarily in order to
(A) prevent clotting.
(B) scavenge free radicals.
(C) inhibit glycolysis.
(D) inhibit bacterial growth.

7- With hemolytic anemia, which of the following is a compensatory metabolic change in the red blood cells?
(A) Increased 2,3-bisphosphoglycerate.
(B) Increased insulin sensitivity.
(C) Increased oxygen consumption.
(D) Decreased oxygen consumption.

8- With R-L cycle in red blood cells, net ATP production from one glucose molecule is
(A) 0-1.
(B) 1-2.
(C) 0-2.
(D) 0-8.

9- Glucose 6-phosphate dehydrogenase deficiency in red blood cells leads to
(A) increased resistance to oxidant stress.
(B) decreased ability to synthesize fatty acids.
(C) increased reduced glutathione.
(D) increased $NADP^+$/NADPH ratio.

10- Hemolysis in G6PD-deficient patients is attributed to decreased
(A) reduced glutathione.
(B) $NADP^+$.
(C) oxidizing agents.
(D) fava beans intake.

11- Which of the following may be useful for management of a case of G6PD deficiency?
(A) Ribose and deoxyribose.
(B) Tocopherol.
(C) Hydrogen peroxide.
(D) Iron supplements.

12- Hemolytic anemia may result from deficient energy production secondary to deficiency of which enzyme?
(A) Transketolase.
(B) Glucose 6-phophate dehydrogenase.
(C) Pyruvate kinase.
(D) Glucokinase.

13- Respiratory burst takes place in which cells?
(A) Red blood cells.
(B) Neutrophils.
(C) Alveolar cells.
(D) Capillary endothelial cells.

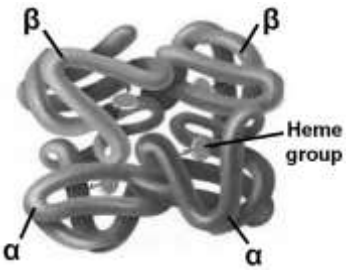
14- Hypochlorous acid is produced by which cells?
(A) Gastric mucosal cells.
(B) Renal tubular cells.
(C) White blood cells.
(D) Red blood cells.

15- Thromboxane is an important product of
(A) red blood cells.
(B) white blood cells.
(C) blood platelets.
(D) endothelial cells.

16- Which of the following about cell membrane is important for leukocyte function?
(A) Spectrin-cytoskeleton interaction.
(B) Integrin-cytoskeleton interaction.
(C) Exposure of negatively charged phospholipids.
(D) Providing arachidonic acid for thromboxane synthesis.

Hemoglobin

Hemoglobin (about 68,000 daltons) is the main protein of red blood cells. Its intracellular concentration is about 30 g/dL. In the whole blood, hemoglobin is about 15 g/dL. It is slightly higher in healthy men and lower in women and children. It is the major known heme protein. It is a tetramer, formed of four globin chains, each with a heme group.

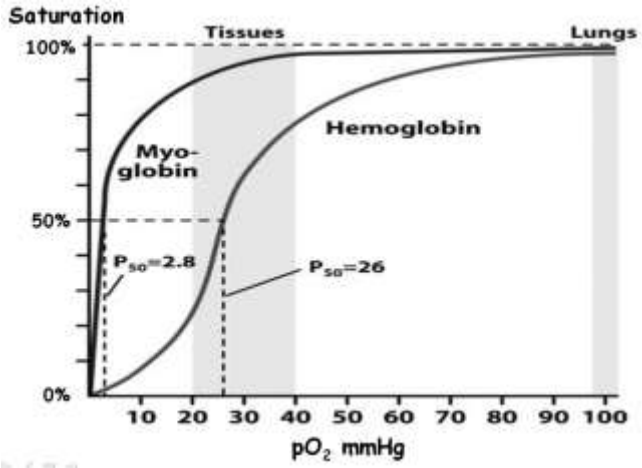


Adult hemoglobin (Hb A_1) has 2 alpha (141 aminoacids) and 2 beta (146 aminoacids) subunits (α_2,β_2). Fetal hemoglobin (Hb F) has 2 alpha- and 2 gamma-subunits (α_2,γ_2). It is replaced completely six months after birth, leaving less than 1% of total hemoglobin in a normal adult. Minor adult hemoglobin (Hb A_2), which should be less than 3.5% in a normal adult, has 2 alpha- and 2 delta-subunits (α_2,δ_2). Glycated hemoglobin (Hb A_{1c}) results from glucose attachment to terminal valine. Its value varies according to average blood glucose during the erythrocyte life span (less than 5.7% in healthy people). It is used clinically as a measure of diabetes control, and recently for diagnosis of type 2 diabetes mellitus. [Embryonic hemoglobins have epsilon (ε)- and zeta (ζ)-chains: $\zeta_2\varepsilon_2$, $\alpha_2\varepsilon_2$, and $\zeta_2\gamma_2$. Hb F predominates after six months of gestation].

Each globin chain has eight alpha helices, numbered A to H. The hydrophobic groups of aminoacids are hidden in the interior of this tertiary structure. The E-helix contains the distal histidine residue, His E7. The F-helix contains proximal histidine, His F8, which binds the heme group. Heme is located in a central hydrophobic pocket. Iron is in the ferrous state. It forms four coordination bonds with porphyrin nitrogen, a fifth bond with proximal histidine, and a sixth bond with oxygen. Distal histidine protects Fe^{2+} against oxidation and attachment of the toxic carbon monoxide.

Hemoglobin functions as a transporter of oxygen from the lungs to different tissues. It also carries about 15% of CO_2 produced by the tissues, and it buffers the acidifying effect of CO_2 (carbonic acid) and lactic acid. Oxygen is carried on Fe^{2+}. Carbon dioxide is carried by the terminal amino groups of globin chains, forming carbamate. Protons are buffered by the imidazole groups of histidine residues.

The quaternary structure of hemoglobin is like four myoglobin molecules grouped together. Myoglobin is a monomer, present in skeletal and cardiac muscles, where it functions as a reservoir of oxygen. Myoglobin structure suits this function. At oxygen partial pressure equal to that in muscle capillaries, myoglobin is 100% saturated with O_2. Myoglobin leaves oxygen only at very low oxygen pressure, i.e., during severe exercise. Hemoglobin on the other hand should be saturated with oxygen at the lungs to unload it in tissue capillaries. Hemoglobin is completely saturated with oxygen at the lung (pO_2 = 100 mmHg). At tissue capillaries (pO_2 = 20 mmHg), hemoglobin is only 20% saturated, thus oxygen is delivered to the tissues.



The sigmoid shape of hemoglobin's oxygen saturation/dissociation curve results from co-operative binding of oxygen to hemoglobin (wrongly called heme-heme interaction). Binding of an O_2 molecule to one heme group increases the oxygen affinity of the remaining heme groups of the hemoglobin molecule. The four subunits of hemoglobin are arranged as two identical dimers $(\alpha\beta)_1$ and $(\alpha\beta)_2$. The two subunits of each dimer are held tightly by interaction of hydrophobic groups present on the surface of the subunits. The two dimers are less strongly held by ionic and hydrogen bonds. Deoxy-hemoglobin is called T (taut, tense). It is a low oxygen affinity form. Binding of oxygen to hemoglobin causes rupture of some ionic and hydrogen bonds between the two dimers. The two dimers become less strongly held, forming R (relaxed) form, a high oxygen affinity form. The trigger is the movement of iron when binding oxygen. Iron pulls the F helix and causes conformational change transmitted to other subunits. This conformational change facilitates binding of oxygen to other subunits. This cooperativity in oxygen binding is a positive allosteric effect of oxygen.

Allosteric activators shift the oxygen dissociation curve to the left. Allosteric inhibitors shift it to the right. These include increased CO_2, increased hydrogen ion concentration (decreased pH), increased temperature and increased 2,3-BPG (2,3,-bisphosphoglycerate). These are the conditions that accompany tissue activity and signal the requirement for oxygen to be delivered from hemoglobin.

Bohr effect, named after the Danish physiologist Christian Bohr (1855-1911), describes a decreased O_2 affinity of hemoglobin by increase of CO_2 and hydrogen ions. This is explained by increase of salt bridges that stabilize T form due to formation of negative charges of carbamate and positive charges of protonated imidazole groups of histidine residues. The protonation of histidine residues results from the increased acidity due to ionization of lactic acid and carbonic acid formed by carbonic anhydrase catalysis. Haldane effect, named after the Scottish physician John Scott Haldane, states that deoxygenating hemoglobin increases its ability to carry CO_2, while oxygenation decreases the capacity for CO_2.

The binding of 2,3-bisphoshoglycerate (2,3-BPG) to a positively charged pocket formed by the two β-units leads to stabilization of T form and decreased oxygen affinity of hemoglobin. Hemoglobin F has no β-chains and does not bind 2,3-BPG, which enables fetal hemoglobin to take oxygen released by maternal hemoglobin in the placenta. Chronic hypoxia or anemia increases 2,3-BPG, ensuring more oxygen delivery to the tissues. Stored blood has low concentration of 2,3-BPG, with enhanced oxygen affinity of transfused blood that may decrease oxygenation of patient’s tissues. This problem may be solved by adding inosine (hypoxanthine-ribose) to the stored blood to produce 2,3-BPG.

Carbon monoxide intoxication

Carbon monoxide binds ferrous heme of hemoglobin (and cytochrome oxidase) with a 200 times higher affinity than oxygen, in spite of the steric hindrance by distal histidine. It also shifts the hemoglobin oxygen dissociation curve to the left, causing more tissue anoxia. Chemical treatment is usually not satisfactory and oxygen therapy remains necessary.

Inherited hemoglobinopathies

Hemoglobin polypeptides are encoded by separate genes. Chromosome 11 carries the gene for β-chain. Chromosome 16 carries two copies of α-chain gene. This means that the cell has two copies of β-chain gene and four copies of α-chain gene. Inherited hemoglobinopathies are either in the form of abnormal hemoglobin chains, e.g., Hb M and Hb S, or insufficient synthesis of hemoglobin chains (thalassemia).

Beta-thalassemia results from defective synthesis of β-chain, with normal synthesis of α-chain. Gamma chain synthesis increases and persists after birth leading to higher levels of Hb F. There is also increased production of Hb A_2 (unless delta-thalassemia coexists). Homozygous state, known as thalassemia major, is characterized by severe anemia (Cooley anemia, Mediterranean anemia) that needs regular blood transfusion. Stunted growth, iron overload, splenomegaly, bone deformity and fractures are common complications. Bone marrow transplantation is reportedly the most successful treatment. Few reports showed a success of gene therapy. Premarital screening and counselling showed a high success rate in preventing the disease in the implementing countries.

Heterozygous state, named thalassemia minor or thalassemia trait, is a milder condition that usually needs no specific treatment, but should not be confused with the more common iron deficiency anemia. Chemical methods for validation of iron deficiency are superior to hematological indices when hemoglobin electrophoresis is not convenient.

Alpha-thalassemia results from defective synthesis of α-chain. It is less frequently seen than β-thalassemia since there are four copies of α-chain gene. In addition, the α-chain is essential in hemoglobin, and there could be no living baby without α-chain. Silent carriers have only one of the four gene copies defective. Alpha-thalassemia trait with mild symptoms results when two copies are defective. Hemoglobin H (β_4), an unstable tetramer and Hb Barts (γ_4, nickname of St Bartholomew's Hospital in London), which shows no Bohr effect, are seen with defective three copies of the α-gene, with mild to severe anemia. Hydrops fetalis and fetal death result from defective four copies, where severe anemia causes heart failure.

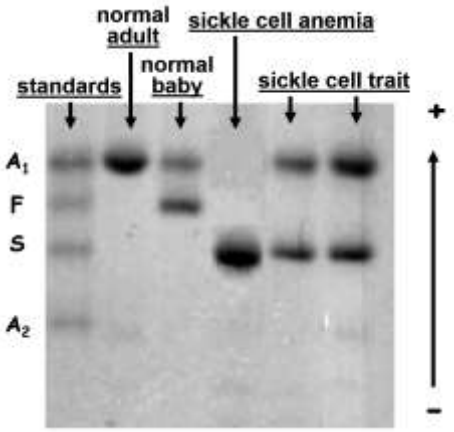
Hemoglobin S (Hb S) results from a point mutation affecting the β-chain gene. Valine replaces glutamate at position 6 of the β-chain. Two sticky patches (protrusions) appear on the surface of hemoglobin molecule. These sticky patches bind to complementary sites on deoxy-Hb S, forming fibers that precipitate causing sickling of red blood cells. Unlike squeezable discoid cells, sickled cells are rigid and angular. They are stuck in small capillaries causing capillary occlusion, ischemia and infarction with attacks of severe pain. Sickled cells are destroyed by the reticuloendothelial system, causing hemolytic anemia (sickle-cell anemia). These characteristics of the homozygous sickle cell disease start with the switch from fetal to adult Hb and ensue from factors that increase sickling, i.e., increase deoxygenation, e.g., high altitude, diseases of hypoventilation, infections, fevers, increased carbon dioxide and low pH.

In the treatment of sickle-cell disease, hydroxyurea (hydroxycarbamide) increases nitric oxide, cGMP, gamma globin gene expression and the production of Hb F, which does not polymerize and deform red blood cells. It also suppresses white blood cells (by inhibiting nucleoside diphosphate reductase) that contribute to the general inflammatory state in sickle cell patients.

Heterozygous sickle cell disease or sickle cell trait is a milder condition. Patients are resistant to malignant malaria caused by *plasmodium falciparum*, because of the shorter than normal life span of their red blood cells.

More than 200 variant and abnormal hemoglobins have been described. Hemoglobin electrophoresis, high performance liquid chromatography (HPLC) and capillary electrophoresis are useful techniques for establishing a definitive diagnosis of abnormal hemoglobin conditions.

In alkaline electrophoresis, Hb H is the fastest in migration toward the anode, followed by Barts. Hb S is slower than Hb A_1 since an acidic glutamate is replaced by a neutral valine. In heterozygous sickle cell disease, both types of hemoglobin appear.



In Hb C, Hb E, and Hb O, a glutamate residue is replaced by lysine, a basic aminoacid. Therefore, these types of hemoglobin migrate even slower (with Hb A_2, before carbonic anhydrase). These mutations usually cause no harm or only mild hemolysis.

In Hb M, there is a mutation of α- or β-globin whereby tyrosine replaces histidine, with formation of methemoglobin. Heme is oxidized to hemin, with ferric iron that cannot carry oxygen. The sixth coordination position is occupied by water. Patients suffer from chocolate cyanosis and tissue hypoxia.

Study Questions

Choose one best answer for every question of the following:

1- Regarding hemoglobin and cytochromes,
(A) they are important mitochondrial proteins.
(B) they perform an antioxidant function.
(C) they are involved in respiration, but at different levels.
(D) their iron oscillates between the ferric and ferrous state for their physiological function.

2- A globin chain is characterized by which of the following?
(A) Hydrophobic groups are generally located on the surface.
(B) The secondary structure contains 8 α-helices: A-H.
(C) Heme is present in a hydrophilic pocket in the center.
(D) Heme is attached by its iron to distal histidine.

3- Which of the following globin chains is most essential in hemoglobin structure?
(A) Alpha.
(B) Beta.
(C) Gamma.
(D) Delta.

4- Heme is bound to globin by
(A) hydrophobic and a coordination bonds.
(B) electrostatic attraction.
(C) hydrogen bonds.
(D) ionic bonds.

Anemia

Anemia is the most common blood disorder, affecting about one third of the global population. It is diagnosed when hemoglobin value is less than 13.5 g/dL in a man or less than 12.0 g/dL in a woman. Normal values for children vary with age, generally 11.0 g/dL or above. Anemia can be caused by decreased red blood cell production, increased red blood cell breakdown, blood loss, or fluid overload.

Impaired production:

- Aplastic anemia, pure red cell aplasia, and space-occupying lesions in the bone marrow.
- Insufficient production of the hormone erythropoietin due to kidney failure.
- Pernicious anemia, vitamin B_{12} nutritional deficiency, and folate deficiency.
- Iron deficiency, vitamin B_6 deficiency, and lead poisoning.
- Thalassemias.

Increased destruction:

- Hereditary spherocytosis and elliptocytosis due to defect in membrane/skeleton proteins.
- Enzyme deficiencies:
    - Glucose-6-phosphate dehydrogenase and glutathione synthetase.
    - Pyruvate kinase and hexokinase.
- Hemoglobinopathies:
    - Sickle cell anemia.
    - Hemoglobinopathies causing unstable hemoglobins.
- Autoimmune hemolytic anemia and paroxysmal nocturnal hemoglobinuria.
- Infections such as malaria.
- Mechanical trauma as with heart surgery and hemodialysis.

Blood loss:

- Trauma or surgery, causing acute blood loss.
- From menstruation and gynecologic disturbances.
- Gastrointestinal tract lesions, causing either acute bleeds, e.g., variceal lesions and peptic ulcers, or chronic blood loss, e.g., angiodysplasia.
- Many types of cancer, including colorectal cancer and cancer of the urinary bladder.
- Infection by intestinal nematodes such as hookworms and the whipworm *Trichuris trichiura*.
- Iatrogenic anemia, blood loss from repeated blood draws and medical procedures, e.g., anemia of prematurity, from frequent blood sampling for laboratory testing, combined with insufficient RBC production due to lack of erythropoietin.

Fluid overload (hypervolemia):

- excessive sodium or fluid intake and sodium or water retention.
- Pregnancy.

Treatment

Treatment of anemia should be directed to the treatment of the cause whenever possible. Dietary supplementation, without determining the specific cause, is not recommended. The use of blood transfusion is typically based on a person's signs and symptoms. Generally, it is not recommended unless hemoglobin level is less than 6-8 g/dL. Erythropoiesis-stimulating medications are only recommended in those with severe anemia. Administration of oxygen helps blood of a low hemoglobin value to deliver oxygen to the needy tissues by increasing the oxygen dissolved in the plasma. Hyperbaric oxygen may even be needed when blood transfusion is not feasible, e.g., with no compatible blood or for religious reasons.

Study Questions

Choose one best answer for every question of the following:

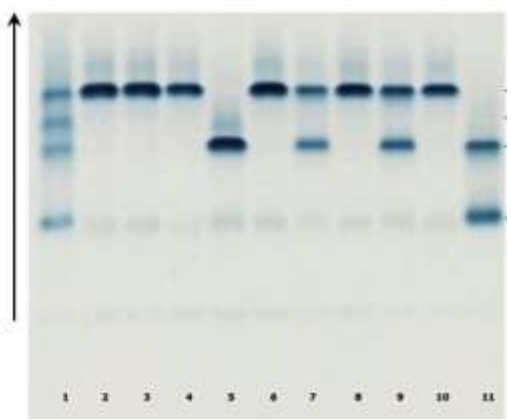
5- Carbon dioxide is carried by the blood mainly as
(A) carbaminohemoglobin.
(B) carboxyhemoglobin.
(C) reduced hemoglobin.
(D) bicarbonate.

6- Bohr effect is due to
(A) increased pH.
(B) stabilizing the R form of hemoglobin.
(C) increased salt bridges between hemoglobin dimers.
(D) shifting the hemoglobin oxygen saturation curve to the left.

7- Decreased oxygen affinity of hemoglobin by muscle activity is partly due to
(A) increased pH.
(B) increased temperature.
(C) both (A) and (B).
(D) neither (A) nor (B).

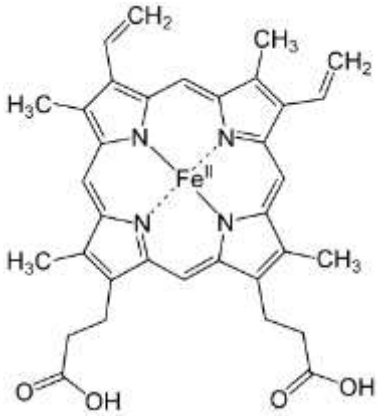
8- Why is β-thalassemia more common than α-thalassemia?
(A) More copies of α-globin gene than β-globin gene.
(B) Absolute necessity of β-globin chain for hemoglobin.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

9- In this alkaline electrophoresis gel, lane 1 is the standards, what is your diagnosis for lane 5?
(A) Normal adult.
(B) Normal baby.
(C) Sickle cell trait.
(D) Sickle cell disease.



10- What is your diagnosis for lane 11?
(A) Normal baby.
(B) Sickle cell trait.
(C) Hemoglobin SC.
(D) Beta-thalassemia.

Heme Synthesis

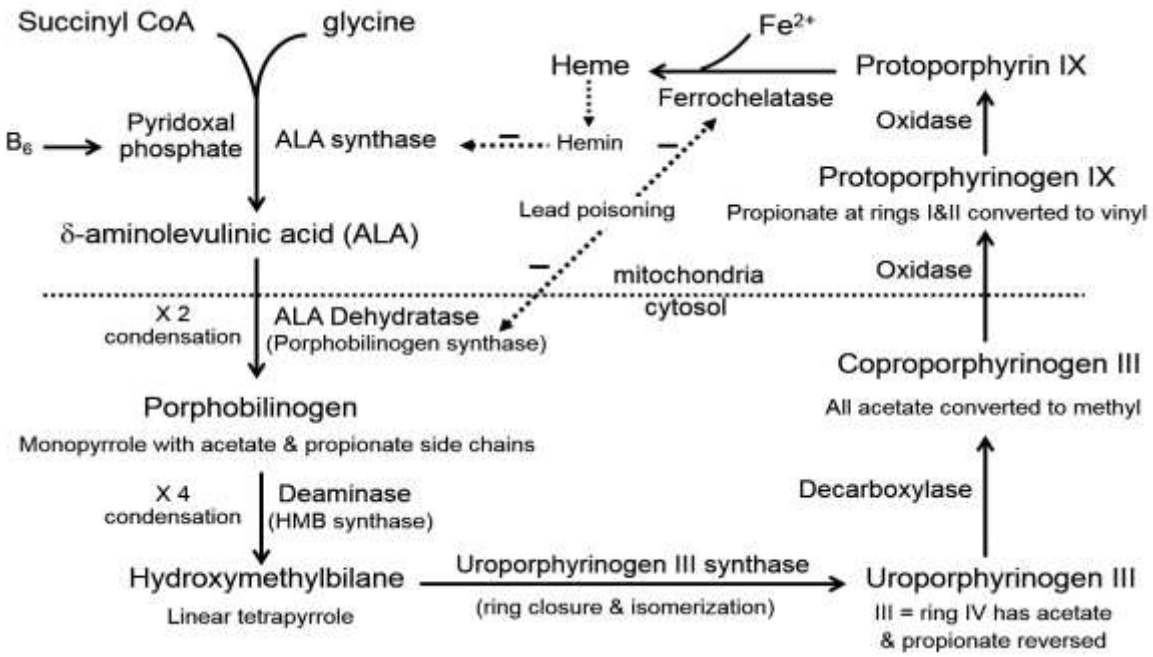


Heme is the prosthetic group of hemoglobin, myoglobin, cytochromes, hydroperoxidases, tryptophan pyrrolase, and cytosolic guanylate cyclase, which is activated by nitric oxide. Heme consists of protoporphyrin IX with one iron atom in its center chelated to the four pyrrole nitrogen atoms.

Porphyrins are cyclic molecules composed of four linked pyrrole rings with eight side chains. Heme is quantitatively the most important porphyrin in humans. Porphyrins are colored compounds, while porphyrinogens are reduced (hydrogenated) porphyrins and are colorless.

Heme biosynthesis occurs mainly in the bone marrow and liver cells, as required for hemoglobin and cytochromes, especially cytochrome P450 (hydroxylase). Porphyrins are synthesized from glycine and succinyl CoA. The key enzyme of porphyrin synthesis is the mitochondrial enzyme delta-aminolevulinic acid (ALA) synthase (ALAS), which is feedback inhibited by hemin (oxidized heme) through inhibition of the enzyme synthesis and its incorporation into the mitochondria. Pyridoxal phosphate is a coenzyme of this key reaction, thus deficiency of vitamin B_6 may be a cause of anemia.

There are two forms of ALAS. ALAS1 is expressed in all cells. Its gene is located on chromosome 3. ALAS2 is erythroid-specific and is expressed only in fetal liver and adult bone marrow. Its gene is located on the X chromosome. Deficiencies of ALAS2 result in hereditary or congenital hypochromic anemia (X-linked sideroblastic anemia, XLSA). Lead poisoning inhibits two enzymes of heme synthesis: ALA dehydratase and ferrochelatase. Heme synthesis begins and ends in the mitochondria. Therefore, mature red blood cells cannot synthesize heme.



Porphyrias

Porphyrias result from defects in heme synthesis. At least eight different types of porphyria have been classified according to the deficient enzyme and the tissue of origin, erythropoietic or hepatic. A genetic defect of an enzyme of heme synthesis leads to lack of feedback inhibition and accumulation of intermediates. The clinical picture is therefore a result of lack of the final product, heme, and accumulation of the intermediates.

A defect of a late step in heme synthesis results in increased blood uroporphyrin and coproporphyrin. Porphyrins can capture light energy and activate ground state oxygen to singlet oxygen, a reactive oxygen species that initiates a destructive peroxidative chain reaction, which leads to photosensitivity. Porphyria cutanea tarda is the most common porphyria. It is caused by deficiency of uroporphyrinogen decarboxylase. The urine is red, especially on standing in the air due to oxidation of uroporphyrinogen to uroporphyrin. Patients should avoid sun light and use antioxidants like beta-carotene for skin care. They should also avoid drugs that induce cytochrome P450, such as phenobarbital and corticosteroids. Hemin, i.v., may be used to suppress ALA synthase.

A defect of an early step in the synthetic pathway does not lead to photosensitivity. Acute intermittent porphyria results from deficiency of porphobilinogen deaminase (HMB synthase). Its symptoms are neurological and visceral.

Study Questions

Choose one best answer for every question of the following:

1- Porphyrins are
(A) heterocyclic compounds.
(B) metalloproteins.
(C) inorganic pigments.
(D) modified proteins.

2- Deficiency of aminolevulinic acid synthase leads to which of the following?
(A) Red color of the urine.
(B) Photosensitivity.
(C) Anemia.
(D) Jaundice.

3- Isoniazid therapy of tuberculosis may lead to which of the following?
(A) Iron deficiency anemia.
(B) Sideroblastic anemia.
(C) Ferrochelatase inhibition.
(D) Accumulation of porphyrins in the liver and bone marrow.

4- Patients with porphyria cutanea tarda may benefit from
(A) iron supplements.
(B) exposure to sun.
(C) using antioxidants like beta-carotene in skin care preparations.
(D) using drugs that induce cytochrome P450 such as phenobarbital and corticosteroids.

5- Intravenous hemin may be used for
(A) porphyria.
(B) iron overload.
(C) lead poisoning.
(D) induction of cytochrome P450.

Heme Catabolism

Red blood cells are destroyed at the end of their life span, which is about 120 days. Hemoglobin in the circulation dissociates to hemoglobin dimers, which can escape in the renal glomeruli. Hemoglobin is carried on haptoglobin, a high molecular weight protein that prevents the escape of hemoglobin in the glomerular filtrate, thus conserving the body iron and protecting the renal tubules against hemoglobin precipitation. Hemoglobin, on haptoglobin, and damaged red blood cells are taken by phagocytic cells of the reticuloendothelial system. The globin part is broken down to its constituent aminoacids. Heme ($C_{34}H_{32}N_4O_4Fe$) is oxidized by heme oxygenase, which breaks the link between pyrrole rings I and II. This reaction produces the linear tetrapyrrole, biliverdin ($C_{33}H_{34}N_4O_6$) and carbon monoxide; iron is recycled. As its name indicates, biliverdin is green in color. It is then reduced to bilirubin ($C_{33}H_{36}N_4O_6$), the yellow to red bile pigment.

Bilirubin is water-insoluble. It is carried in the circulation on plasma albumin, gaining the name “hemobilirubin”. It is taken by the liver cells and conjugated with glucuronic acid, forming bilirubin diglucuronide, cholebilirubin, which is excreted in the bile.

In the intestine, free bilirubin is liberated and reduced by intestinal bacteria to urobilinogen ($C_{33}H_{44}N_4O_6$). Some urobilinogen is absorbed and passes to the liver to be excreted again in the bile. Some escapes to the kidney where it is excreted in the urine in trace amounts. Urobilinogen is colorless. Its oxidized form, urobilin ($C_{33}H_{42}N_4O_6$) contributes to urochrome, the yellow pigment of urine.

Urobilinogen that remains in the colon can either be reduced to stercobilinogen (fecal urobilinogen, $C_{33}H_{48}N_4O_6$) and finally oxidized to stercobilin ($C_{33}H_{46}N_4O_6$) or it can be directly reduced to stercobilin, which contributes to the brown color of stool, with other bilirubin derivatives, e.g., dipyrroles. Stool color becomes darker on standing in the air probably due to oxidation of remaining stercobilinogen.

Normally, plasma bilirubin concentration is less than 1 mg/dL, mostly of the non-conjugated type. Conjugated bilirubin is a water-soluble bilirubin, which reacts with diazotized sulfanilic acid with no need for adding an organic solvent. It is therefore called direct bilirubin. Hemobilirubin, on the other hand, needs an organic solvent to react with the reagent, so it is indirect bilirubin. Although direct bilirubin can pass to the glomerular filtrate, its renal threshold is higher than its normal plasma concentration, therefore it does not normally appear in the urine.

Abnormal bilirubin metabolism

Increased bacterial reduction of bilirubin, e.g., with biliary tract infection can increase the production of urobilinogen and stercobilinogen. A substantial amount of stercobilin was found to be present brown pigment gallstones in infants. This suggests that brown pigment gallstones could form spontaneously with bacterial infections of the biliary tract.

Taking antibiotics that kill the intestinal bacteria can stop the production of urobilinogen. Bilirubin passes in the stool in this case and may be oxidized to biliverdin, giving a greenish stool.

Abnormalities of bilirubin metabolism may lead to increased plasma bilirubin concentration. If plasma bilirubin rises above 2 mg/dL, it infiltrates into the tissues causing yellow discoloration seen in the skin and mucous membrane (jaundice).

Physiological jaundice of neonates is the most common jaundice. There is a high rate of red blood cell destruction together with immaturity of the enzyme glucuronyl transferase and probably other enzymes that provide its substrate, UDP-glucuronate. Plasma indirect bilirubin is high. Plasma albumin can carry about 20-25 mg of bilirubin per deciliter. Above this level, indirect bilirubin can cross the blood brain barrier and precipitate in the basal ganglia (kernicterus), causing encephalopathy. Bilirubin inhibits several important enzymes. The most serious toxic action of bilirubin is probably its acting as an uncoupler of oxidative phosphorylation and inhibition of mitochondrial ATPase. Phenobarbital is used to induce the liver enzymes. The baby is routinely given glucose solution, which is the substrate for forming UDP-glucuronate. Breast milk may contain substances that compete with bilirubin for the same excretory mechanism, therefore it is withdrawn. Phototherapy may convert bilirubin to more water-soluble isomers or more easily excreted products. Exchange blood transfusion should be considered for critical cases.

Hemolysis, with increased red cell destruction that exceeds the efficient excretory mechanism, leads to increased indirect bilirubin in the plasma. Hemolytic jaundice has a wide range of causes. It is characterized by being acholuric (no bilirubin in the urine). Urobilinogen characteristically increases in urine due to its overproduction, with no change of urine color. The stool color is expected to become darker due to increased stercobilin. Plasma haptoglobin decreases. The urine color turns brownish only with hemoglobinuria, which accompanies the depletion of plasma haptoglobin. Increased urinary urobilinogen is a marker of hemolysis, but it may also indicate liver disease that halts the excretion of urobilinogen coming from the intestine or increased bacterial reduction of bilirubin, e.g., with biliary tract infection.

Obstruction to the bile flow leads to regurgitation of conjugated bilirubin, producing obstructive (cholestatic) jaundice characterized by increased plasma direct bilirubin. Direct bilirubin appears in the urine. Urine color becomes deep yellow, seen in the foam, to reddish brown (tea color). Urinary urobilinogen decreases or may even be absent with complete obstruction. With decrease of the brown pigment, the stool becomes clay-colored. Bile salts escaping to the plasma cause the itching symptom, and can be detected in the frothy urine.

Hepatitis leads to decreased capacity of hepatocytes to conjugate bilirubin, together with an element of obstruction to biliary flow. A rise of both types of plasma bilirubin is seen. The urine is colored by direct bilirubin and the stool is under-pigmented. Urinary urobilinogen may decrease or increase as described before.

Some genetically inherited diseases affect bilirubin metabolism. Gilbert's disease is characterized by defect in uptake of bilirubin by liver parenchymal cells and possibly glucuronyl transferase. In Crigler-Najjar syndrome there is deficiency of bilirubin conjugation. In Dubin-Johnson syndrome and Rotor syndrome, there is deficient excretion of conjugated bilirubin. Plasma bilirubin of the corresponding type rises with development of jaundice.

Study Questions

Choose one best answer for every question of the following:

1- Hemoglobin is broken down by which cells?
(A) Red blood cells.
(B) Erythropoietic cells.
(C) Intestinal cells.
(D) phagocytic cells.

2- Which of the following molecules is least expected to result from catabolism of heme?
(A) Bilirubin.
(B) Stercobilin.
(C) Urobilinogen.
(D) Porphobilinogen.

3- Heme oxygenase produces
(A) oxyhemoglobin.
(B) bilirubin.
(C) carbon dioxide.
(D) iron.

4- Which pathway of glucose metabolism is directly linked to bilirubin excretion?
(A) HMP pathway.
(B) Uronic acid pathway.
(C) Polyol pathway.
(D) Glycolytic pathway.

5- Urobilinogen is
(A) yellow.
(B) red.
(C) green.
(D) colorless.

6- Biliverdin is
(A) yellow.
(B) red.
(C) green.
(D) colorless.

7- Haptoglobin is important for carrying which of the following?
(A) Iron.
(B) Hemoglobin.
(C) Bilirubin.
(D) Biliverdin.

8- Deficiency of aminolevulinic acid synthase leads to which of the following?
(A) Anemia.
(B) Red color of the urine.
(C) Hemolytic jaundice.
(D) Hepatic jaundice.

9- Anemia is most commonly due to deficiency of
(A) glucose 6-phosphate dehydrogenase.
(B) glucuronyl transferase.
(C) heme oxygenase.
(D) iron.

10- Jaundice in a newborn is probably due to
(A) sickle hemoglobin.
(B) high plasma carotene.
(C) immaturity of the enzyme heme oxygenase.
(D) breast milk interfering with bilirubin excretion.

11- Which of the following is true about physiological jaundice?
(A) There is high plasma bilirubin, mostly of the direct type.
(B) Bilirubin can inhibit cellular energy production.
(C) Bilirubin concentration higher than 2-3 mg/dL exceeds the capacity of plasma albumin.
(D) Direct bilirubin crosses the blood-brain barrier and causes kernicterus.

12- What is the benefit of phenobarbital given for physiological jaundice?
(A) Induction of the liver enzymes.
(B) Conversion to UDP-glucuronate.
(C) Stabilizing the red blood cell membrane.
(D) Inhibition of haptoglobin catabolism.

13- Hemolytic jaundice is characterized by
(A) high plasma indirect bilirubin.
(B) high plasma haptoglobin.
(C) clay-colored stool.
(D) low or absent urinary urobilinogen.

14- Which of the following is a manifestation of hemolytic anemia?
(A) Bilirubin in the urine.
(B) Increased urobilinogen in the urine.
(C) High serum iron binding capacity.
(D) Low serum iron.

15- In hemolytic jaundice, the urine color may turn brownish due to
(A) high urobilinogen.
(B) direct bilirubin.
(C) indirect bilirubin.
(D) hemoglobin.

16- Urinary urobilinogen increases with
(A) intravascular hemolysis.
(B) viral hepatitis.
(C) obstructive jaundice.
(D) leaving the urine in air.

17- Which of the following is a marker of intravascular hemolysis?
(A) Low plasma haptoglobin.
(B) Low serum ferritin.
(C) Decreased urobilinogen in urine.
(D) Bilirubin in the urine with clay-colored stool.

18- Greenish stool that may accompany the intake of antibiotics is probably due to
(A) conversion of stercobilin to biliverdin.
(B) oxidation of bilirubin to biliverdin.
(C) increased conversion of bilirubin to stercobilinogen.
(D) formation of green compounds from stercobilin.

19- In obstructive jaundice:
(A) There is high plasma total bilirubin but low direct bilirubin.
(B) Stool is darker in color.
(C) Bilirubin passes in the urine.
(D) Glucuronyl transferase is not working.

20- Brownish discoloration of the urine seen with biliary obstruction is due to
(A) hemoglobin.
(B) urobilin.
(C) direct bilirubin.
(D) indirect bilirubin.

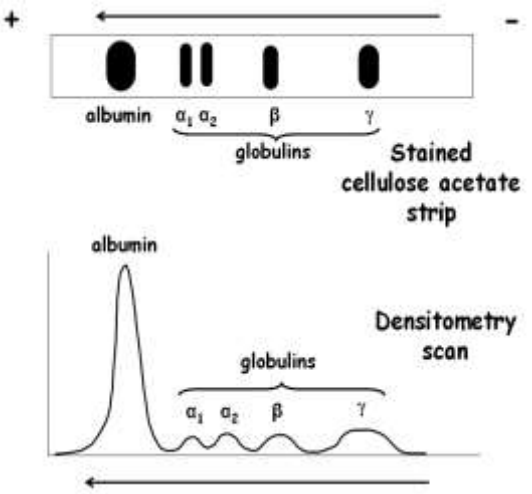
21- Which of the following may be seen with hepatitis?
(A) Increased conjugation of bilirubin.
(B) Obstruction of bile flow.
(C) High plasma total and low direct bilirubin.
(D) Darkly pigmented stool.

22- In Dubin-Johnson syndrome and Rotor syndrome, there is
(A) defect of uptake of bilirubin by liver cells.
(B) glucuronyl transferase deficiency.
(C) deficiency of bilirubin conjugation.
(D) deficient excretion of conjugated bilirubin.

Plasma Proteins

Plasma proteins are normally about 7.0 g/dL. The major plasma protein is albumin, which is about 4.0 g/dL. Globulins are about 2.7 g/dL. Fibrinogen is about 0.3 g/dL. Other proteins are present as traces. Serum is the blood fluid remaining after coagulation. Its composition is like the plasma, but it lacks fibrinogen. It is commonly used in the clinical lab instead of plasma for certain analyses. Plasma proteins are generally synthesized by the liver cells. Gamma globulins are synthesized by plasma cells, derived from B-lymphocytes.

Plasma or serum proteins can be separated by electrophoresis. It is usually performed on cellulose acetate strip in alkaline buffer, above isoelectric point of all the proteins. All proteins then carry a net negative charge and move toward the anode.



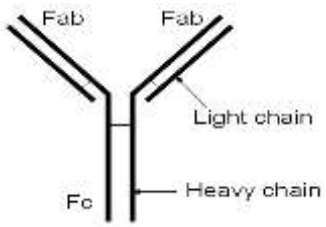
Hypoproteinemia is usually a hypo-albuminemia. This results from reduced synthesis, e.g., with liver disease or protein malnutrition, or from abnormal loss, e.g., renal disease and nephrotic syndrome characterized by albuminuria.

Plasma proteins perform different functions. Osmotic pressure, or oncotic pressure, generated by plasma proteins is about 25 mmHg. This is a small value, but of great significance for exchange of interstitial fluid with plasma. It is mainly due to albumin, since it has the highest mass concentration and a relatively low molecular weight, meaning a high molar concentration.

Plasma proteins act as pH buffers in the plasma. Also, substances that are not water-soluble are transported in the blood carried on plasma proteins, e.g., bilirubin, calcium and fatty acids on albumin, thyroxine on thyroid hormone binding globulin, iron on transferrin, and copper on ceruloplasmin in addition to many drugs too.

Alpha 1-globulin band is formed mainly of α_1-antitrypsin, which is important as anti-elastase in the lung protecting the lung alveoli from the proteolytic effect of neutrophil elastase. Its deficiency leads to development of emphysema (Lecture 6).

Defense function is exerted by immunoglobulins (gamma globulins, antibodies). These glycoproteins bind specifically, non-covalently, to antigens. A gamma-globulin molecule is typically formed of two identical light chains, κ (kappa) or λ (lambda) and two identical heavy chains: α (alpha) in IgA, γ (gamma) in IgG, δ (delta) in IgD, ε (epsilon) in IgE, or μ (mu) in IgM. These are connected by inter-chain S-S bonds. IgA are present in dimers in secretions. IgM are present as pentamers in blood, and they are responsible for the primary immune response. (IgG) are the highest in concentration, are responsible for secondary immune response, and can cross the placenta. IgE are responsible for hypersensitivity reactions.



Coagulation of blood is a function of plasma proteins: fibrinogen, prothrombin and other coagulation factors. These interact to produce the blood clot that stops bleeding.

Study Questions

Choose one best answer for every question of the following:

1- A sharp gamma-globulin band indicates which of the following?
(A) Liver cell failure.
(B) Chronic inflammation.
(C) Monoclonal antibodies of myeloma cells.
(D) Liability to develop lung emphysema.

2- Which of the following plasma proteins may be synthesized by cells other than hepatocytes?
(A) Fibrinogen.
(B) Albumin.
(C) Alpha globulin.
(D) Gamma globulins.

3- The oncotic pressure of the plasma is normally
(A) higher than arterial blood pressure.
(B) lower than venous blood pressure.
(C) higher than venous and lower than arterial blood pressure.
(D) higher than diastolic and lower than systolic blood pressure.

4- Which of the following is a major marker of liver cell failure?
(A) Low plasma albumin.
(B) High serum transaminases.
(C) Low blood ammonia.
(D) High gamma-globulin fraction.

5- A low or absent alpha 1-globulin may be seen most probably with
A) liver cirrhosis and emphysema.
B) generalized edema.
C) hyper-coagulation state.
D) nephrotic syndrome.

6- Immunoglobulins are classified into
A) alpha, beta, and gamma globulins.
B) many serotypes according to Fab.
C) five types according to light chains.
D) two types according to heavy chains.

7- Hyperventilation may lead to development of tetany because
A) hyperventilation leads to respiratory acidosis.
B) plasma proteins bind more hydrogen ions.
C) plasma proteins bind more calcium ions.
D) washing out of carbon dioxide causes more bicarbonate binding to albumin.

8- On hydrolyzing immunoglobulins with pepsin, the crystalline (constant) fragment is formed by parts of
A) the heavy chains.
B) the light chains.
C) heavy and light chains.
D) mixed kappa and lambda light chains.

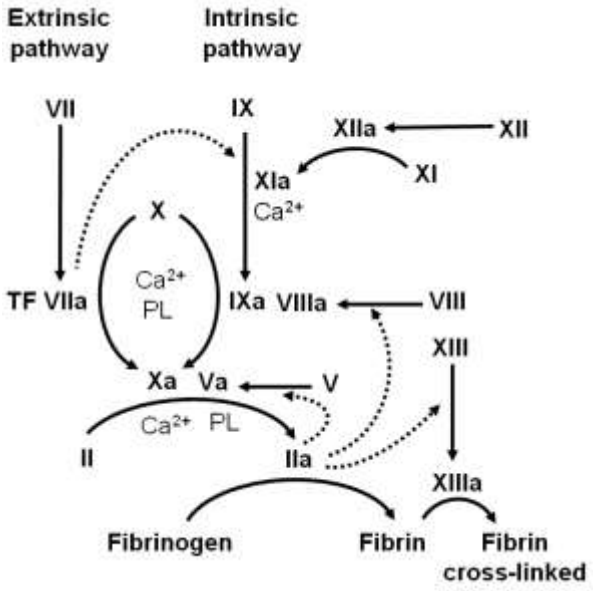
9- A specific protein that passes in the urine in cases of multiple myeloma originates as
A) albumin.
B) an antiprotease.
(C) immunoglobulin light chain.
(D) immunoglobulin heavy chain.

10- Monoclonal antibodies are prepared using
C) hepatocytes.
D) lymphocytes.
(C) myeloma cells.
(D) hybridoma cells.

Blood Coagulation

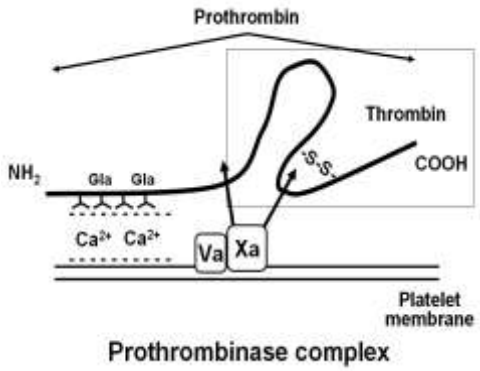
The physiological events of hemostasis in response to tissue injury include vaso-constriction, formation of a platelet plug (thrombus) then the formation of a fibrin clot that entangles blood cells. This is followed later by dissolution of this blood clot (fibrinolysis).

Formation of the fibrin clot results from a cascade of reactions, which activates the co-agulation factors in a sequence. This cascade involves amplification in each step. Zymogens, generally synthesized by the liver, are activated by proteolytic cleavage. The intrinsic pathway has all its factors in the blood, while the extrinsic pathway needs a tissue factor (TF). Both lead to activation of factor ten (X to Xa), which in turn cleaves prothrombin (factor II), yielding the active thrombin (IIa). Thrombin cleaves fibrino-gen, yielding fibrin which polymerizes and is cross-linked by factor XIIIa. Thrombin also activates factors V, VIII (positive feedback), and XIII. Factor XIIIa cross-links glutamine and lysine by transglutaminase activity.



Factors Va, VIIIa are not enzymes but protein cofactors needed by factors Xa and IXa for activation of factors II (prothrombin) and X respectively.

Calcium ions and platelet phospholipids (PL) are needed especially for the activation complexes of factors II (prothrombin) and X. Adhesion of platelets to exposed collagen, helped by von Willebrand factor, is followed by the release of their granule contents. Thromboxane and ADP released cause more platelet aggregation. Activation of the platelets exposes the inner surface of their plasma membrane, with its negative charges (of phosphatidyl serine and phosphatidyl inositol). This phospholipid (PL) membrane is important for the assembly of the coagulation complexes. Calcium ions bridge prothrombin with its negatively charged gamma-carboxyglutamate (Gla) residues, formed with the help of vitamin K, to the negatively charged phospholipids of the platelet membrane. Factor Va binds Xa to the membrane, thus completing the prothrombinase complex. Factor Xase complex has a similar configuration. Factor X is bridged to membrane by Ca^{2+}, and its proteolytic enzyme is factor IXa in the intrinsic pathway, with VIIIa as the cofactor, or factor VIIa in the extrinsic pathway with the tissue factor (TF).



Prothrombinase complex

Fibrinogen is formed of three types of aminoacid chains, $(A\alpha,B\beta,\gamma)_2$, and is soluble due to the negative charges on A and B regions of the molecule. Thrombin cleaves off the two fibrinopeptides A and B, leaving fibrin $(\alpha,\beta,\gamma)_2$ which spontaneously polymerizes.

Coagulation factors with Gla residues, which are dependent on vitamin K for post-translational modification include prothrombin and factors VII, IX, and X, as well as protein C (PC) and protein S (PS).

Hemophilia

Classical hemophilia (hemophilia A) is due to X-linked hereditary defect of factor VIII. Hemophilia B is due to X-linked defect of factor IX. Hemophilia C is due to autosomal defect of factor XI. The intrinsic pathway is inhibited. The extrinsic pathway cannot compensate for this defect because of the tissue factor pathway inhibitor (TFPI) that rapidly inhibits TF-VIIa complex in presence of Xa. Treatment is by the factor replacement therapy. The factor can now be prepared by recombinant DNA technology. Gene therapy has been recently introduced.

Physiological anticoagulants

Antithrombin III binds heparin and acts as a serpin (serine protease inhibitor), inhibiting thrombin and also factors XIIa, XIa, IXa, and Xa. It accounts for 75% of plasma antithrombin activity. α_2-Macroglobulin accounts for about 25% of plasma antithrombin activity. α_1-Anti-trypsin has a minor antithrombin activity.

Thrombin binds to thrombomodulin, a protein of the endothelial cell membrane. The complex activates protein C (PC), which in presence of its cofactor protein S (PS) inactivates factors Va and VIIIa. This is an anticoagulant action of thrombin.

Tissue factor pathway inhibitor is another serpin synthesized by endothelial cells and inhibits the extrinsic Xase complex.

Anticoagulant therapy

Heparin (or heparin-like) injection gives an instant anticoagulant effect, but needs antithrombin III for its action. Coumarin-based oral anticoagulants, e.g., warfarin are vitamin K antagonists, which have a delayed action. Novel oral anticoagulants (NOAC) are designed to inhibit thrombin or factor Xa. Aspirin, by inhibition of cyclooxygenase inhibits thromboxane synthesis and platelet aggregation. Eicosapentaenoic acid of fish oil gives thromboxane A_3, which is far less potent than thromboxane A_2 from arachidonic acid.

In-vitro anticoagulants

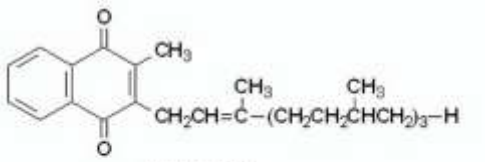
Coagulation of blood can be inhibited by heparin, precipitating calcium ions by oxalate, or binding calcium in a soluble form using citrate or EDTA (ethylene diamine tetra-acetate).

Fibrinolysis

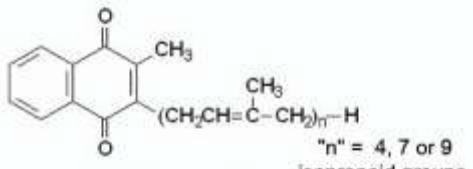
Plasmin (fibrinolysin), a serine protease, degrades fibrin and fibrinogen. It is present in the plasma as plasminogen that is activated by tissue plasminogen activator, another serine protease secreted by vascular endothelium. This activator is active only when bound to fibrin, i.e., it acts locally to dissolve the blood clot. It is inhibited by plasminogen activator inhibitor. From the fibrin degradation products (FDPs) in the plasma, D-dimer (D dimer) is the test of choice to rule out thrombotic disease, and to assess the severity of COVID-19.

Urokinase is another activator of plasminogen formed by many cells. Streptokinase is a bacterial enzyme that is used therapeutically, e.g., in cases of coronary occlusion. It has the disadvantage of non-localized action besides being a foreign protein leading to tolerance and probably immune reaction. Tissue plasminogen activator is now synthesized by recombinant DNA technology to replace streptokinase for therapeutic uses.

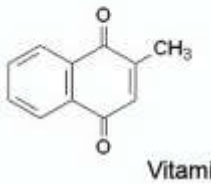
Vitamin K



Vitamin K_1



Vitamin K_2



Vitamin K_3

Vitamin K exists naturally as the vitamers: K_1 (phylloquinone, phytomenadione, phytonadione) in green vegetables and K_2 (menaquinone, MK-n) produced by bacteria. MK-4, MK-7, and MK-9 are the most well-studied menaquinones. Vitamin K is a fat-soluble vitamin that needs bile for its absorption, stored in the liver, and distributed in plasma lipoproteins.

Vitamin K_3 is synthetic menadione. When administered, it is alkylated in the liver to one of the vitamin K_2 forms. Due to its toxicity, it is no longer used for humans in developed countries.

The major function of vitamin K is maintenance of normal levels of the blood clotting proteins: factors II, VII, IX, and X and also protein C and protein S (K is from koagulation in Danish/German). These vitamin K-dependent proteins are synthesized in the liver and require a posttranslational carboxylation of specific glutamate residues to γ-carboxyglutamate (Gla). The enzyme responsible needs vitamin K as a cofactor. The Gla residues are important for binding calcium ions, which bridge the protein to the phospholipids of platelet membranes.

During the carboxylation reaction, reduced hydroquinone form of vitamin K is converted to a 2,3-epoxide form. The regeneration of the hydroquinone form requires two-step reduction. Vitamin K epoxide reductase is competitively inhibited by the coumarin-based anticoagulants such as warfarin.

Other Gla-proteins include osteocalcin needed for proper bone calcification, hence the suggested role of vitamin K in management of osteoporosis, and matrix Gla-protein investigated for its role in preventing calcification of arteries and coronary heart disease.

Adequate intake of the vitamin is 60-120 µg/day for adults (1 µg/kg/day). Main sources of vitamin K_1 are leafy vegetables and plant oils. Animal sources are second and include mainly poultry and eggs. Vitamin K_2 is obtained in fermented foods. People taking warfarin and similar anticoagulants need to maintain a consistent intake of vitamin K from food and supplements to maintain a steady anticoagulant effect. Administration of ezetimibe inhibits vitamin K absorption together with cholesterol and can potentiate the warfarin effect.

Fat malabsorptive diseases can result in vitamin K deficiency. This is manifested as hemorrhagic tendency. Since the vitamin K_2 form is synthesized by intestinal bacteria, deficiency of the vitamin in adults is rare. However, long term antibiotic treatment can lead to vitamin K deficiency. The intestine of newborn infants is sterile, the placental transfer of the vitamin is poor and the breast milk is poor in the vitamin. Therefore, infants are susceptible to vitamin K deficiency. The primary manifestation of a deficiency in infants is a hemorrhagic syndrome. It is a common practice to give vitamin K supplement for all newborns.

No upper tolerable intake level was set for vitamin K. Toxicity may be seen in infants taking high doses of the vitamin. The main manifestation is hemolytic anemia.

Study Questions

Choose one best answer for every question of the following:

11- Poor response of a patient to heparin may be due to
(A) High plasma fibrinogen.
(B) Deficiency of a functional antithrombin III.
(C) The use of aspirin as a platelet inhibitor.
(D) Excess vitamin K in the diet.

12- Placing a blood sample in a glass test tube leads to formation of a blood clot due to the action of which of the following?
(A) The intrinsic and common pathways of blood coagulation.
(B) The extrinsic and common pathways of blood coagulation.
(C) Cyclooxygenase enzyme.
(D) Thrombin-thrombomodulin complex.

13- Which of the following is the nature of cross-links made by factor XIIIa?
(A) Covalent bonds.
(B) Hydrogen bonds.
(C) Ionic bonds.
(D) Hydrophobic interaction.

14- Factor V Leiden is named after the Dutch city where it was first identified in 1994 and is characterized by a mutation that makes it resistant to activated protein C leading to.
(A) hereditary hypercoagulability.
(B) hemorrhagic tendency.
(C) inability to activate prothrombin.
(D) prolonged clotting time.

15- A difference between clotting time (or partial thromboplastin time) and prothrombin time tests is:
(A) Clotting time measures the intrinsic and common pathways.
(B) Prothrombin time measures the extrinsic and common pathways.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

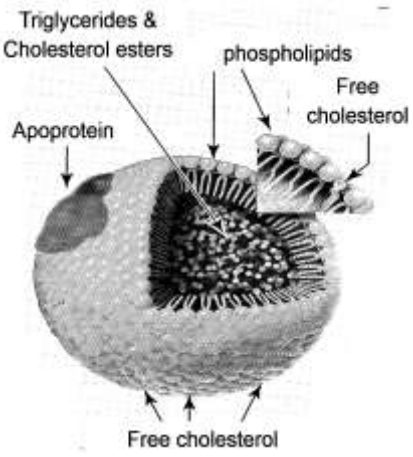
16- Serum lacks which of the following?
(A) Albumin.
(B) alpha 1- globulin.
(C) Fibrinogen.
(D) Thrombin.

17- Warfarin overdose is treated by
(A) heparin.
(B) antihemophilic globulin.
(C) tissue plasminogen activator.
(D) vitamin K.

18- Which of the following is measured in the plasma to help the diagnosis of disseminated intravascular coagulation?
(A) D-dimer.
(B) Plasmin.
(C) Thromboxane.
(D) Protein C.

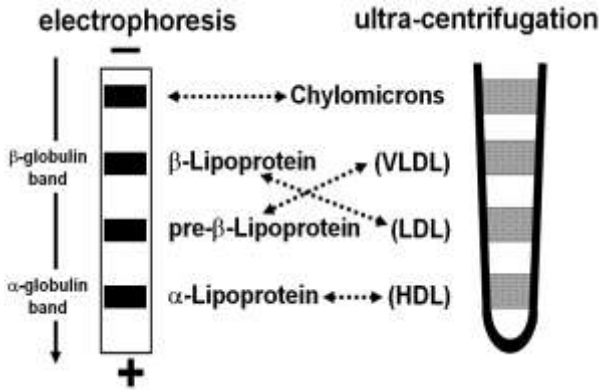
Plasma Lipoproteins

Lipids circulate in the blood combined with proteins in the form of lipoprotein particles. A lipoprotein particle is composed of the hydrophobic lipids, triacylglycerols and cholesterol esters, in the center, surrounded by amphipathic lipids (phospholipids and free cholesterol) and proteins (apolipoproteins). This arrangement ensures the miscibility of lipids with the aqueous plasma. The lipid/protein ratio and the differential lipid content vary between different types of lipoproteins. The higher the lipid content, the lower the density of the lipoprotein is.



Apolipoproteins are synthesized in the liver except ApoB-48, which is synthesized by the intestine. ApoB-48 constitutes 48% of ApoB-100 encoded by the same gene. In the intestinal mucosal cells, an RNA editing complex alters one base in ApoB-100 mRNA, producing a stop codon, which leads to the production of the smaller protein.

Apolipoproteins are important for transporting the lipids in the aqueous plasma and for activation of enzymes: LCAT by ApoA-I and lipoprotein lipase by ApoC-II. They are also important for identification of the lipoprotein particles by cell receptors.



Plasma lipoproteins can be separated by electrophoresis or ultracentrifugation. The major plasma lipoproteins are:

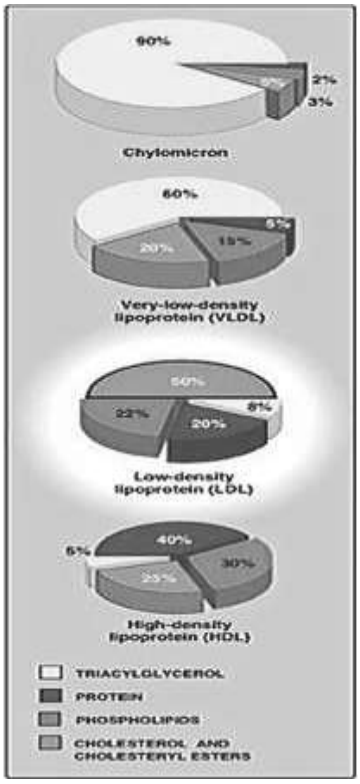
- Chylomicrons
- Very Low Density Lipoproteins (VLDL)
- Low Density Lipoproteins (LDL)
- High Density Lipoproteins (HDL)

Chylomicrons

Chylomicrons are the largest, the highest in fat, and the lowest density among all lipoproteins. Chylomicrons carry exogenous triacylglycerols from the intestine to various tissues. They also carry some cholesterol esters. They pass from the intestine to the lymph, and via the thoracic duct to the general circulation. They are responsible for the lipemia that follows the ingestion of fatty meals. Their triacylglycerols are hydrolyzed by lipoprotein lipase, which is induced by insulin and expressed on the luminal surface of the endothelial cells of tissue capillaries. Free fatty acids are taken by the tissues for storage and glycerol passes to the liver. Their apo-lipoprotein is ApoB-48, which is synthesized by the intestinal cells. They also accept from HDL ApoC-II, which activates lipoprotein lipase, and ApoE, which helps the uptake of chylomicron remnants by liver cells.

VLDL

VLDL carries triacylglycerols from the liver to provide fatty acids as a cell fuel to muscle and heart cells. They also carry cholesterol esters. Their apoprotein is ApoB-100, but they also acquire ApoC-II and ApoE from HDL in the circulation. By the action of lipoprotein lipase, they are converted to intermediate density lipoproteins (IDL), which are either taken by the liver cells with the help of ApoE receptors, or converted to LDL. Lipoprotein lipase of the heart capillaries has a lower K_m than that of the adipose tissue capillaries, allowing the heart cells to use fatty acids as a fuel. If hepatic triacylglycerol synthesis exceeds the VLDL packing, fatty liver develops. This is observed in cases of increased fatty acid mobilization from adipose tissue, e.g., in diabetes. Fatty liver is treated by lipotropic drugs, e.g., choline, which increases phospholipid synthesis, thus helping the packing of VLDL. Ethanol oxidation results in acetaldehyde, which has a toxic effect on tissue protein. Formation of covalent adducts of acetaldehyde with tubulin causes the inability of hepatocytes to secrete VLDL, and development of fatty liver.



LDL

LDL carries cholesterol from the liver to the tissues. It is the main cholesterol carrier, and it has the highest cholesterol content among all plasma lipoproteins. Its apoprotein is ApoB-100. LDL is formed from VLDL, through IDL. It (and other ApoB-100-containing lipoproteins) accepts cholesterol esters from HDL in exchange for triglycerides by cholesterol ester transfer protein (CETP) bound to HDL. Most of LDL particles are taken by the liver cells, the rest by other cells. Uptake is through ApoB-100 receptor-mediated endocytosis. The expression of the receptors increases with the utilization of intracellular cholesterol for biosynthetic functions. Increased intracellular cholesterol down regulates the LDL receptors. LDL is the mostly blamed for the pathogenesis of atherosclerosis, especially altered LDL (by oxidation, glycation, or thiolation). Altered LDL is taken by macrophages, which are then converted to foam cells that start the atherosclerosis process. Uptake of altered LDL by macrophages is through the scavenger receptor SR-A1, which is not down regulated by intracellular cholesterol.

HDL

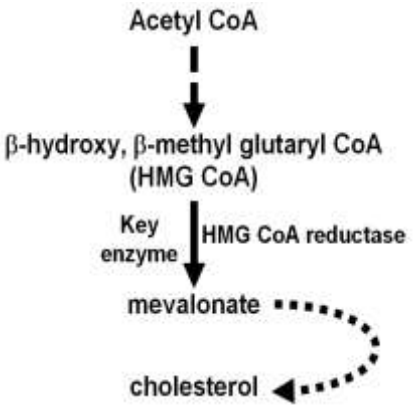
HDL carries cholesterol from the tissues to the liver (reverse transport). The liver is the main organ that excretes cholesterol. The apoprotein of HDL is ApoA-I, which activates the enzyme lecithin:cholesterol acyl transferase (LCAT). This enzyme esterifies cholesterol, taking the fatty acid from position 2 of lecithin, which is converted to lysolecithin. The esterified cholesterol moves from the surface of the HDL particle to its hydrophobic core. The particle can then accept more cholesterol from the tissues, helped by ABCA1 transporter. The nascent discoid particle swells as it accepts more cholesterol to become a mature HDL. Scavenger receptors SR-B1 facilitate the uptake of cholesterol esters from HDL by liver cells. Plasma HDL is considered protective against atherosclerosis owing to its reverse cholesterol transport function. Its antioxidant power (paraoxonase activity) may add to this beneficial effect.

Cholesterol Metabolism and Transport

Cholesterol has important functions in the body. It is the parent steroid, acting as a precursor of all other steroids including the bile salts, steroid hormones and vitamin D. The most important function of cholesterol is its sharing in the membrane structure. Cholesterol is essential for every cell in this sense. It is provided in food of animal source only, since it is an animal sterol, not present in plants. Plant sterols in the diet are not used. However, with this extreme importance of cholesterol, food is not its only or primary source. Cholesterol is synthesized by all tissues, especially the liver, adrenal cortex, and reproductive tissues.

Cholesterol synthesis

Cholesterol is synthesized from acetyl CoA in the cytosol. Reducing equivalents are provided by NADPH. Increased intracellular cholesterol inhibits cholesterol synthesis and down regulates LDL receptors. The key enzyme, HMG CoA reductase, is down regulated by cholesterol. This is achieved by cholesterol inhibition of the release of the transcription factor, sterol regulatory element binding protein (SREBP), from the endoplasmic reticulum. Cholesterol is esterified inside the cells for storage by the enzyme acyl CoA:cholesterol acyl transferase (ACAT).



Cholesterol excretion

Cholesterol is not degraded to acetyl CoA. It is not a cell fuel. Cholesterol is excreted as an intact steroid. The main route of excretion is through the bile to the stool. It is excreted by the liver cells either as cholesterol per se or changed to bile salts. Cholesterol is kept in solution in bile thanks to bile salts and lecithin. Low bile salts/cholesterol ratio can lead to formation of biliary stones. Some of the cholesterol in the intestine is reduced by intestinal bacteria to cholestanol and coprostanol. Other minor routes for cholesterol elimination from the body are in the urine as catabolites of steroid hormones and through menstrual blood and shed cells.

Cholesterol transport

Plasma cholesterol is carried mainly by LDL. LDL carries cholesterol from the liver to peripheral tissues, while HDL carries cholesterol from peripheral tissues to the liver. High plasma cholesterol is a risk factor for atherosclerosis. The risk is higher with a high LDL/HDL cholesterol ratio. HDL cholesterol is high in pre-menopausal women, justifying their apparent immunity against coronary heart disease.

The favorable concentration of cholesterol in the plasma is below 200 mg/dL (LDL cholesterol < 130 mg/dL). Plasma cholesterol above 240 mg/dL (LDL cholesterol > 160 mg/dL) carries a high risk for coronary heart disease. It is recommended that HDL cholesterol in an adult male be at least 40 mg/dL. LDL cholesterol may be measured or calculated by Friedewald formula in a fasting sample: LDL = Total – HDL – TG/5 (providing that TG<200 mg/dL).

Dyslipoproteinemia

Hyperlipidemia

Primary (genetic) hyperlipoproteinemia can result from different mutations affecting lipoprotein metabolism. These are reflected in abnormal concentration of serum cholesterol and triglycerides (TG) and the appearance of serum. Complications may be fatal. The most common types are types II and IV. The following table is based on Fredrickson classification.

Type	Pathology	Cholesterol	TG	serum
Type I: Familial lipoprotein lipase deficiency	↓ LPL or Apo CII ↑ chylomicron & VLDL Chidlhood, abdominal pain, pancreatitis, xanthoma, hepatomegaly No risk of CHD	N or ↑	↑ ↑ ↑ ↑	Creamy
Type IIa: Familial hypercholesterolemia	Defective or ↓ LDL receptors ↑ ↑ risk of CHD	↑ ↑ ↑	Normal	Clear
Type IIb:	↑ LDL & VLDL ↑ risk of CHD	↑	↑	S. turbid
Type III: dysbetalipoproteinemia (Broad β band)	Apo E abnormalities ↑ IDL, xanthomas ↑ risk of CHD	↑ ↑	↑ ↑	Turbid
Type IV: CHO induced hypertriacylglycerolemia	↑ VLDL glucose intolerance. ↑ risk of CHD	↑	↑ ↑	turbid

Familial hypercholesterolemia (type IIa) is due to defective LDL receptors. Deficient uptake leads to deficient inhibition of cholesterol synthesis. The disease is inherited in an autosomal dominant pattern. Plasma cholesterol is dangerously high. If homozygous, the patient may die at childhood from myocardial infarction.

Some families have lipoprotein (a), which consists of LDL and Apo(a). Apo(a) shows structural homology to plasminogen (profibrinolysin). Familial lipoprotein (a) excess is associated with coronary heart disease (due to atherosclerosis + inhibition of fibrinolysis).

Hypercholesterolemia may be secondary to hypothyroidism, nephrotic syndrome, diabetes mellitus, or obstructive jaundice. Secondary hypertriglyceridemia is seen with diabetes mellitus, obesity, alcoholism, and some drugs, and looks similar to type IV hyperlipidemia. Insulin is important for induction of lipoprotein lipase, and the lack of insulin leads to deficient clearance of chylomicrons and VLDL. The favorable plasma triglyceride is below 150 mg/dL (above 200 mg/dL is risky).

Lowering blood cholesterol

Plasma cholesterol concentration may be lowered by taking low-calorie diets with low-animal fat and high-fiber content. Vegetable oils have very low cholesterol content, practically considered zero cholesterol. In addition, vegetable oils are rich in unsaturated fatty acids required for cholesterol esterification in HDL, hence cholesterol excretion. Besides diet control, blood cholesterol is lowered by exercise. Pharmaceutical preparations targeting different points of cholesterol metabolism have been used to lower blood cholesterol.

Hypolipidemic drugs

Drug therapy aims at lowering the total and LDL cholesterol, and raising the HDL cholesterol. A total to HDL cholesterol of 3.5 or less is strongly favored. With history of a heart attack (secondary prevention), the target LDL cholesterol is as low as 70 mg/dL.

Statin group of drugs are competitive inhibitors of the key enzyme of cholesterol synthesis, HMG CoA reductase. Inhibition of cholesterol synthesis leads to up regulation of LDL receptors and elimination of more cholesterol from the blood.

Inhibition of synthesis of mevalonate will also inhibit the synthesis of CoQ for the mitochondrial electron transport chain and the synthesis of dolichol pyrophosphate, a required cofactor in N-glycosylation of proteins in the endoplasmic reticulum. The most common side effects of statins include myalgia, headache, dizziness, and elevated plasma transaminases. Myopathy and rhabdomyolysis are rare with statin monotherapy at the approved dose ranges, but the risk increases with use of higher doses, interacting drugs and genetic predisposition. Statins are metabolized by a cytochrome P450 system that is inhibited by grapefruit juice often taken by patients going on a weight reduction diet, thus precipitating statin toxicity.

Normally, about 95% of secreted bile salts is reabsorbed (enterohepatic circulation). Cholestyramine and other resins that increase elimination of bile salts force the liver to use more cholesterol to synthesize bile salts, thus lowering the internal level of cholesterol in the hepatocyte. Decreased cholesterol within the cell increases LDL receptor expression allowing the hepatocyte to remove more LDL cholesterol from the blood. Care should be taken for fear of fat-soluble vitamin deficiency.

Ezetimibe decreases intestinal cholesterol absorption by inhibiting its transport protein. The same transporter may be needed for absorption of vitamins E and K. Phytosterols (plant sterols) also decrease the absorption of cholesterol from the intestine.

Niacin activates lipoprotein lipase. It also decreases fatty acid mobilization by inhibiting the fat cell lipase, thus decreasing hepatic production of VLDL. It also increases HDL synthesis.

Fibrates, e.g., clofibrate and gemfibrozil increase fatty acid oxidation, decrease the synthesis of VLDL by the liver, and increase their degradation by lipoprotein lipase. They also increase HDL production.

PCSK-9 is a protein that binds to LDL+ LDL receptor and marks the internalized receptor for degradation. It is upregulated by the administration of statins, limiting their efficacy in the treatment of hypercholesterolemia. Families having defective function of this protein have lower cholesterol levels and very low rates of coronary heart disease. Monoclonal antibodies, evolocumab and alirocumab, target this protein and inhibit LDL receptor degradation. More recently, a small interfering RNA (siRNA), inclisiran, has been introduced to block the production of PCSK-9 in liver cells using the RNA silencing mechanism.

Antisense therapy, mipomersen, was approved in 2013 in the United States to manage cases of homozygous familial hypercholesterolemia. It is a synthetic oligonucleotide that targets ApoB-100 mRNA, thus inhibiting VLDL synthesis. However, it has a high toxicity.

Gene therapy, Glybera (trade name), was introduced in 2012 in Europe for treatment of type I hyperlipidemia. It was discontinued in 2017 due to its high cost and rarity of the disease.

Cholesterol ester transfer protein (CETP) has been targeted by new research. It was observed that certain mutations of this protein gene lead to higher HDL cholesterol and lower cardiovascular risk, thus inhibitors of this protein are investigated for possible beneficial effect.

Other targets under investigation include diacyl glycerol acyl transferase, ATP citrate lyase, ACAT, microsomal triglyceride transfer protein (MTTP), HMG CoA synthase, lanosterol synthase, and squalene epoxidase. These proteins are involved in synthesis and metabolism of triglycerides, cholesterol, and VLDL.

For secondary hyperlipidemia, treatment of the disease that causes hyperlipidemia comes first. Other risk factors should also not be ignored.

Surgical interference

Surgical shortening of the intestine may be considered in life threatening conditions. Liver transplantation is also considered for provision of working LDL receptors in homozygous cases of familial hypercholesterolemia.

Hypolipoproteinemia

Abetalipoproteinemia is a rare genetic deficiency of ApoB-containing lipoproteins. Little or no chylomicrons, VLDL or LDL are found in the blood. Lipids accumulate in the liver and intestinal cells. This condition may be attributed to deficient microsomal triglyceride transfer protein (MTTP) responsible for incorporation of triglycerides in the lipoprotein particle.

Tangier disease is a defect of HDL. This is due to defect in the membrane cholesterol transporter to HDL (ATP-binding cassette transporter, ABCA1). In homozygous individuals, there is near absence of HDL. Cholesterol accumulates in the tissues. Hypertriglyceridemia results from absence of ApoC-II required for lipoprotein lipase.

Low cholesterol and triglyceride can be seen secondary to thyrotoxicosis, advanced liver cirrhosis, malnutrition, and malabsorption. Hypolipidemia is actually a laboratory marker of these conditions. The lowest normal plasma cholesterol is 140 mg/dL. A value of 100 mg/dL may be used as an indicator of malnutrition/malabsorption.

Sitosterolemia

Sitosterolemia (Phytosterolemia) is a rare autosomal recessive disease, characterized by hyperabsorption and decreased biliary excretion of dietary sterols, especially phytosterols. Dietary phytosterols passively enter intestinal cells, and normally are pumped back into the gut lumen by transporter proteins (ABCG5 and ABCG8). Mutations in either of the two genes result in sitosterolemia. There is hypercholesterolemia, tendon and tuberous xanthomas, premature development of atherosclerosis, and abnormal hematologic and liver function test results.

Lipoprotein X

This is an abnormal lipoprotein found in the plasma in cases of cholestasis. It is also found with familial LCAT deficiency. It is characterized by its high content of phospholipids and unesterified cholesterol, and its low content of protein, cholesterol esters, and triglycerides. The protein component is dominated by albumin located in the core, and apolipoprotein C on the surface of the particle.

Homocysteine

Elevated homocysteine in the plasma is an independent risk factor for atherosclerosis and coronary heart disease. It causes alteration of LDL by thiolation. Elevated plasma homocysteine may be prevented and treated by the vitamins: pyridoxine (B_6), cobalamin (B_{12}), and folic acid. This is explained with aminoacid metabolism (Lecture 14).

Study Questions

Choose one best answer for every question of the following:

1- What is the most important function of plasma lipoproteins?
(A) Keeping the osmotic pressure (oncotic pressure) of the plasma.
(B) Transporting lipids in the circulation in a water-miscible form.
(C) Predisposing to atherosclerosis and coronary heart disease.
(D) Adding turbidity to the plasma.

2- Which of the following may be located in the core of a lipoprotein particle?
(A) Cholesterol and lecithin.
(B) Cholesterol esters and triglycerides.
(C) Proteins and phospholipids.
(D) Cholesterol and proteins.

3- Fasting plasma does not contain
(A) chylomicrons.
(B) Very-low-density lipoproteins (VLDL).
(C) Low-density lipoproteins (LDL).
(D) High-density lipoproteins (HDL).

4- Free fatty acids are carried in the circulation on
(A) LDL.
(B) HDL.
(C) VLDL.
(D) albumin.

5- Lipoprotein lipase converts chylomicrons to
(A) chylomicron remnants.
(B) LDL.
(C) cholesterol and free fatty acids.
(D) apolipoproteins.

6- What is the fate of fatty acids released from chylomicrons?
(A) Triglyceride and phospholipid synthesis.
(B) Oxidation for energy production.
(C) Conversion to ketone bodies.
(D) Storage as glycogen.

7- The ability of HDL to take cholesterol from the tissues depends on
(A) esterification of surface cholesterol by LCAT.
(B) movement of free cholesterol from the particle surface to its core.
(C) ApoB-100 receptors.
(D) ApoE and ApoC.

8- What is meant by “good cholesterol”?
(A) Cholesterol of plant oils.
(B) HDL cholesterol.
(C) Cell membrane cholesterol.
(D) Non-esterified cholesterol.

9- Which of the following is not true about diet cholesterol?
(A) It is present in food from animal source.
(B) It is absent in food from plant source.
(C) It is absent in corn and olive oils.
(D) It is essential in diet.

10- Cholesterol is synthesized from
(A) Cortisol.
(B) Bile salts.
(C) Vitamin D.
(D) Acetyl CoA.

11- Which of the following is not a function of cholesterol?
(A) Formation of cell membrane.
(B) Biosynthesis of vitamin D.
(C) Bile salt production.
(D) Oxidation for energy production.

12- What is the main route for excretion of cholesterol?
(A) Urine.
(B) Stool.
(C) Sweat.
(D) Menstrual blood.

13- Which of the following carry cholesterol for excretion?
(A) Chylomicrons.
(B) VLDL.
(C) LDL.
(D) HDL.

14- Plasma cholesterol concentration may be lowered by taking diets characterized by
(A) High calorie.
(B) Low fiber.
(C) High lipid.
(D) Low animal fat.

15- The favorable plasma cholesterol concentration is
(A) 150-250 mg/dL.
(B) Below 200 mg/dL.
(C) 200-240 mg/dL.
(D) Above 240 mg/dL.

16- Which of the following is most dangerous?
(A) High plasma cholesterol.
(B) High HDL/LDL cholesterol.
(C) High HDL/total cholesterol.
(D) Low LDL/total cholesterol.

17- Low level of cellular HMG CoA reductase is most likely due to
(A) a vegetarian diet.
(B) use of bile acid sequestering resin.
(C) familial hypercholesterolemia.
(D) long-term high-cholesterol diet.

18- Which of the following enzymes is most important for cholesterol excretion?
(A) Lecithin:cholesterol acyltransferase (LCAT).
(B) Acyl CoA:cholesterol acyltransferase (ACAT).
(C) Pancreatic cholesterol esterase.
(D) HMG CoA reductase.

19- The risk of coronary heart disease is high with
(A) total cholesterol above 240 mg/dL.
(B) LDL cholesterol below 130 mg/dL.
(C) HDL cholesterol above 40 mg/dL.
(D) total/HDL cholesterol below 3.5

20- Familial hypercholesterolemia is characterized by all the following except
(A) Autosomal dominant.
(B) Deficient inhibition of HMG CoA reductase.
(C) Death of homozygous children from myocardial infarction.
(D) Low lipoprotein lipase activity.

21- Secondary hypertriglyceridemia is not characterized by
(A) association with diabetes, obesity, alcoholism, and some drugs.
(B) similarity to type IV hyperlipidemia.
(C) turbid plasma, glucose intolerance and increased risk of coronary heart disease.
(D) good response to treatment with statins.

22- Hypercholesterolemia may be treated by drugs that inhibit all the following except
(A) cholesterol absorption.
(B) cholesterol synthesis.
(C) LDL receptor catabolism.
(D) LCAT.

Brain Metabolism

Despite its relatively low weight, the brain consumes about 20% of all oxygen and about 25% of glucose consumed by all the body. The brain is dependent on aerobic oxidation of glucose. Glycogen store in the brain is limited. Long chain fatty acids are not used as energy source. Brain cells can use lactate in periods of intense physical activity. On prolonged fasting, the brain starts using ketone bodies. Up to two thirds of energy requirements are then to be obtained from ketone bodies. This diminishes the need for gluconeogenesis on the expense of muscle proteins and saves glucose for more needy cells, e.g., red blood cells.

Blood-brain barrier

The blood–brain barrier (BBB) is a highly selective semipermeable border of blood capillaries that prevents solutes in the circulating blood from non-selectively crossing into the extracellular fluid of the central nervous system. The blood-brain barrier is formed by endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillaries, and pericytes embedded in the capillary basement membrane. This system allows the passage of some molecules by passive diffusion, as well as the selective transport of various nutrients, ions, organic anions, and macromolecules that are crucial to neural function.

Different metabolites pass from the blood capillaries to brain cells via specific transporters. The glucose transporter GLUT1 is found on the capillary endothelial cells as well as astrocytes and neurons, while GLUT3 is associated with neurons. Monocarboxylic acid transporters MCTs transport lactic acid and ketone bodies: acetoacetic acid and β-hydroxy-butyric acid out of the capillaries and into the brain cells.

Astrocyte-neuron lactate shuttle

Astrocytes (glial cells) can store glycogen to be used for immediate energy needs, while the glycogen synthesizing machinery in neurons is kept inactive through proteasomal-dependent mechanisms. Astrocytes exhibit active glycolysis, producing lactate, which is preferentially used by neurons (rather than glucose) for aerobic energy production. Neurons oxidize glucose via the HMP pathway, which is necessary in mature neurons for its antioxidant function. Astrocytes preferentially degrade glucose anaerobically due to:

- Highly active phosphofructokinase (activated by fructose 2,6-bisphosphate).
- Low expression of mitochondrial glutamate/aspartate carrier, which is a component of the malate shuttle for cytosolic reducing equivalents.
- Low expression or phosphorylation-mediated inhibition of pyruvate dehydrogenase.

Glutamic acid-glutamine

Astrocytes express pyruvate carboxylase enzyme, a key enzyme in the main anaplerotic pathway in the brain, and can synthesize glutamate from glucose. They also capture the glutamate released by excitatory neurons into the synaptic clefts. They shuttle glutamate to neurons in the form of glutamine as a precursor for glutamate and γ-aminobutyric acid (GABA). Glutamine synthesis is also the way adopted by the brain for detoxifying ammonia.

Neurotransmitters: See lecture 9.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is an absolute requirement for the brain at all times?
(A) Glucose and oxygen.
(B) Glucose and ketone bodies.
(C) Ketone bodies and oxygen.
(D) Glucose and fatty acids.

2- In fasting conditions, glucose is provided for the brain by
(A) absorption from the intestine.
(B) glycogenolysis in the muscles.
(C) gluconeogenesis in the liver.
(D) glycogenolysis in the neurons.

3- Brain cells uptake glucose by
(A) GLUT1 and GLUT2.
(B) GLUT1 and GLUT3.
(C) GLUT4.
(D) SGLT2.

4- Glucose uptake by the brain is
(A) insulin-dependent.
(B) sodium-dependent.
(C) ATP-dependent.
(D) insulin-independent.

5- Astrocytes degrade glucose mainly to
(A) lactate.
(B) ketone bodies.
(C) carbon dioxide.
(D) carbon dioxide and water.

6- In brain cells, glucose is phosphorylated by
(A) glucokinase.
(B) hexokinase.
(C) glucose 6-phosphatase.
(D) phosphohexose isomerase.

7- Glucose 6-phosphate is mainly utilized in which pathway in neuronal cells?
(A) Aerobic glycolysis.
(B) Anaerobic glycolysis
(C) Glycogenesis.
(D) Pentose phosphate pathway.

8- What is the main function of HMP pathway in the mature brain cells?
(A) Mitochondrial transfer of reducing equivalents.
(B) Nucleic acid synthesis.
(C) Lipogenesis.
(D) Antioxidant.

9- Ammonia is normally detoxified in brain cells by synthesis of
(A) urea.
(B) glutamate.
(C) glutamine.
(D) α-ketoglutarate.

Diet importance and composition

The diet provides the materials required for building up the tissues, homoeostasis, and energy production. One nutritional Calorie (Cal) equals 1 kcal, i.e., the amount of energy required to raise the temperature of 1 kg of water 1^{o}C. The daily energy requirement can be calculated as 24 Cal/kg body weight, which represents the basal metabolic rate, with adding 30% for those leading a sedentary life or 50% for those doing hard work. For example, the energy requirement for a 70 kg doctor would equal 70 x 24 x 130/100, i.e., about 2200 Cal/day. The energy requirement per kilogram body weight is higher in children than in adults due to the higher relative surface area in children. The basal metabolic rate decreases during starvation and in persons going on a low-calorie diet for long periods, which may cause failure of weight reduction attempts.

Carbohydrates provide the main source of energy in the diet. The caloric value of carbohydrate or protein is 4 Cal/g, and that of fat is 9 Cal/g. It is recommended that the contribution of fat to the total caloric value of a balanced diet be 10-30%. The majority of fat should be unsaturated. The recommended daily allowance of proteins is 1 g/kg for adults, which increases to 2 g/kg or more for children. High biological value proteins are recommended. Vegetarians can get all the essential aminoacids by varying the source of plant protein in their food. The rest of the caloric value of the diet is then covered by carbohydrates. The balanced diet should also contain the recommended daily allowances of the micronutrients: vitamins and minerals.

Fibers in the diet are very important. Fibers add to the bulk of undigested food, stimulate peristalsis and prevent constipation. By filling the stomach, they help reduce the caloric intake. Taking complex carbohydrate, containing the food fiber, leads to slower gastric emptying and slower rise of blood glucose after food intake (lower glycemic index). Food fibers may also adsorb certain materials like cholesterol and carcinogens, in addition to their enhancement of getting rid of these materials by stimulating peristalsis. Therefore, increased dietary fiber is linked to lowering of blood cholesterol and protecting against colon cancer. An added value is that fibers are usually associated with other beneficial dietary components, e.g., vitamins and antioxidants.

Excess calories are stored as body fat. To lose weight, one should take less than the daily caloric need according to body weight. The recommended daily allowance of proteins and vitamins should be fully covered. One kilogram of adipose tissue is considered to contain 90% fat. This means that to lose 1 kg of body weight during a specified period, one should take about 8000 Calories less than that calculated according to body weight. There should be no restriction of fiber or water. In fact, taking more fiber and water is recommended.

Alcohol provides 7 Cal/g. Alcohol is empty calories, with no vitamins or other beneficial nutrients. The claimed antioxidant value of red wine can be easily, and better, obtained from fresh fruits. Chronic alcoholics are prime candidates for malnutrition diseases in developed societies.

Dietary Reference Intakes (DRI) is the general term for a set of reference values used to plan and assess nutrient intakes of healthy people. These values vary by age and sex. Recommended Dietary Allowance (RDA) is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97.5%) healthy people. Adequate Intake (AI) is established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy. Tolerable Upper Intake Level (UL) is the maximum daily intake unlikely to cause adverse health effects.

Typical Western diets are high in saturated fats, sugar, and refined grains. They are causally associated with development of cardiovascular disease, type 2 diabetes, and some types of cancer, including breast and colorectal cancer.

The Mediterranean diet, which is claimed to improve various clinical outcomes including a reduction in total mortality, is described as fruit- and vegetable-rich, high in monounsaturated fats (30-40% of total daily calorie intake), olive oil in particular, and high in legumes and fish, with a low to moderate intake of dairy and meat products.

DASH (Dietary Approaches to Stop Hypertension) diet includes vegetables, fruits and low-fat dairy foods, with moderate amounts of whole grains, fish, poultry and nuts, and low intake of saturated fat, cholesterol and total fat and limited sodium salt.

Special diets are mandatory for certain clinical conditions, e.g., low phenylalanine diet for phenylketonuria patients, lactose-free formula for babies with galactosemia, gluten-free diet for celiac disease, and lactose-free diet for lactose intolerance. Intestinal mucosal disaccharidase deficiency following enteritis may necessitate withholding milk and other disaccharide-containing food. Low FODMAPs (fermentable oligo-, di-, and monosaccharides and polyols) diet is recommended for the management of irritable bowel syndrome (IBS).

Dairy products have been a key component of dietary guidance, since milk is an excellent source of protein and calcium. The latest “MyPlate” reserves a cup of milk or yogurt (low-fat is preferred) next to the plate that is divided to a half: fruits and vegetables and a half: grains and proteins. However, a substantial proportion of the world’s adult population (65–70%) exhibits lactase nonpersistence, leading to lactose intolerance. For this reason, calls have been made for removal of dairy as a separate food group in dietary guidance.

Type 2 diabetic patients benefit from a weight-losing diet. A blood cholesterol lowering diet is low-calorie, low-fat, especially animal fat, and high-fiber. A low-carbohydrate diet (Atkins diet) is thought to reduce weight by decreasing the secretion of insulin. A ketogenic diet (high fat, low carbohydrate) may be helpful for controlling epilepsy. A high-fat, low-carbohydrate diet is also thought to reduce weight due to the need for gluconeogenesis from protein with the energy provided by fat oxidation.

Numerous fad diets are propagated, with no solid evidence of association with a better health. These include macrobiotic diet, paleolithic diet (paleo, caveman, or stone-age diet), and high-carbohydrate/low-fat diets.

Fad diets may lead to nutritional deficiencies. Adherence to a vegan diet, for example, leads to deficiency of vitamin B_{12}. Contrary to essential aminoacids, this vitamin cannot be secured by varying the types of plants in the diet.

It is important to avoid malnutrition when planning a special diet for the diseased. Patients with end-stage liver failure and hepatic encephalopathy, for example, are maintained on a low-protein and sodium diet. Malnutrition is a common complication in these patients. The supplementation of the diet with amino acids, antioxidants, vitamins as well as probiotics, in addition to meeting energy and protein requirements may improve nutritional status, liver function, and hepatic encephalopathy.

There are no standard methods for screening and diagnosing patients with malnutrition. Laboratory markers include plasma albumin, prealbumin, which is preferred due to its shorter half-life, and cholesterol among others. Laboratory markers are not reliable by themselves. They are subject for interference by liver, kidney and other diseases. They could be used as a complement to a thorough nutrition-focused physical examination (NFPE).

Obesity

Body mass index (BMI) is a measure of body fat. A normal value lies between 18.5 and 25 kg/m^2. Below 18.5 is underweight. From 25 to 30 is overweight. A value of 30 or above means obesity. Forty or above is morbid obesity.

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2}$$

In certain individuals, such as body builders, who have a large muscle mass, a high BMI does not relate to fat content.

For children and teens (2-20 years), charts of age versus BMI for boys and girls are used. Normal weight is 5-85 percentile. above 95 percentile is obese.

Obesity is associated with several serious and life-threatening complications resulting in significant increases in morbidity and mortality relative to lean individuals. These complications include insulin resistance (IR), type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), atherosclerosis, degenerative disorders such as dementia, and also in many instances cancers.

The combination of abdominal (central, apple shaped, android) obesity, hyperlipidemia, insulin resistance, pro-inflammatory status, and hypertension is clinically referred to as the metabolic syndrome. Adipose tissue in the visceral area relaeases its fatty acids to the portal circulation, i.e., directly to the liver, which may explain the liver pathology.

Individuals whose BMI is normal but have any one of the disorders of metabolic syndrome are considered metabolically obese, normal-weight (MONW) individuals. It was found that individuals in the upper normal-weight and slightly overweight BMI range are at increased risk of having the metabolic syndrome. Reducing weight substantially lowers all metabolic syndrome components, and risk of type 2 diabetes and cardiovascular disease. Other cardiovascular disease risk factors such as smoking should be corrected as a priority. Anti-diabetic agents that improve insulin resistance and reduce blood pressure, lipids and weight should be preferred for diabetic patients with metabolic syndrome. Bariatric surgery offers an alternative treatment for those with BMI ≥ 40 or 35–40 kg/m2 with other significant co-morbidity. Greater emphasis should be given to effective early weight-management to reduce risk in pre-symptomatic individuals with large waists.

Metabolic syndrome manifestations are believed to result from insulin resistance. Insulin resistance is thought to be caused by serine phosphorylation of insulin receptors and insulin receptor substrates: IRS-1 and IRS-2, by isoforms of protein kinase C (PKC) activated by elevated diacylglycerols (DAG) and acetyl CoA.

Metformin, besides inhibiting gluconeogenesis, causes partial inhibition of NADH oxidation by the respiratory chain, which leads to deficient ATP production and increased intracellular AMP. AMP-activated protein kinase recruits the glucose transporters GLUT4 to muscle cell membrane and inhibits acetyl CoA carboxylase, decresing malonyl CoA, thus enhances fatty acid oxidation and inhibits lipogenesis.

The improvement in insulin resistance seen with thiazolidinediones, e.g., Avandia (trade name) is thought to be mediated through their interaction with the peroxisome proliferator-activated receptor PPARγ. Clofibrate action is mediated by binding PPARα. PPARs are transcription factors that bind to DNA response elements (PPREs) and control multiple aspects of lipid metabolism.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is recommended to lose weight?
(A) Less fat and more carbohydrate.
(B) More fiber and less water.
(C) Less water and salt.
(D) Less sugar and fat.

2- To reduce weight, one should take more
(A) fiber.
(B) natural fruit juice.
(C) unsaturated fat.
(D) high biological value protein.

3- Basal metabolic rate is
(A) the energy expenditure in post-absorptive state and complete rest.
(B) roughly 48 Cal/kg/day for adults.
(C) relatively lower in children.
(D) measured during sleeping.

4- Failure of weight reduction for those going on a low-calorie diet for long periods is due to
(A) increased energy demands.
(B) decreased basal metabolic rate.
(C) increased utilization of food components.
(D) increased water intake.

5- Adherence to a vegan diet leads to deficiency of
(A) cholesterol.
(B) essential aminoacids.
(C) vitamin A.
(D) vitamin B_{12}.

6- How can vegans (strict vegetarians) ensure intake of all required essential aminoacids?
(A) Taking only plant proteins of high biological value.
(B) Adding animal proteins to their food.
(C) Varying the source of plant protein.
(D) Taking supplementary aminoacids.

7- The healthy food plate should contain
(A) the recommended daily allowances of vitamins and minerals.
(B) enough fiber.
(C) half the plate: fruits and vegetables.
(D) all the above.

8- Milk intake is avoided in which conditions?
(A) Phenylketonuria.
(B) Galactosemia.
(C) Lactose intolerance.
(D) All the above.

9- Kwashiorkor is distinguished from marasmus by
(A) edema.
(B) caloric deficiency.
(C) adequate intake of proteins.
(D) insufficient supply of carbohydrate.

10- Terminal events that issue upon extended fasting do <u>not</u> include
(A) regeneration of fat stores.
(B) depletion of tissue proteins leading to organ failure.
(C) acidosis and electrolyte imbalance.
(D) effects of lack of essential nutrients, e.g., vitamins and minerals.

Blood Glucose

Normal fasting blood glucose equals 70-110 mg/dL. It rises after meals, but is always below 180 mg/dL, the renal threshold. If blood glucose rises above the renal threshold, glucose appears in urine. Glucose is not detected in the urine of normal persons. Blood glucose concentration is controlled by hormones, mainly insulin and glucagon.

Sources of blood glucose:

1. Diet carbohydrate: Glucose is the main monosaccharide resulting from digestion of different carbohydrates. Fructose taken in the diet or resulting from digestion of sucrose can be converted to glucose. Galactose resulting from the digestion of lactose is also converted to glucose by the liver cells.
2. Liver glycogen: After meals, glucose is stored as glycogen mainly in the liver and muscle cells. In periods of fasting, glucose is obtained from the breakdown of liver glycogen. This process is activated by the hormone glucagon, which increases with fasting, and epinephrine (adrenaline) in emergencies.
3. Gluconeogenesis: This pathway is activated by fasting.

Absorptive (fed) state

This state begins with feeding. It is the period during which digestion and absorption occurs. Substrates for cell metabolism are provided by the diet. Insulin/glucagon ratio is high. All tissues produce energy by oxidation of glucose, which is available from intestinal absorption. Excess glucose and fatty acids are stored as glycogen and triacylglycerols.

Action of insulin:

- Facilitation of glucose uptake by muscle and adipose tissue (by GLUT4).
- Enhancement of glycolysis (glucose degradation) and acetyl CoA production.
- Activation of glycogenesis and inhibition of glycogenolysis and gluconeogenesis.
- Activation of HMP pathway for utilization of glucose and NADPH production.
- Activation of lipogenesis from glucose (acetyl CoA and NADPH) by activation of acetyl CoA carboxylase. It also inhibits lipolysis (hormone-sensitive lipase) and fatty acid oxidation (by malonyl CoA action on carnitine shuttle).
- Activation of protein synthesis from aminoacids, using glucose for energy.

Post-absorptive (fasting) state

This state begins with the completion of digestion and absorption. Insulin/glucagon ratio is low. Substrates for cell metabolism are provided by breaking down of cellular stores. Glycogenolysis in the liver cells, activated by the hormone glucagon, provides the immediate source of blood glucose. Liver cells maintain blood glucose by gluconeogenesis, which is activated by the hormones glucagon and cortisol. A period of 12 hours after a meal, e.g., overnight fasting ensures a post-absorptive state. Prolonged fasting (starvation) starts after 2-4 days of fasting. Liver glycogen is depleted in 10-16 hours. The end of glycogenolysis and beginning of gluconeogenesis overlap so as to prevent a drop of blood glucose below the physiological level. Glucose is no longer taken by muscle and fat cells. Skeletal muscles provide the aminoacids needed for gluconeogenesis. Lipolysis in adipose tissue provide fatty acids as a fuel for all but nerve and red blood cells. Ketone bodies generated by the liver cells are utilized by other cells including nerve cells, but not red blood cells.

Study Questions

Choose one best answer for every question of the following:

1- Which of the following is true about a high insulin/glucagon ratio?
(A) It is seen in post-absorptive period.
(B) It leads to increased utilization and storage of glucose.
(C) It enhances glycogenolysis and gluconeogenesis.
(D) It prevents drop of blood glucose on fasting.

2- A rapid uptake of glucose by the liver and muscle after a meal, followed by a drop of the uptake by the liver more than by the muscle indicates
(A) normal function of the liver and muscle cells.
(B) decreased K_m of glucokinase.
(C) prediabetes.
(D) abnormal response of muscle cells to glucagon.

3- Glucose given i.v. to hospitalized patients has which beneficial effect?
(A) Sparing tissue proteins.
(B) Increasing insulin secretion.
(C) Conversion to fat.
(D) Inhibiting ketone body production.

4- Glucose is the only cell fuel at all times for
(A) liver.
(B) brain.
(C) skeletal muscle.
(D) red blood cells.

5- Which of the following can use glucose, fatty acids and ketone bodies as cell fuel?
(A) brain.
(B) liver.
(C) muscles.
(D) red blood cells.

6- In the fasting state, aminoacids are used for formation of
(A) glucose.
(B) ketone bodies.
(C) proteins.
(D) both (A) and (B).

7- What fuel is used by adipocytes during fasting?
(A) Glucose.
(B) Fatty acids and glycerol.
(C) Fatty acids from the plasma lipoproteins.
(D) Fatty acids from the cell's own store.

8- In the fasting state, what fuel is initially used by muscle cells for an intense exercise?
(A) glucose.
(B) glycogen.
(C) fatty acids.
(D) aminoacids.

9- The rate of muscle proteolysis decreases by long-term fasting due to
(A) utilization of ketone bodies by the brain and decreased need for gluconeogenesis.
(B) availability of fatty acids as an energy source.
(C) inability to get rid of glutamine.
(D) saturation of the urea cycle.

10- Insulin is released from the pancreas in response to
(A) rise of blood glucose.
(B) aminoacids, particularly arginine.
(C) incretins (intestinal hormones).
(D) all the above.

11- Which of the following pathways is least expected to be active during fasting?
(A) TCA cycle.
(B) Lipolysis.
(C) Gluconeogenesis.
(D) HMP pathway.

Diabetes Mellitus

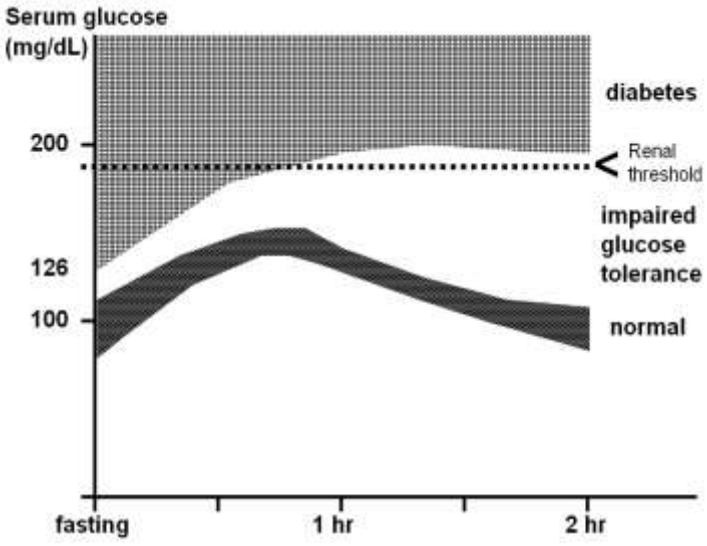
Diabetes mellitus is a disorder of the metabolism of carbohydrates, lipids, proteins and minerals. It affects all body systems. This is a disease due to lack of insulin, or the action of insulin. It is characterized by hyperglycemia and glucosuria. The clinical picture of diabetes includes polyuria, polydipsia, and polyphagia, with loss of weight.

Types of diabetes:

- Type 1, juvenile diabetes: Antibodies are formed against pancreatic islet cells. Insulin is absent. The disease appears in children or older individuals.
- Type 2, adult onset, non-insulin-dependent diabetes (NIDD): There is insulin resistance, usually with higher than normal insulin in blood. A drop of insulin secretion can then occur due to failure of pancreatic islet cells. The disease usually appears in obese adults. Insulin resistance probably results from action of isoforms of protein kinase C activated by elevated diacylglycerols (DAG) and acetyl CoA, leading to serine phosphorylation of insulin receptors and insulin receptor substrates: IRS-1 and IRS-2.
- Maturity onset diabetes of the young (MODY): There are no islet antibodies. It results from a number of mutations affecting different proteins. One of these is a mutated pancreatic glucokinase necessary for release of insulin from beta-cells. Glucokinase normally acts as glucose sensor for the beta-cells.
- Gestational diabetes: Hyperglycemia is due to the effect of anti-insulin hormones during pregnancy. It usually disappears after delivery. There is a high possibility of developing frank diabetes afterwards.

Oral glucose tolerance test (OGTT)

This is a standardized test of the body's capacity to deal with a glucose load. After overnight fasting, the patient is given a glucose load. Blood and urine samples are collected before taking glucose and every half hour. Glucose is measured in these samples and a curve that represents blood glucose concentration versus time is plotted.



In normal people, fasting blood glucose is in the normal range. Blood glucose rises after taking the glucose load and returns to fasting level within 2 hours. It does not exceed the renal threshold at any time, and glucose does not appear in urine. Higher levels of blood glucose indicate impaired glucose tolerance (pre-diabetes) or frank diabetes mellitus.

Diagnosis of diabetes

A diagnosis of diabetes is established if fasting blood glucose is at least 126 mg/dL, or two-hour or random blood glucose is more than 200 mg/dL. The measurement should be repeated if positive, unless the clinical picture is strongly suggestive of diabetes. Glycated hemoglobin may be used for diagnosis of type 2 diabetes.

Glycated hemoglobin

Glycated hemoglobin (HbA_{1c}) results from non-enzymatic glucose binding to terminal valine. It is measured as percentage of total hemoglobin. Its concentration depends on the average blood glucose during the erythrocyte life span. It is used clinically as a measure of diabetes control, and for diagnosis of type 2 diabetes.

Hemoglobin A_{1c} measurement gives an indication to the average glucose concentration in the previous 2-3 months, which reflects the effectiveness of treatment and the patient's compliance. It has the advantage of not needing fasting, and since it relates to the average blood glucose, it shows no day to day variation.

When using HbA_{1c} for diagnosis of diabetes, a healthy person should have a level below 5.7%. A level of 6.5% or above indicates diabetes. A level of 5.7-6.4% indicates pre-diabetes. The identification of individuals with pre-diabetes is important for their treatment with exercise and diet control before they progress to frank diabetes.

The use of HbA_{1c} is subject to certain precautions. It is lower with a shorter life span of red blood cells, e.g., sickle cell disease and with increased competing reactants, e.g., urea. It gives no indication to the fluctuation of blood glucose concentration. Relying on it for adjusting the dose of insulin carries the risk of occurrence of hypoglycemia. It is not useful for diagnosis and monitoring of gestational diabetes and is not recommended in pregnancy after the first visit.

Complications of diabetes:

- Hyperglycemic coma: ketoacidosis, hyper-osmolarity, electrolyte imbalance and dehydration.
- Abnormal glycation of proteins and formation of advanced glycation end products (AGE): involving collagen, LDL, and proteins of the lens, glomerular basement membrane, and nerve cells. This leads to diabetic microangiopathy, atherosclerosis, early cataract, nephropathy, and neuropathy.
- Osmotic effect of accumulated sorbitol: leading to cataract, nephropathy and neuropathy. Inhibitors of aldose reductase are being tried for prevention of diabetic complications.
- Decreased NADPH: duo to overconsumption by aldose reductase and decreased formation by HMP pathway. This leads to decreased antioxidant capacity.
- Elevated VLDL: due to increased hormone-sensitive lipase activity and decreased lipoprotein lipase. This leads, in combination with the glycation and oxidant stress, to atherosclerosis.

Glucosuria:

- Diabetes mellitus.
- Alimentary glucosuria.
- Gestational glucosuria.
- Renal glucosuria.

Hypoglycemia:

- Iatrogenic: insulin injection, especially with a missed meal or with muscular exercise.
- Neonatal: especially with a diabetic mother (high baby's insulin).
- Glycogen storage disease.
- Hereditary fructose intolerance.
- Deficient fatty acid oxidation: deficient energy and deficient allosteric effect of acetyl CoA.
- Alcoholism: inhibition of gluconeogenesis plus malnutrition.
- Vitamin deficiency, e.g., biotin.
- Advanced liver disease.
- Insulinoma.
- Cancer.
- Hypo-secretion of anti-insulin hormones.

Study questions

Choose one best answer for every question of the following:

12- Normal fasting blood glucose concentration in an adult is
(A) 40-100 mg/dL.
(B) 70-110 mg/dL.
(C) up to 140 mg/dL.
(D) up to 180 mg/dL.

13- A diagnosis of diabetes mellitus is accepted if blood glucose:
(A) fasting (8 hours) > 110 mg/dL.
(B) two-hour > 140 mg/dL.
(C) random sample > 200 mg/dL.
(D) any of the above.

14- Which of the following is true about blood glucose concentration in a normal person?
(A) It rises after meals to above 180 mg/dL.
(B) It returns to normal by the effect of glucagon hormone.
(C) Its rise triggers the release of glucagon from the pancreas.
(D) It does not reach up to the renal threshold.

15- With high insulin levels, diabetes mellitus is caused by which of the following?
(A) Insulin resistance.
(B) High food intake.
(C) Low glucagon level.
(D) Low cortisol secretion.

16- Glucose may be detected in the urine in which of the following conditions?
(A) Diabetes mellitus.
(B) Lactating females.
(C) All normal people following meals (alimentary glucosuria).
(D) All cases of normal pregnancy (gestational glucosuria).

17- Renal glucosuria is caused by
(A) high renal threshold for glucose.
(B) rapid absorption of glucose following meals.
(C) lactosuria mistaken for glucosuria.
(D) none of the above.

18- In a normal person undergoing oral glucose tolerance test,
(A) fasting blood glucose is higher than 125 mg/dL.
(B) blood glucose exceeds 180 mg/dL after 1 hour.
(C) glucose is detected in urine after 2 hours.
(D) glucose is absent in all urine samples.

19- In a normal person undergoing oral glucose tolerance test,
(A) blood glucose is always below 126 mg/dL.
(B) blood glucose reaches 200 mg/dL and drops before 2 hours.
(C) blood glucose reaches its peak after 2 hours.
(D) blood glucose returns to fasting level, or below it, in the 2-hour sample.

20- A diabetic oral glucose tolerance curve is characterized by
(A) high fasting blood glucose.
(B) rise of blood glucose to above the renal threshold.
(C) blood glucose not returning to fasting level by 2 hours.
(D) all the above.

21- In oral glucose tolerance test, blood glucose of fasting sample was 120 mg/dL, reached a peak of 180 mg/dL and dropped to 150 mg/dL by 2 hours. Glucose appeared in one urine sample. What is the most probable diagnosis of this case?
(A) Normal.
(B) Impaired glucose tolerance (pre-diabetes).
(C) Diabetes mellitus.
(D) Renal glucosuria.

22- Hypoglycemia is usually encountered in neonates, but is more severe when the mother is diabetic because of
(A) high insulin in the mother.
(B) high glucagon in the mother.
(C) high insulin in the neonate.
(D) high tolerance of the placenta to glucose.

23- Hypoglycemia due to deficient gluconeogenesis is usually encountered with
(A) alcoholism.
(B) biotin deficiency.
(C) deficiency of fatty acid oxidation.
(D) all the above.

24- Deficiency of fatty acid oxidation leads to hypoglycemia due to
(A) overconsumption of glucose.
(B) lack of energy for gluconeogenesis.
(C) lack of allosteric effect of acetyl CoA.
(D) all the above.

25- Hemoglobin A_{1c} (HbA_{1c})
(A) is glycated adult hemoglobin.
(B) has glucose attached to the iron of hemoglobin.
(C) decreases in diabetic patients.
(D) when elevated in early pregnancy indicates gestational diabetes.

26- Hemoglobin A_{1c} test is <u>not</u> recommended in which condition?
(A) After the first visit in pregnancy.
(B) Renal failure.
(C) Some hemoglobin variants and hemolytic anemia.
(D) All the above.

27- Hemoglobin A_{1c} measurement is <u>not</u> useful for
(A) diagnosis of type 2 diabetes.
(B) follow up of type 1 and type 2 diabetic patients.
(C) diagnosis of and monitoring gestational diabetes.
(D) adjusting the dose of anti-diabetic medication.

28- Type 1 diabetes is characterized by
(A) high insulin secretion.
(B) high C-peptide concentration.
(C) normal insulin receptors.
(D) obesity.

29- Type 1 diabetes is characterized by
(A) higher incidence of ketosis due to complete absence of insulin.
(B) being more common than type 2.
(C) absence in adult populations.
(D) poorly responding to insulin.

30- Insulin injection is used for treatment of patients with
(A) type 1 diabetes who did not respond to oral hypoglycemic drugs and diet control.
(B) type 2 diabetes with exhausted pancreatic β-cells.
(C) hypoglycemic coma.
(D) renal glucosuria.

31- Concentration of C-peptide in the plasma may be measured as
(A) an indicator of insulin production since it has a longer half-life than insulin.
(B) a differentiator between hypoglycemia due to endogenous insulin secretion and that due to insulin injection.
(C) a tumor marker of insulinoma.
(D) all the above.

Xenobiotic Metabolism

Xenobiotics (Greek: *xenos* means stranger) include drugs, insecticides, industrial chemicals, etc., which make more than 200,000 compounds. Toxic effects of xenobiotics include over-inhibition of intended enzymes or other enzymes, DNA changes leading to cancer, and oxidant effect leading to various pathological changes.

Most xenobiotics are metabolized, but some are excreted unchanged. Xenobiotics are metabolized primarily in the liver. Other organs involved include the kidneys, intestine and skin. Xenobiotic metabolism may inactivate a drug or a carcinogen but may also activate a prodrug or a procarcinogen. The terms “detoxification” and “biotransformation” have been used as synonyms for xenobiotic metabolism.

Approximately thirty different enzymes catalyze reactions involved in xenobiotic metabolism. These include cytochrome P450 enzymes, aldehyde oxidase, hydrolases, e.g., esterases, and conjugating enzymes, e.g., glucuronyl transferase. They usually make the foreign substance more water-soluble, and hence easier to excrete. They are not unique to foreign substances and may share in metabolism of endogenous compounds as well.

Cytochrome P450 proteins (CYPs) are so called since they show a distinct absorbance peak at 450 nm when reduced then exposed to carbon monoxide. They are a superfamily of heme-containing enzymes. Humans have 57 genes divided among 18 families of cytochrome P450 genes and 43 subfamilies. Each family has 40% or more sequence identity and a subfamily has more than 55% sequence identity. About 150 isoforms have been discovered. CYP1A1 means cytochrome P450 of family 1, subfamily A, the first member of the subfamily (It functions as aryl hydroxylase).

CYPs metabolize thousands of endogenous and exogenous chemicals, more than 50% of drugs and various pollutants and carcinogens as well as endogenous compounds. Human CYPs are primarily membrane-associated proteins located either in the inner membrane of mitochondria or in the smooth endoplasmic reticulum. Some CYPs metabolize only one (or very few) substrates, such as CYP19 (aromatase), while others may metabolize multiple substrates. They play important roles in hormone synthesis and breakdown, cholesterol synthesis, vitamin D metabolism, and metabolism of polyunsaturated fatty acids, eicosanoids, drugs, and endogenous metabolites.

Aldehyde oxidase is a molybdo-flavo-protein located in the cytosolic compartment of the liver and other organ cells. It catalyzes the oxidation of aldehydes into carboxylic acids, in addition to the hydroxylation of some heterocyclic compounds. It can also catalyze the oxidation of intermediate products of CYPs and monoamine oxidase (MAO). It plays an important role in the metabolism of several drugs.

Phase 1

The major reaction in phase 1 of xenobiotic metabolism is hydroxylation catalyzed by CYP monooxygenases (hydroxylases, mixed function oxidases). One atom of the oxygen molecule is inserted into an organic substrate (RH); the other atom is reduced to water:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Other reactions catalyzed by CYP enzymes include deamination, dehalogenation, desulfuration, epoxidation, peroxidation, and reduction. Phase 1 also includes hydrolysis and some other reactions not catalyzed by CYPs.

Phase 2

Phase 2 reactions include conjugation with glucuronte, sulfate, glutathione, certain aminoacids, e.g., glycine and glutamine, or acetyl or methyl groups. Excretion of bilirubin and salicylic acid requires conjugation with glucuronate, which is provided by UDP-glucuronate. Sulfation of phenols and hydroxylated steroid hormones requires 3'-phosphoadenosine, 5'-phsphosulfate (PAPS). Acetylation of aromatic amines or amides, e.g., aniline, sulfanilamides, histamine, and isoniazid requires acetyl CoA. Catechol O-methyltransferase for metabolism of catecholamines needs S-adenosylmethionine. Benzoic acid taken as a food additive or resulting from intestinal bacterial fermentation is converted to hippuric acid by conjugation with glycine. Glutathione S-transferases link different substrates to the cysteinyl part of glutathione. A mercapturic acid is formed by conjugation with glutathione, removal of glycine and glutamate, and N-acetylation of cysteine.

Drug interaction

Most of cytochrome P450 enzymes are inducible. Drug interaction may result from induction or inhibition of a drug-metabolizing enzyme by another drug or a natural product. This is especially important to consider when using drugs of vital importance to the patient, drugs with significant side-effects, or drugs with a narrow therapeutic index. Phenobarbital, a classical enzyme inducer, enhances the metabolism of warfarin, thus reducing its anticoagulant efficacy. Smoking induces aromatic hydrocarbon hydroxylases, which activate procarcinogens. Grape fruit inhibits the metabolism of statins, thus increasing their toxicity.

Varied drug metabolism

Any drug may be subject to an altered plasma concentration due to altered drug metabolism. The dose of some drugs should be adjusted individually for every patient according to their plasma level because of gene polymorphism of CYPs and aldehyde oxidase that leads to varied metabolism of drugs from one individual to another.

Study Questions

Choose one best answer for every question of the following:

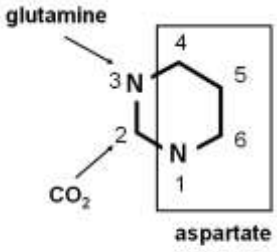
1- Which of the following reactions makes the substrate molecule most hydrophilic?
(A) Hydroxylation.
(B) Methylation.
(C) Acetylation.
(D) Sulfation.

2- Detoxification may not be an exact synonym of xenobiotic metabolism because
(A) not all xenobiotics are metabolized.
(B) metabolism may render a xenobiotic more toxic.
(C) both (A) and (B).
(D) neither (A) nor (B).

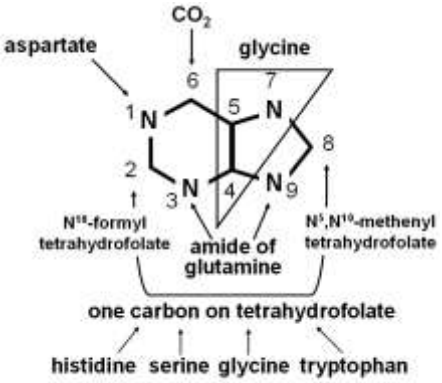
3- Which pathway of carbohydrate metabolism is most linked to bilirubin excretion?
(A) Glycolysis.
(B) Pentose phosphate pathway.
(C) Uronic acid pathway.
(D) Polyol pathway.

Purine and Pyrimidine Metabolism

Purine and pyrimidine bases are essential constituents of nucleic acids (Lecture 29). A nucleic acid is a polynucleotide. A nucleotide is a nucleoside-phosphate. A nucleoside is a base-pentose. Nucleic acids in the diet are digested by pancreatic enzymes, producing free bases. This is not the main source of purine and pyrimidine bases for cellular nucleic acid synthesis. Nucleotides are synthesized de novo. Aminoacids provide all the nitrogen and most of the carbon atoms of the purine and pyrimidine rings.



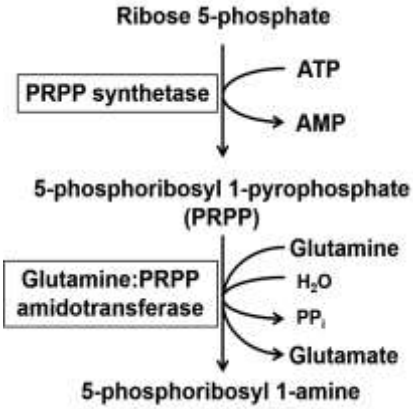
De novo synthesis of pyrimidines



De novo synthesis of purines

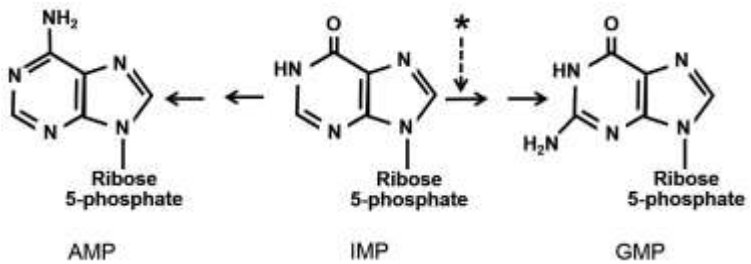
Purine nucleotide synthesis

The starting material for purine nucleotide de novo synthesis is 5-phosphoribosyl 1-pyrophosphate (PRPP), which is synthesized by the reaction of ribose 5-phosphate produced by HMP pathway with ATP. This reaction is inhibited by formed purine and pyrimidine nucleotides. This reaction is not unique for purine nucleotide synthesis.



The committed step in purine nucleotide de novo synthesis is the following reaction in the pathway, the glutamine:PRPP amidotransferase, which is activated by PRPP and inhibited by IMP, AMP, and GMP (shown below). In this reaction, the amide nitrogen of glutamine becomes N^9 of the purine ring attached to $C^{1'}$ of the 5'-phosphoribose.

A sequence of reactions leads to the formation of the first purine nucleotide, inosine monophosphate (IMP), in which the purine base is hypoxanthine.

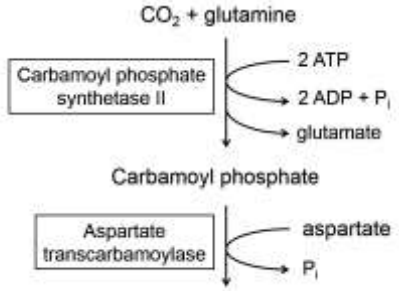


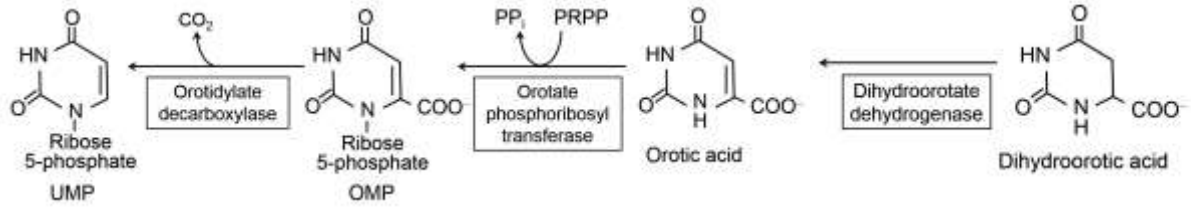
IMP is then converted to adenosine monophosphate (AMP), with the 6-amino group provided by aspartate, and guanosine mono-phosphate (GMP), with the 2-amino group provided by the amide of glutamine. AMP formation needs GTP, while formation of GMP needs ATP as energy source.

IMP dehydrogenase, required for GMP synthesis (first step, oxidizing hypoxanthine to xanthine) is a target for inhibition by the immunosuppressant mycophenolate and potential antiviral therapy. Mutations of its gene are associated with a type of autosomal dominant retinitis pigmentosa and Leber's congenital amaurosis.

Pyrimidine nucleotide synthesis

The synthesis of pyrimidine nucleotides is different in that the pyrimidine ring is synthesized first then phosphoribosylated. The initial and committed step is the formation of carbamoyl phosphate by the cytosolic carbamoyl phosphate synthetase II. This enzyme is different from that of the mitochondria in having the amide of glutamine, not ammonia, as the nitrogen source and not needing activation by N-acetyl glutamate. It is activated by PRPP and inhibited by uridine triphosphate (UTP). The second reaction, aspartate transcarbamoylase, is inhibited by cytidine triphosphate (CTP).





The pyrimidine ring formed is orotic acid, which is phosphoribosylated by PRPP to produce orotidine monophosphate (OMP). The latter is decarboxylated to give uridine monophosphate (UMP). Uridine triphosphate (UTP) is 4-aminated to cytidine triphosphate (CTP) by glutamine and ATP.

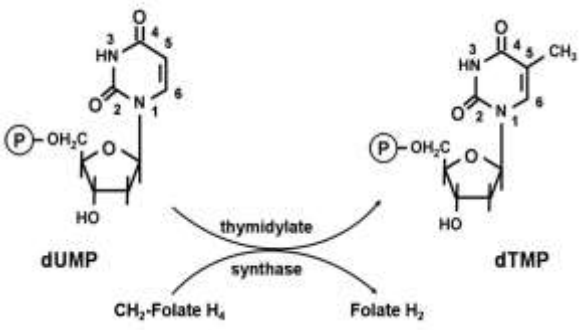
Inhibitors of the mitochondrial enzyme dihydroorotate dehydrogenase are immunosuppressants and antiviral drugs. The enzyme gene mutations cause Miller syndrome.

Hereditary orotic aciduria is an autosomal recessive disorder caused by a deficiency in the enzyme UMP synthase, a bifunctional protein that includes the enzyme activities of orotate phosphoribosyl transferase and orotidylate decarboxylase. Infants show failure to thrive, megaloblastic anemia, and orange crystalluria (orotic aciduria). This condition can be treated by giving uridine in the diet.

Allopurinol competes with orotic acid for orotate phosphoribosyl transferase, and the unusual nucleotide formed inhibits orotidylate decarboxylase, thus producing orotic aciduria. Orotic aciduria is seen with ornithine transcarbamoylase deficiency due to leaking of excess carbamoyl phosphate from the mitochondria (Lecture 14).

Deoxyuridine monophosphate (dUMP) is 5-methylated to deoxythymidine monophosphate (dTMP) by thymidylate synthase and N^5,N^{10}-methylene tetrahydrofolate. Thymidylate synthase reduces methylene to methyl and oxidizes tetrahydrofolate to dihydrofolate.

Folic acid deficiency leads to deficient synthesis of DNA, with megaloblastic anemia.



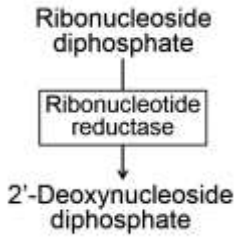
Thymidylate synthase is inhibited by 5-fluorouracil (after being activated to 5-fluoro-dUMP). Folate antagonists, e.g., methotrexate and trimethoprim inhibit dihydrofolate reductase. Leucovorin (formyl tetrahydrofolate) ameliorates the action of methotrexate and potentiates that of 5-fluorouracil. Sulfonamides inhibit folic acid synthesis in bacteria. Vitamin B_{12} deficiency leads to functional folate deficiency (Lecture 15).

Nucleoside di- and triphosphates

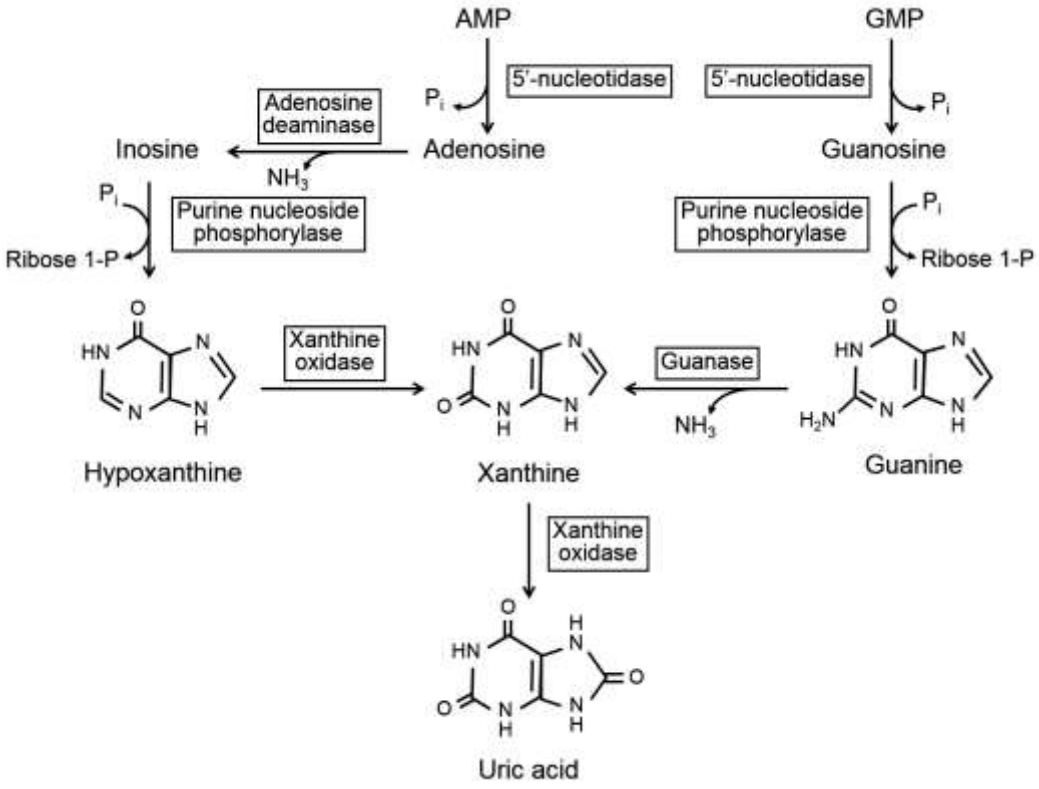
Kinase enzymes phosphorylate nucleoside monophosphates to nucleoside di- and triphosphates, using ATP as the phosphate donor. Adenylate kinase (myokinase) has a specific role in cell energy storage and utilization (Lectures 10,17).

Deoxy-nucleotides

2'-Deoxynucleotides required for DNA synthesis are obtained by reduction of their corresponding ribonucleoside diphosphates. The enzyme ribonucleotide reductase is inhibited by the deoxynucleotides dATP and dGTP. It is also inhibited by hydroxyurea used to induce the synthesis of γ-chain of hemoglobin in cases of sickle cell disease.



Purine nucleotide catabolism



The end product of catabolism of purine nucleotides is uric acid, which is excreted in the urine. Serum uric acid concentration is considered a kidney function test. Uric acid has a low solubility, and may precipitate forming uric acid crystals. These crystals if formed in the tissues can cause inflammation, typically manifested as gouty arthritis. Urinary urate stone formation is another problem that can occur with high excretion of uric acid.

Uric acid is the main excreted nitrogenous product in uricotelic organisms, e.g., birds and reptiles. Man is ureotelic, excreting nitrogen mainly as the highly soluble urea. In lower primates and other mammals, not in man, uricase enzyme hydrolyzes uric acid to allantoin, a highly water-soluble product.

Pyrimidine nucleotide catabolism

Degradation of pyrimidines results in the formation of water-soluble compounds.

Adenosine deaminase deficiency

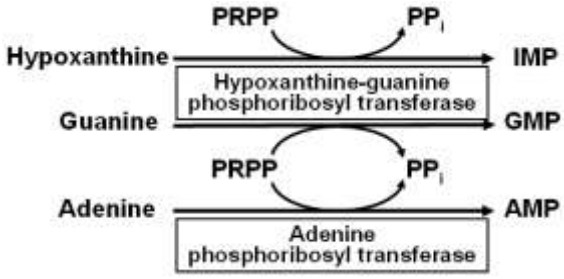
Adenosine deaminase deficiency results in severe combined immunodeficiency (SCID), involving both T and B lymphocyte dysfunction (bubble babies). It is characterized by large buildup of dATP in red blood cells. The accumulation of dATP can inhibit ribonucleotide reductase, hence DNA synthesis. Affected children usually die before the age of two years from overwhelming infection. Treatment is by enzyme replacement therapy, matched hematopoietic cell transplant, or gene therapy. This disease was the first to be treated by gene therapy in 1990.

Purine nucleoside phosphorylase deficiency

The deficiency of purine nucleoside phosphorylase leads to accumulation of dATP and dGTP. This inhibits ribonucleotide reductase, hence DNA synthesis. However, only T cells are affected, producing a less severe immune deficiency with normal production of antibodies. Increased dGTP may activate apoptosis in T lymphocytes.

Free base salvage

Free purine bases: hypoxanthine, guanine and adenine are phosphoribosylated using PRPP. The utilization of PRPP in this salvage pathway may exceed its utilization in de novo purine nucleotide synthesis. Two enzymes are involved as shown.



Lesch-Nyhan syndrome is an X-linked recessive disease associated with a virtually complete deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase. There is increased utilization of PRPP in de novo purine synthesis, resulting in hyperuricemia. Characteristic neurologic features include cerebral palsy, self-mutilation and involuntary movements. The disease is fatal in a few years.

Deficiency of adenine phosphoribosyl transferase leads to accumulation of adenine, which is oxidized to 2,8-dihydroxy-adenine, which precipitates as renal stones.

Free pyrimidines are not salvaged, except for orotic acid in the de novo synthesis pathway by the enzyme orotate phosphoribosyl transferase. The same enzyme can phospho-ribosylate the pro-drug 5-fluorouracil.

Nucleoside salvage

Of the purine nucleosides, only adenosine and 2'-deoxyadenosine are phosphorylated by adenosine kinase to AMP and dAMP, with ATP as the phosphate donor. Pyrimidine nucleosides and deoxynucleosides are efficiently salvaged. Deoxycytidine kinase, in addition to phosphorylating deoxycytidine, can also phosphorylate 2'-deoxyadenosine and 2'-deoxy-guanosine. Pyrimidine analogue drugs are usually given as nucleosides.

Uric acid

Uric acid is the final catabolite of purine bases. It is formed by the enzyme xanthine oxidase that catalyzes oxidation of hypoxanthine to xanthine and xanthine to uric acid. Uric acid has a pK_a of 5.75 (for N^9). At the physiological plasma pH, uric acid is present mainly as sodium urate, which is seventeen times more soluble than the protonated uric acid. Deposition of sodium urate crystals in soft tissues forms the characteristic tophi and ignites an inflammatory process manifested as gouty arthritis. At an acidic pH of urine, below the pK_a of uric acid, the protonated species is the predominant form. Uric acid crystals deposit easily, with the possibility of urinary stone formation.

Hyperuricemia (plasma uric acid more than 7 mg/dL) may result from overproduction or under-excretion. Plasma uric acid concentration is a renal function test, and hyperuricemia may be caused by renal insufficiency as seen with chronic renal failure. Overproduction may be observed with primary gout, where no solid reason for hyperuricemia can be identified. This overproduction may be attributed to hyperactive PRPP synthetase, with a higher V_{max}, a lower K_m for ribose 5-phosphate, or lower responsiveness to feedback inhibition by the formed nucleotides.

Overproduction hyperuricemia may be secondary to a defined disease. Cancerous conditions like leukemia may be the cause. Enzyme defects like deficient hypoxanthine-guanine phosphoribosyl transferase, either partial or complete (Lesch-Nyhan syndrome), can cause hyperuricemia. Glucose 6-phosphatase deficiency (Von Gierke disease) leads to shifting of glucose 6-phosphatase to the HMP pathway, with overproduction of ribose 5-phosphate that goes for production of PRPP and purine nucleotides. Deficiency of glucose 6-phosphatase also leads to lactic acidosis, which antagonizes the excretion of uric acid by the kidneys.

Purines in the diet may be another contributor to hyperuricemia in susceptible individuals. Food rich in purines includes meat, especially organ meat, e.g., liver, kidney, etc. Alcohol intake can exacerbate gouty arthritis by causing lactic acidosis that interferes with uric acid excretion and decreases urate solubility in the tissues.

Remember that hyperuricemia is not gout. Gouty arthritis, especially of the metatarso-phalangeal joint of the big toe is usually associated with hyperuricemia. Treatment of gout is centered on stopping the inflammation by colchicine and anti-inflammatory agents, then controlling hyperuricemia.

Uricosuric drugs and urine alkalinization increase urate excretion. Xanthine oxidase inhibitors, e.g., allopurinol and febuxostat decrease the formation of uric acid. The accumulated hypoxanthine is phosphoribosylated and forms purine nucleotides, thus inhibiting more purine and uric acid synthesis. Other factors like diet and alcohol intake should also be controlled.

One good aspect of uric acid is its antioxidant power. It is to be noted that the ability to synthesize ascorbic acid is lost in humans, together with the uricase machinery that converts uric acid to allantoin. Therefore, uric acid may be partly replacing ascorbic acid as an anti-oxidant in humans.

Pseudogout (false gout) results from deposits of calcium pyrophosphate crystals in the cartilage (calcium pyrophosphate dihydrate crystal deposition disease, CPPD). It commonly affects the knees and wrists. Less often, it can involve the hips, shoulders, elbows, knuckles, toes or ankles. Rarely it affects the neck and causes neck and shoulder pain, headache and in some cases fever. CPPD affects both men and women, more frequently over age 60. It is more common with disturbed calcium and phosphorus metabolism.

Study Questions

Choose one best answer for every question of the following:

1- Nucleotides function as
(A) components of nucleic acids.
(B) coenzymes and regulators of metabolism.
(C) phosphate donors and energy currency.
(D) all the above.

2- A nucleotide should have a base such as
(A) adenine.
(B) nicotinamide.
(C) flavin.
(D) any of the above.

3- Which of the following is not correct?
(A) Purine and pyrimidine bases are essential constituents of nucleic acids.
(B) A nucleotide is a nucleoside-phosphate.
(C) A nucleoside is a base-pentose.
(D) A nucleic acid is a polynucleoside.

4- Thioredoxin is
(A) an enzyme that reduces xanthine to hypoxanthine.
(B) a component of the salvage pathway.
(C) the cofactor for thymidylate synthesis.
(D) the source of reducing equivalents for deoxynucleotide production.

5- Immunosuppressant drugs may target enzymes of nucleotide synthesis such as
(A) inosine monophosphate dehydrogenase.
(B) dihydroorotate dehydrogenase.
(C) dihydrofolate reductase.
(D) all the above.

6- Xanthine oxidase converts
(A) hypoxanthine to xanthine.
(B) uric acid to xanthine.
(C) xanthine to hypoxanthine.
(D) xanthine to allantoin.

7- Which of the following is true about uric acid?
(A) It is the end product of protein catabolism in man.
(B) It has a limited solubility in the urine and body fluids.
(C) It precipitates with increase in pH.
(D) Hyperuricemia is clinically known as gout.

8- At the physiological plasma pH, uric acid is present mainly as
(A) oxidized uric acid.
(B) albumin conjugate.
(C) uric acid crystals.
(D) sodium urate.

9- Urinary uric acid stones are treated by
(A) acidification of urine.
(B) decreasing water and fluid intake.
(C) high doses of ascorbic acid.
(D) none of the above.

10- A good aspect about uric acid in man may be its
(A) conversion to ascorbic acid.
(B) conversion to allantoin.
(C) antioxidant power.
(D) all the above.

11- Purine-rich food includes
(A) meat, especially organ meat, e.g., liver, kidney, etc.
(B) fruits and vegetables.
(C) both (A) and (B).
(D) neither (A) nor (B).

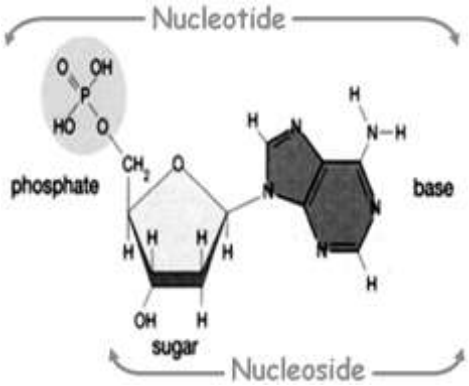
Molecular Biology

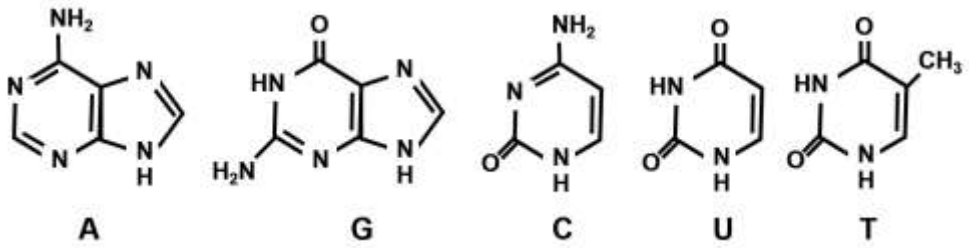
Central dogma of molecular biology

Proteins constitute a major component of living tissues. By their catalytic and regulatory functions, proteins control the composition and function of all tissue components. Although all proteins are formed from the same twenty aminoacids, they differ from one species to another and from one individual to another. Protein structure is determined by a code that is unique to each individual. This coded information is stored in the cell nucleus (little in the mitochondria) in the form of DNA (deoxy-ribonucleic acid). RNA (ribonucleic acid) is the means of using this coded information to synthesize specific proteins by the cell ribosomes. Thus, the genetic information is stored in DNA, transferred to RNA, then to proteins. The flow of genetic information in a living cell is in the direction: DNA $\rightarrow$ RNA $\rightarrow$ protein.

Nucleic acid structure

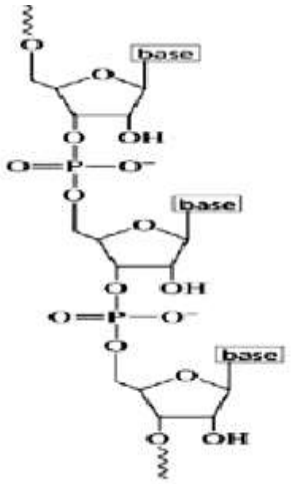
A nucleic acid molecule is a chain of nucleotides. A nucleotide is a nucleoside phosphate. A nucleoside is a nitrogenous base connected to a pentose. The pentose is ribose in RNA and 2'-deoxy-ribose in DNA. Nitrogenous bases are purine bases: adenine (A) and guanine (G), and pyrimidine bases: uracil (U), cytosine (C) and thymine (T). The bases in RNA are A, G, U, and C. The bases in DNA are A, G, T, and C.



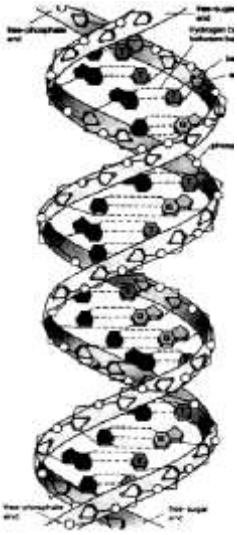


RNA

RNA molecule is a single strand of nucleotides connected by phosphodiester bonds between the 3' and 5' carbons of successive ribose moieties. Note that numbers without a prime denote the carbon and nitrogen atoms of the base ring structure. Carbon 1' of ribose is connected by a glycosidic bond to nitrogen 1 of a pyrimidine base or nitrogen 9 of a purine base. The nucleotide chain has a 5'-phosphate on one end and a free 3'-hydroxyl group on the other end.



DNA



DNA is double stranded; each strand is a chain of deoxy-nucleotides connected by phosphodiester bonds. The deoxyribose-phosphate chains forms the backbone of DNA strands and are located on the outside of the molecule. The two strands are coiled forming a double helix (Watson and Crick model). Two spiral grooves are seen along the DNA molecule: a major and a minor grooves. The two strands are anti-parallel: one strand in a 5'→3' direction, the other in the 3'→5' direction. The two strands are complementary to each other: A faces T and C faces G. Therefore, in DNA the number of A equals the number of T, and C equals G (Chargaff rule). Two hydrogen bonds (Lecture 1) between the bases A and T and three hydrogen bonds between the bases C and G of the opposite strands keep the two strands together. The specific sequence of nucleotides of DNA constitutes the genetic information of the cell.

The human genome (total DNA) is two meters long, packed into 23 pairs of chromosomes. Each chromosome is formed of two chromatids joined at the centromere; each chromatid contains one DNA molecule. DNA is packed with the help of nuclear proteins, mainly histones, forming the cellular chromatin.

Histones are basic proteins, rich in arginine and lysine, which facilitate their binding to the acidic DNA (phosphate groups). The basic packed structures are the nucleosomes, which by further coiling form chromatin fibers, and finally the chromosomes. This structure is stabilized by attachment to non-histone protein scaffold (the nuclear matrix). Other nuclear proteins include enzymes, transcription factors and hormone receptors, making more than 1000 non-histone DNA-binding proteins.

Denaturation-Reannealing

Denaturation of DNA is the separation of the two strands by disruption of the hydrogen bonds. This is achieved by changing the pH (thus changing base ionization) or heating. G-C pair is more resistant to denaturation than A-T due to more hydrogen bonds. Denaturation can be monitored by increased absorbance at 260 nm. The temperature at which the DNA is half denatured is called melting temperature (Tm). Reannealing is the reformation of the double helix, e.g., by cooling.

Study Questions

Choose one best answer for every question of the following:

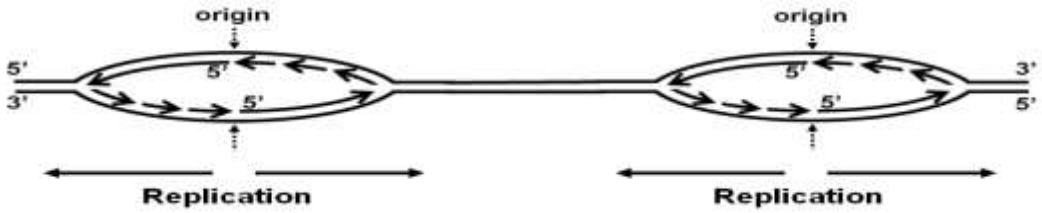
1- Which of the following is the most proper description of RNA structure?
(A) A double stranded molecule.
(B) A chain of nucleosides.
(C) A ribose-containing molecule.
(D) A polypeptide.

2- If one strand of a piece of DNA contains: 20 As , 25 Gs, 30 Cs, and 22 Ts, then the double-stranded DNA contains
(A) 40 As, 50 Gs, 60 Cs, and 44 Ts.
(B) 44 As, 60 Gs, 50 Cs, and 40 Ts.
(C) 45 As, 45 Gs, 52 Cs, and 52 Ts.
(D) 42 As, 55 Gs, 55 Cs, and 42 Ts.

Replication

For the cell to divide into two daughter cells, its DNA should be doubled. This takes place during the S (synthesis) phase of cell cycle. The synthesis of new DNA is called replication, since the new DNA is exactly the same or a replica of the old one. In fact, the new DNA molecule contains one strand from the old molecule that acts as a template for synthesizing a new complementary strand according to the base pairing rule: A-T and C-G. Thus, DNA replication is semi-conservative.

Initiation of the replication of a long human DNA molecule occurs at multiple replication origins called autonomous replicating sequences (ARS), which are rich in A-T to facilitate their melting. The two strands separate forming a replication bubble at each site. For the circular chromosome of prokaryotes, there is only one replication origin. Replication proceeds in both directions, forming a replication fork in each direction, which extends away from the origin of replication.



Disruption (denaturation) of the double helix is catalyzed by the enzyme helicase. The replication fork is stabilized by single strand binding proteins, which also prevent intra-chain hydrogen bonding and protect the single strands against the action of nuclease enzymes. Bloom syndrome, attributed to helicase deficiency, is a rare hereditary disease characterized by short stature, delayed growth, skin lesions and readiness to develop cancer. Intercalators like doxirubicin, actinomycin D, and cisplatin bind to DNA, interfering with replication. They are used in different cancers.

The unwinding of the helix creates a tension ahead and tends to form super-coils. Topoisomerases are enzymes that relieve this tension by cutting and resealing one strand (topoisomerase I) or the two strands (topoisomerase II) of DNA. Topoisomerase II can also introduce negative supercoils ahead of the replication fork to neutralize the positive supercoils introduced later by helicase. Gyrase (topoisomerase II of bacteria) is the target for inhibition by the quinolone antibiotics (e.g., nalidixic acid and ciprofloxacin). Topoisomerases in humans may be the target of inhibition by anti-cancer drugs.

The substrates for building the new strand are the deoxy-nucleoside triphosphates. There should be a primer that receives the first added deoxy-nucleotide. This primer is a short (about 10) ribonucleotide sequence (RNA) formed by primase enzyme. The enzyme DNA polymerase (DNA polymerase III in prokaryotes) adds a nucleoside 5'-phosphate that matches the template to the 3'-OH of the preceding nucleotide of the new chain, with release of pyrophosphate (thus using two high energy units). AZT (3'-azido-2',3'-dideoxythymidine) used for chemotherapy of HIV provides a nucleotide with no free 3'-hydroxyl group, which upon incorporation in the newly synthesized strand stops replication.

The new strands are synthesized in the direction 5'$\rightarrow$3'. Since the two template strands are anti-parallel, it results that at each replication fork, one strand is synthesized in the direction of the fork movement and the other in the opposite direction. The first is called the leading strand and is synthesized continuously. The second is the lagging strand and is synthesized in pieces called Okazaki fragments, about 1000 deoxy-nucleotides each.

RNase H by its 5'→3' exonuclease activity (an exonuclease splits off one nucleotide at a time from the end of a chain) removes the nucleotides of the primers, and DNA polymerase adds deoxy-nucleotides instead (DNA polymerase I in prokaryotes performs both functions). A ligase enzyme then binds the loose ends of deoxy-nucleotides pieces.

Meeting of the replication forks moving towards each other from neighboring origins plus the ligase enzyme finish the replication of the whole molecule. Energy required for unwinding and ligation is provided by ATP hydrolysis. It takes only nine hours to replicate the whole $3X10^9$ base pair human genome.

DNA polymerase rarely makes mistakes while inserting a matching nucleotide into the newly synthesized strand. DNA polymerase has the ability to recheck the already inserted nucleotide before inserting a new one. If the last inserted nucleotide is mismatched to the template, DNA polymerase removes it by its 3'→5' exonuclease activity and adds a correct one, a proofreading capability of the enzyme.

Eukaryotic DNA Polymerases:

- DNA polymerases α (alpha), δ (delta), and ε (epsilon) synthesize the leading and lagging strands during replication. Primase is a subunit of DNA polymerase α (pol α/primase complex).
- DNA polymerases β (beta) and ε (epsilon) participate in DNA repair.
- DNA polymerase γ (gamma) replicates mitochondrial DNA.

Telomeres and telomerase

Telomeres are non-coding tandem repeats of hexameric sequence at the ends of linear DNA molecules in eukaryotic chromosomes. DNA polymerase cannot complete synthesis of the 5'-end of each strand since it can only add to an existing polynucleotide or poly-deoxynucleotide. Therefore, with each round of replication in most normal cells, the telomeres are shortened. This contributes to the aging of cells, because eventually the telomeres become so short that the chromosomes cannot function properly and the cells die.

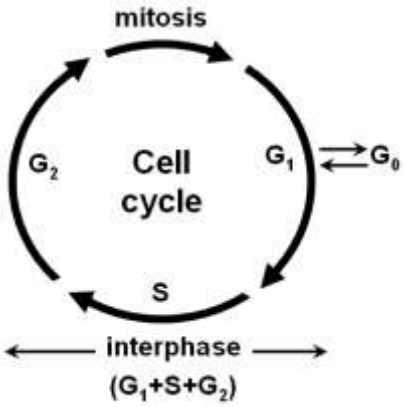
Telomerase is an enzyme in eukaryotes used to maintain the telomeres. It contains a short RNA template complementary to the tandem repeats of the 3'-end telomere, as well as telomerase reverse transcriptase (RNA-dependent DNA polymerase) activity. It binds by base complementarity to the already longer 3'-end and elongates it by its reverse transcriptase activity. A primer and a new DNA can be synthesized on the elongated telomere and ligated to the pre-existing 5'-end of the short strand. Telomerase is thus able to replace telomere sequences that would otherwise be lost during replication. Prokaryotes, with their circular DNA need no telomerase.

Normally telomerase activity is present only in embryonic cells, germ (reproductive) cells, and stem cells, but not in somatic cells. Cancer cells often have relatively high levels of telomerase, preventing the telomeres from becoming shortened and contributing to the immortality of malignant cells. Targeting telomerase may be useful for cancer treatment.

Histones

Histones are associated with one of the old strands. New histones are synthesized and associated with the other DNA molecule. Thus histones are conservedly doubled during the semiconservative DNA replication.

DNA repair



Mismatch repair: Replication errors may escape the proofreading system. These should be corrected in G_2 (gap 2) phase. A mismatch repair system identifies mismatches of replication, removes a sequence that contains the error and replaces it with a properly matched sequence. Mutations of the proteins involved in mismatch repair may lead to hereditary non-polyposis colon cancer (HNPCC, Lynch syndrome). It is responsible for about 5% of cases of colon cancer, and is associated with other cancers as well.

Base excision repair: Abnormal bases may result from deamination of cytosine (producing uracil) or adenine (producing hypoxanthine), which if not repaired would be paired during replication to A and C instead of G and T, leading to a permanent mutation. Base deamination can be spontaneous or chemically induced, e.g., by nitrous oxide. Bases can also be lost spontaneously. DNA glycosylase removes the wrong base by cleaving the N-glycosidic bond, creating an AP (apurinic, apyrimidinic) site. Spontaneously losing a base also creates an AP site. AP endonuclease (an endonuclease hydrolyzes the phosphate ester bond within a chain) cuts the strand at the 5'-side of the AP site. AP lyase removes the empty sugar-phosphate residue. DNA polymerase fills the gap and ligase seals the DNA.

Repair of thymine dimer: Thymine dimer results from ultraviolet (UV) light-induced covalent linking of two adjacent thymine bases. This halts replication of DNA. The damage is corrected in eukaryotes by nucleotide excision repair. A UV-specific endonuclease multienzyme complex (excinuclease) identifies the dimer, unwinds DNA by its helicase activity and cuts the DNA strand on both sides of the damage, removing an oligonucleotide that includes the thymine dimer. DNA polymerase and DNA ligase fill the gap and seal the strand. Mutations affecting this repair mechanism can lead to xeroderma pigmentosum, an autosomal recessive disease characterized by abnormal sensitivity to sunlight and multiple skin lesions including cancer.

Double-strand breaks: In general, DNA repair depends on excision of the damaged area and filling the gap using the other sound strand as a template. Double-strand breaks, induced by radiation or free radicals, cannot be repaired by this mechanism and are potentially lethal to the cell. Repair by homologous recombination (HR) uses the enzymes that normally perform genetic recombination between homologous chromosomes during meiosis. Non-homologous end-joining (NHEJ) is error prone, may lead to loss of DNA, and is mutagenic. Mutations of the BRCA1 and BRCA2 involved in HR increase the risk of developing breast cancer.

The tumor suppressor p53 is a 53 kDa protein that plays a key role in G_1 and G_2 checkpoint controls for monitoring and repairing DNA. It is an important transcription factor that helps in arresting cell division and providing enzymes needed for DNA repair. If DNA damage is beyond repair, the affected cell undergoes apoptosis (programmed cell death). Alteration of p53 may lead to cancer. Over 50% of human tumors have developed an inactivating mutation of p53. This contributes to tumor cell growth via increased DNA mutation rates. Li-Fraumeni syndrome, characterized by development of multiple cancers is attributed to mutation of p53.

Study Questions

Choose one best answer for every question of the following:

3- Replication takes place in which of the following?
(A) Nucleus.
(B) Rough endoplasmic reticulum.
(C) Mitochondria.
(D) Nucleus and mitochondria.

4- The substrates required for DNA synthesis are
(A) dATP, dGTP, dCTP, and dTTP.
(B) dAMP, dGMP, dCMP, and dTMP.
(C) dATP, dGTP, dCTP, and dUTP.
(D) ATP, GTP, CTP, and TTP.

5- The enzyme primase
(A) is a DNA polymerase.
(B) requires a DNA template.
(C) has a proofreading function.
(D) has an exonuclease activity.

6- Helicase is an enzyme that
(A) catalyzes DNA polymerization.
(B) increases hydrogen bonding.
(C) catalyzes the denaturation of DNA
(D) causes Bloom syndrome.

7- The energy for binding a new d-nucleotide to the growing chain is provided by
(A) hydrolysis of ATP.
(B) the high-energy d-nucleoside triphosphate substrate.
(C) the unwinding of the two DNA strands.
(D) breaking the hydrogen bonds between the two strands.

8- Adding a 2',3'-dideoxynucleoside triphosphate to the replication mixture causes
(A) replication arrest.
(B) cytosine deamination.
(C) chain break.
(D) mismatch error.

9- The antimetabolite methotrexate (an anti-folate) inhibits DNA replication by
(A) interfering with the formation of phosphodiester bond.
(B) inhibiting the synthesis of thymidylate.
(C) causing covalent bonding of adjacent thymines.
(D) intercalating with DNA (major groove).

10- The prodrugs cytosine arabinoside (araC) and AZT (3’-azido-2’,3’-dideoxythymidine) are activated by
(A) phosphorylation.
(B) hydroxylation.
(C) glycosylation.
(D) methylation.

11- What is the function of RNA in telomerase?
(A) Binding the 5'-end of the chromosome.
(B) Template for elongation of the 3'-end of the chromosome.
(C) Primer for DNA polymerase.
(D) Protection against exonucleases.

Transcription

The synthesis of RNA on a DNA template is called transcription. One strand of DNA acts as a template for the synthesis of RNA. Transcription follows the base pairing rule: T, C, G, and A in DNA are paired to A, G, C, and U respectively in RNA.

RNA polymerase separates the two DNA strands and moves along the template strand in the 3' to 5' direction as it synthesizes the RNA product in the 5' to 3' direction using nucleotide triphosphates (ATP, GTP, CTP, UTP) as substrates. No primer is required. Transcription ends when RNA polymerase reaches a termination signal.

RNA polymerase does not have a proofreading activity. This causes no problem since RNA is short-lived and a faulty RNA is compensated for by the production of new sound RNA molecules. However, this lack of proofreading of RNA polymerase may be the cause of high mutation rate of RNA viruses.

Types of RNA

There are three major types of RNA: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA):

- Messenger RNA carries a transcript of the specific information encoded by a specific region of DNA (gene) to the ribosomes for the synthesis of a specific protein.
- Transfer RNA is a small RNA molecule that acts as an adaptor for an aminoacid sharing in protein synthesis. It carries its activated aminoacid to the ribosomes, the site of protein synthesis.
- Ribosomal RNA is the most abundant type of RNA. Together with ribosomal proteins, it forms the machinery for protein synthesis.
- In eukaryotic cells: the primary RNA script and all intermediates in the synthesis of mature mRNA are called heterogeneous nuclear RNA (hnRNA).
- Small nuclear RNA (snRNA) shares in splicing of the primary script in eukaryotes.
- MicroRNA (miRNA) is involved in RNA interference.
- Long non-coding RNA (lncRNA) is emerging as important regulator in gene expression networks by controlling nuclear architecture and transcription in the nucleus and by modulating mRNA stability, translation and post-translational modifications in the cytoplasm.

Sense and antisense strands of DNA

The sense strand is the coding strand that carries the same base sequence of the transcribed RNA, except for T instead of U. The antisense strand is the template strand. The RNA product is complementary and antiparallel to the template strand. The coding (anti-template) strand is not used for transcription. By convention, the base sequence of a gene is given from the coding strand (5' to 3').

Numbering system

The first base transcribed as RNA is defined as the +1 base of that gene region. To the left (5', or upstream) of this starting point for transcription, bases are -1, -2, -3, etc. To the right (3', or downstream) of this point, bases are +2, +3, etc. There is no zero base.

RNA polymerases

There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell. The core polymerase is a tetramer, with the subunit structure $\alpha_2\beta\beta'$. A protein factor called sigma (σ) is required for the initiation of transcription at a promoter. Sigma factor is released immediately after initiation of transcription. Termination of transcription sometimes requires a protein called rho (ρ) factor. Rifampin inhibits this enzyme. Actinomycin D (dactinomycin) binds to the DNA, preventing transcription.

There are three eukaryotic RNA polymerases, distinguished by the particular types of RNA they produce: RNA polymerase I, II, and III. Ribosomal RNAs are primarily synthesized by polymerase I. Polymerase II synthesizes hnRNA (and mRNA), snRNA and miRNA. Polymerase III synthesizes tRNA and one rRNA. Transcription factors, such as TFIID for RNA polymerase II, help to initiate transcription. Transcription factors are named TFIX, TFIIX, and TFIIIX, where X is a variable letter. The requirements for termination of transcription in eukaryotes are not well understood. All transcription can be inhibited by actinomycin D. RNA polymerase II is also inhibited by α-amanitin (a peptide toxin from certain mushrooms).

Production of mRNA

In eukaryotes, all mRNA is monocistronic (one gene) in contrast to some prokaryotic polycistronic mRNA. Messenger RNA constitutes about 5% of the total cell RNA and it is the most heterogeneous type of RNA. In eukaryotes, most genes are composed of coding segments (exons) interrupted by noncoding segments (introns). Both exons and introns are transcribed in the nucleus. The primary transcript must undergo extensive post-transcriptional processing to form the mature mRNA molecule.

A 7-methylguanosine cap is added to the 5' end by guanylyl transferase in the nucleus and methyl transferase in the cytosol. This cap is attached head to head (5’ to 5’) through a triphosphate linkage. The cap structure serves as a ribosome-binding site and helps to protect the mRNA chain from degradation.

A poly-A tail (40-200 As) is added, by polyadenylate polymerase, to the 3' end. The tail is added after an endonuclease cleaves the precursor RNA just downstream of the polyadenylation signal: AAUAAA. The poly-A tail protects the message against rapid degradation and aids in its transport to the cytoplasm. A few mRNAs (for example, histones and interferons mRNAs) have no poly-A tails. This polyadenylate tail is useful for separation of mRNA from total cellular RNA using a poly-dT affinity column.

Introns are removed by splicing. The primary transcript forms spliceosomes by combining with complexes of snRNA and protein (also known as snRNP, or snurps). The intron is excised in the form of a lariat structure and degraded, while the 3’-carbon at the donor site is joined by phosphodiester bond to the 5’-acceptor site of the following exon. A mature mRNA can be under 10 kilobases (10,000 bases), while the primary transcript is as large as 1.7 megabases (1,700,000 bases). The mature mRNA molecule is typically formed of 7-methylguanosine cap, a non-translated region, the translated sequence, a non-translated sequence, and then poly A tail in succession.

Alternative splicing

Alternative splicing of the primary transcript produces different mRNAs, which give two or more protein variants, meaning more than one protein from one gene (100,000 human proteins from about 22,000 genes). Variants of the muscle proteins tropomyosin and troponin T are produced this way. The synthesis of membrane-bound immunoglobulins by unstimulated B lymphocytes, as opposed to secreted immunoglobulins by antigen-stimulated B lymphocytes, also involves alternative splicing.

Splicing and disease

In systemic lupus, there are antibodies against the spliceosomes. Some mutations can introduce an alternative splicing site, which produces a non-functioning protein, causing a disease, e.g., β-thalassemia, Gaucher disease, and Tay-Sachs disease. Introns may carry sequences of miRNAs, whose aberrant expression is associated with many diseases.

RNA editing

In intestinal mucosal cells, an RNA editing complex alters one base in ApoB-100 mRNA (C is deaminated and converted to U), producing a stop codon. This leads to production of a protein that is only 48% of the original protein, the ApoB-48 of chylomicrons. Deamination of adenine (A) in tRNA produces inosine (I), which helps identifying more than one codon (degeneracy of the code). RNA-editing events may regulate cancer development and metabolic dysfunctions.

RNA interference (RNAi)

Gene silencing may be mediated by short (about 20 nucleotides) double-stranded RNAs: small interfering RNAs (siRNAs) and microRNAs (miRNAs); both are produced from longer precursors by Dicer enzyme. The mechanism involves interaction with RNA-induced silencing complex (RISC). The result is promoting the translational repression and degradation of mRNA. The anti-sense strand of siRNA is fully complementary to a single mRNA, while miRNA is partially complementary and targets multiple mRNAs (could be more than 100). This process plays an important role in gene regulation and innate defense against invading viruses. It can be used in therapy. Extracellular miRNAs are potential biomarkers for a variety of diseases.

Post-transcriptional modifications of prokaryotic and eukaryotic tRNA

- Trimming of the 5'- and 3'-ends by RNases.
- Adding CCA to 3'-end by nucleotidyl transferase.
- Removal of an intron from the anticodon loop by endonuclease.
- Modification of bases to produce unusual bases, e.g., dihydrouracil, thymine, and methyl-cytosine. Pseudouridine is characterized by ribose attached to C^5 of uracil instead of N^1.

Post-transcriptional modifications of prokaryotic and eukaryotic rRNA

- They are synthesized from long precursor molecules called pre-ribosomal RNAs. These are divided and the individual subunits are further modified.
- Prokaryotic rRNAs are produced from a single RNA precursor molecule.
- A single RNA precursor molecule synthesized by RNA polymerase I produces most rRNAs in eukaryotes. The 5S rRNA (smallest) is synthesized by RNA polymerase III (like tRNA) and modified separately.

Promoter, enhancers, silencers and response elements

These are DNA sequences to which proteins of specific functions attach:

- The promoter is the binding site for RNA polymerase and basal transcription factors. RNA polymerase locates genes in DNA by searching for promoter regions. Binding determines where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds. The promoter region is usually some distance upstream of the gene. The basal promoter region of eukaryotic genes usually has two consensus sequences called the TATA box (also called Hogness box) and the CAAT box.
- Enhancers are upstream or downstream, near to or thousands base pairs away from the gene and its promoter. They are present on the same chromosome as the gene, and may be on the other strand. They may lie within an intron of the gene they control, e.g., the immunoglobulin heavy chain locus has an enhancer in the large intron separating the coding regions for the variable domain from the coding regions for the constant domain. Enhancers serve as binding sites for activators (a type of specific transcription factors), which increase the rate of transcription.
- Silencer sequences are similar to the enhancers except for binding repressors, which decrease transcription.
- Response elements bind specific transcription factors mediating the response to a signal, e.g., a hormone. They act as enhancers (or silencers).

General and specific transcription factors

In eukaryotes, general (basal) transcription factors are protein factors, common to most genes, which recognize and bind to the consensus sequences of promoter to allow the binding of RNA polymerase for the initiation of transcription. An example is TFIID (the TATA factor), which binds to the TATA box and other core promoter elements. TFIIF brings RNA polymerase II to the promoter. TFIIH melts DNA by its helicase activity, and by its kinase activity phosphorylates the polymerase allowing it to clear the promoter. The Roman numeral II denotes transcription factors for RNA polymerase II.

Specific transcription factors are protein factors: activators or repressors that bind to enhancer or silencer DNA sequences respectively. Through bending of DNA, specific transcription factors can interact with the basal transcription factors and RNA polymerase to modulate the efficiency of initiation of transcription. They can recruit chromatin modifying proteins, e.g., histone acetyl transferases (coactivators) or deacetylases. They regulate transcription in response to different signals (e.g., hormones acting through response element binding proteins). They specify which genes to be or not to be expressed in a particular cell at a particular time.

***Cis*-acting and *trans*-acting elements**

The *cis*-acting elements are regulatory DNA sequences that are located on the same chromosome as the gene they control. These include the consensus promoter sequences, enhancers and silencers.

The *trans*-acting elements are basal and specific protein transcription factors. They are encoded by genes present on different chromosomes. They bind to the *cis*-acting DNA sequences or to other transcription factors to initiate and control transcription.

Regulation of gene expression

Regulation of gene expression in eukaryotes occurs at the level of gene transcription. It occurs at other levels as well: post-transcription processing, translation, and post-translational modification.

Constitutive and regulated genes

Constitutive or housekeeping genes are genes that encode products required for basic cellular functions. They are continually expressed (not regulated). Regulated genes are expressed only under certain conditions. Each regulated gene has a variety of enhancer or silencer sequences in its regulatory region. The exact combination of specific transcription factors available (and active) in a particular cell at a particular time determines which genes will be transcribed and at what rates. Regulated genes may be expressed in all or only a subset of cells (differential cellular expression). Their expression is regulated: varies from one tissue to another and from time to time according to cellular conditions. Their regulated expression is the basis for cellular differentiation, morphogenesis and adaptability of organisms.

Examples of regulation of gene expression

One example of regulation of gene expression is the hormonal regulation of gluconeogenesis by glucagon and cortisol. Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes a critical reaction in gluconeogenesis, which under many conditions is the rate-limiting step in the pathway. A cAMP response element (CRE) and a glucocorticoid response element (GRE) are each located upstream from the transcription start site. Cortisol diffuses into the hepatocyte, where it binds to its receptor. The complex enters the nucleus, and binds to the GRE associated with the PEPCK gene, which increases gene expression. Glucagon binds to a receptor in the cell membrane, which leads to increased cAMP concentration. Protein kinase A becomes active, and then phosphorylates and activates CREB (CRE binding protein), which enters the nucleus and binds to the CRE associated with the PEPCK gene, increasing the gene expression.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that bind to DNA response elements (PPREs) and control multiple aspects of lipid metabolism. Individual members of this family are activated by a variety of natural and xenobiotic ligands, including: fatty acids, prostaglandin derivatives, fibrates, and thiazolidinediones. The improvement in insulin resistance seen with thiazolidinediones is thought to be mediated through their interaction with PPARγ. Clofibrate binds PPARα affecting different aspects of lipid metabolism. Gemfibrozil reduces hypertriglyceridemia by increasing expression of lipoprotein lipase gene and stimulating proliferation of peroxisomes, which oxidize fatty acids especially very long chain and branched fatty acids.

Gene rearrangement

Gene rearrangement is a phenomenon in which a programmed DNA recombination event occurs during cellular differentiation to reconstitute a functional gene from different gene segments separated in the genome. The most studied case is the site-specific recombination of immunoglobulin heavy and light chains and T-cell antigen receptor subunits genes in lymphocytes. It results in the highly diverse repertoire of antibodies (immunoglobulins) and T-cell receptors found in B cells and T cells respectively.

Epigenetic control of gene expression

In eukaryotic cells, DNA is packaged in condensed chromatin structures (heterochromatin). Gene expression typically requires activation to occur, making the promoter region accessible in a relatively relaxed DNA (euchromatin). Chromatin-modifying activities (chromatin remodeling, or epigenetic modifications) do not change DNA sequence.

Histone acetylation/deacetylation is one mechanism of chromatin remodeling. Acetylating lysine residues of histones by acetyl transferase eliminates the positive charge on lysine and thereby decreases the interaction of the histone with the negatively charged DNA. The chromatin thus becomes relaxed (euchromatin) and the gene promoter becomes accessible to the transcription machinery. Removing the acetyl group by histone deacetylase reverses the process and produces the condensed heterochromatin, whereby genes are not accessible for transcription.

DNA methylation is another epigenetic change. Methylation of cytosine of CpG sequence at the promoter region prevents the binding of basal transcriptional factors with the promoter and recruits histone deacetylase, which forms the inactive heterochromatin.

Gene amplification

Gene amplification is an increase in the number of copies of the sequence of a restricted region of a chromosome and is associated with overexpression of the amplified gene(s). It is common in cancer cells. Some amplified genes may promote tumorigenesis and cause cancer cells to grow or become resistant to anticancer drugs. One well-known example is the amplification of the dihydrofolate reductase gene observed in methotrexate-resistant cells.

Study Questions

Choose one best answer for every question of the following:

12- Which of the following mutations decreases the rate of transcription?
(A) ATGCAA… → ATGTAA
(B) ATGAAA… → GTGAAA
(C) TATAAG… → TCTAAG
(D) ATGAAT… → ATGCAT

13- Transcription takes place in which of the following?
(A) Ribosomes.
(B) Cytoplasm.
(C) Nucleus.
(D) Inner mitochondrial membrane.

14- During RNA synthesis, the DNA template sequence TAGC would be transcribed to produce which of the following sequences (both read in the direction 5'→3')?
(A) ATCG
(B) GCTA
(C) GCUA
(D) AUCG

15- Which of the following may result from a mutation that creates a new splice acceptor site 10 nucleotides upstream of the normal splice acceptor site?
(A) Exon 1 is 10 nucleotides shorter.
(B) Exon 1 is 10 nucleotides longer.
(C) Exon 2 is 10 nucleotides shorter.
(D) Exon 2 is 10 nucleotides longer.

16- Response elements are
(A) DNA sequences.
(B) RNA sequences.
(C) Hormone receptors.
(D) *Trans* regulators.

Genetic code

An aminoacid is encoded by a sequence of three bases (triplet) in DNA or mRNA. This triplet is called a codon. Since we have four bases, there are 64 codons, a state of codon redundancy. The twenty aminoacids are encoded by 61 codons. Three (UAA, UGA, UAG) are stop codons (nonsense codons), which terminate translation. They are all written in the 5' to 3' direction. There is one start codon (initiation codon), AUG, coding for methionine. Protein synthesis begins with methionine (Met) in eukaryotes, and formyl-methionine (fmet) in prokaryotes.

UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA, UAG: Stop	UGU, UGC: Cys UGA: Stop UGG: Trp
CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg
AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg
GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly

The genetic code is unambiguous; each codon specifies no more than one amino acid. The code is degenerate; more than one codon can specify a single aminoacid. All aminoacids, except Met and tryptophan (Trp), have more than one codon. For those aminoacids having more than one codon, the first two bases in the codon are usually the same. The code is universal (the same in all organisms), with some minor exceptions in mitochondria. The code is “commaless”; codons on mRNA are contiguous, with no spacers or "commas" in-between. Neighboring codons on a message are non-overlapping. Every codon on mRNA is complementary, by base pairing rule, and antiparallel to an anticodon on tRNA. Every tRNA carries only the aminoacid that corresponds to its anticodon.

Mutation

An alteration of the nucleotide sequence of a gene on DNA is called mutation. A mutation can be spontaneous or caused by radiation or some drugs. A mutated gene passes from one cell to its daughter cells. Gene mutation can result in the synthesis of a faulty (abnormal) protein, which may lead to a disease. A mutation in the germ line is inherited by the offspring, which may produce a genetic disease.

A point mutation is a single base alteration, which may be a transition (purine to purine or pyrimidine to pyrimidine) or a transversion (purine to pyrimidine or vice versa). A point mutation can be silent if the new codon specifies the same aminoacid. A missense mutation is one that produces a codon for a different aminoacid. It may have no, little, or serious effect, e.g., sickle cell disease. A nonsense mutation produces a stop codon, which usually produces a short, non-functional protein.

A frame shift mutation is a deletion or addition of one or two bases, which results in a completely different aminoacid sequence. The product is usually a nonfunctional protein.

Addition or deletion of three nucleotides can lead to one extra or one lacking aminoacid consequently in the final protein product. In cystic fibrosis, a lethal autosomal recessive disease, there is deletion of three nucleotides from the coding region of the gene. This leads to loss of phenylalanine from the final chloride channel protein (cystic fibrosis transmembrane regulator, CFTR). This prevents the proper folding of the protein, leading to its destruction by the cell proteasome.

A large segment deletion can occur during an unequal crossover in meiosis. Crossover or recombination between homologous chromosomes is a normal event of meiosis, which generates genetic diversity in reproductive cells (ovum and sperm). In a normal crossover event, the homologous maternal and paternal chromosomes exchange equivalent segments, and although the resultant chromosomes are mosaics of maternal and paternal alleles, no genetic information has been lost from either one.

On rare occasions, a crossover can be unequal, and one of the two homologous chromosomes loses some of its genetic information. Alpha-thalassemia is a well-known example of a genetic disease in which unequal crossover has deleted one or more α-globin genes from chromosome 16. Cri-du-chat (a characteristic kitten-like cry with mental retardation, microcephaly, and wide-set eyes) results from a terminal deletion of the short arm of chromosome 5.

Trinucleotide repeat expansion is an increase in the number of tandem copies of a trinucleotide in a coding region, which leads to some disease such as Huntington disease and spinobulbar muscular atrophy, or in an untranslated region of the gene, like in fragile X syndrome, and myotonic dystrophy. In the normal Huntington allele, there are five tandem repeats of CAG in the coding region. Affected family members may have 30 to 60 of these CAG repeats. The abnormal protein with 30 or more adjacent glutamine is extremely unstable. Fragile X syndrome, the most common inheritable cause of mental retardation, results from an extended triplet CGG repeat, with gene silencing by hypermethylation of DNA. In myotonic dystrophy, a triplet expansion results in gene silencing by splicing alteration. The increase in the number of repeats, from generation to the following generations, causes more severe and earlier appearance of symptoms. This is called anticipation.

Splice site mutation can lead to abolishing or creating a new splice site, with improper processing of mRNA, e.g., β-thalassemia, Gaucher disease, and Tay-Sachs disease.

Study Questions

Choose one best answer for every question of the following:

17- Unambiguity (specificity) of the genetic code necessitates which of the following?
(A) One codon for every aminoacid.
(B) One aminoacid for a codon.
(C) One transfer RNA for every aminoacid.
(D) One termination codon.

18- Methionine is specified by the codon AUG. What is the anticodon on its tRNA?
(A) AUC
(B) GUA
(C) UAC
(D) CAU

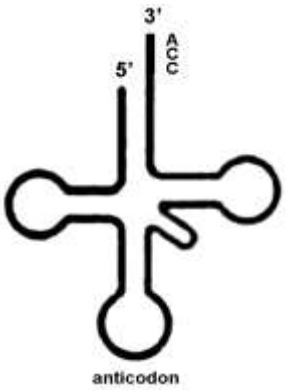
19- A longer than normal protein may be produced by which of the following mutations?
(A) GAU $\rightarrow$ GAC
(B) GCA $\rightarrow$ GAA
(C) UAA $\rightarrow$ CAA
(D) UAA $\rightarrow$ UAG

Translation

The process of protein biosynthesis is called translation. The gene transcript on mRNA (nucleotide sequence) is translated to an aminoacid sequence (polypeptide) by ribosomes. This process is achieved by the participation of mRNA, rRNA, ribosomal proteins, and the various tRNA molecules that carry their activated aminoacids (charged tRNA). Through the complementarity of the codons on mRNA and the anticodons of tRNA, aminoacids are brought together in the specific sequence dictated by the base sequence of mRNA (the transcript of the gene on DNA).

Transfer RNA

Transfer RNA constitutes about 15% of the cellular RNA. Transfer RNA is relatively a small molecule of only 74-95 nucleotide residues. It is folded into three loops, stabilized by intra-chain hydrogen bonding between complementary bases. Transfer RNA is characterized by the presence of unusual or modified bases. The 3'-end of tRNA has the nucleotide sequence CCA, which is the site for attachment of the activated aminoacid. There are at least 20 different species of tRNA for the 20 aminoacids sharing in protein synthesis.



Aminoacid activation

As tRNAs enter the cytoplasm, each combines with its destined aminoacid in a two-step process of aminoacid activation. Each type of aminoacid is activated by a different aminoacyl tRNA synthase, which attaches the aminoacid to the 3'-end (CCA) of the correct tRNA. Aminoacyl tRNA synthases have self-checking functions to prevent formation of incorrectly paired aminoacyl tRNAs.

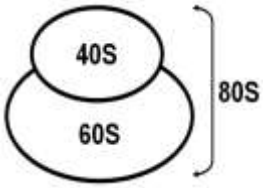
$$\text{Aminoacid} + \text{ATP} + \text{tRNA} \longrightarrow \text{Aminoacyl-tRNA} + \text{AMP} + PP_i$$

Wobble

Many aminoacids are encoded by more than one codon (redundancy, degeneracy). Frequently, a tRNA can translate more than one of these codons, thus decreasing the need for making multiple tRNAs to carry the same aminoacid. For instance, the arg-$tRNA^{arg}$ can translate the CGA and the CGG codons, both of which encode arginine. Correct base pairing is required at the first position of the codon (third of anticodon) and the second position of the codon (second of anticodon). The first position of the anticodon does not always need to be paired with the codon, allowed to "wobble" in some cases, but with no change of the resulting protein.

Ribosomes

Ribosomal RNA constitutes about 80% of cellular RNA. Ribosomes are formed of RNA pieces and proteins. The large 60S subunit (50S in prokaryotes) and small 40S subunit (30S in prokaryotes) make the complete 80S ribosomal particles (70S in prokaryotes). The S (Svedberg) values are determined by the sedimentation speed in an ultracentrifuge. They are a function of both size and shape, and therefore not additive.

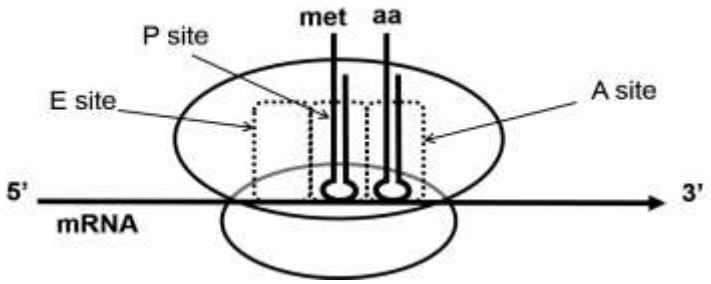


Initiation

In prokaryotes, ribosomes can begin translating the mRNA even before transcription is complete. The prokaryotic mRNA is polycistronic, used for synthesis of more than one protein. In eukaryotes, translation and transcription are completely separated in time and space, with transcription in the nucleus and translation in the cytoplasm. Eukaryotic mRNA carries the message for synthesis of one protein (monocistronic).

Initiation in prokaryotes requires Shine-Dalgarno sequence near to 5'-end of mRNA that is paired to a complementary sequence of an RNA component of 30S subunit. In eukaryotes, the small subunit binds to the 5'-cap, helped by eukaryotic initiation factors, and slides to the first AUG codon. This requires ATP as an energy source. The initiator tRNA, charged with methionine (formyl methionine in prokaryotes), is base-paired by its anticodon to the AUG start codon. This requires GTP as an energy source. The large subunit binds to the small subunit, forming the completed initiation complex.

There are distinct binding sites on the ribosome: P, A, and E sites. The P (peptidyl) site is the site where the initial met-tRNA binds. After formation of the first peptide bond, the P site is the binding site for the tRNA carrying the growing peptide chain. The A (aminoacyl) site binds each new incoming charged tRNA. The third, E (exit), site is for the used uncharged tRNA.



Elongation

During elongation, the ribosome moves in the 5' to 3' direction along the mRNA, synthesizing the protein from amino to carboxyl terminus. Elongation is a three-step cycle that is repeated for each amino acid added. Each cycle uses four high-energy bonds (two from the ATP used in amino acid activation to charge the tRNA, and two from GTP).

The A site binds a charged tRNA complementary to the mRNA codon, needing GTP as energy source. Peptidyl transferase, an enzyme that is a part of the large subunit, forms the peptide bond between the carboxyl end of the growing polypeptide chain and the amino group of the new aminoacid in the A site, uncharging the tRNA in the P site. In a translocation step, the ribosome moves exactly three nucleotides (one codon) along the mRNA, needing eukaryotic elongation factor-2 (eEF-2) and GTP as energy source. This moves the growing peptidyl-tRNA into the P site and aligns the next codon to the vacant A site, while the uncharged tRNA is now in the E (exit) site, and leaves with the following translocation.

Termination

When any of the three stop (termination or nonsense) codons is aligned with the A site, peptidyl transferase (with the help of release factor) hydrolyzes the completed protein from the final tRNA in the P site. The mRNA, ribosome, tRNAs, and translation factors can all be reused for additional protein synthesis.

Inhibitors of protein synthesis

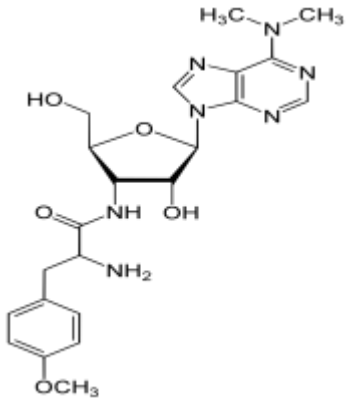
Some well-known inhibitors of prokaryotic translation include clinically used antibiotics. Chloramphenicol inhibits peptidyl transferase of the large ribosomal subunit. Macrolide family antibiotics like clarithromycin, erythromycin and azithromycin bind the 50S ribosomal subunit and block translocation. Tetracyclines inhibit initiation of protein synthesis, while amino-glycosides like streptomycin can lead to misreading of the message and production of faulty non-functional proteins. Rifampin inhibits prokaryotic mRNA synthesis.

Certain antibiotics, e.g., chloramphenicol and erythromycin inhibit mitochondrial protein synthesis, but not cytoplasmic protein synthesis, because mitochondrial ribosomes are similar to prokaryotic ribosomes. Evolutionists believe that mitochondria are originally prokaryotic cells.

Inhibitors of eukaryotic translation include cycloheximide (inhibitor of peptidyl transferase) and diphtheria and pseudomonas toxins (inhibitors of ribosome translocation by inactivating eEF-2 via ADP-ribosylation).

Rapamycin inhibits mammalian initiation of protein synthesis and may be used as immuno-suppressive. It inhibits the phosphorylation of a protein inhibitor of eIF-4E. Ricin, a toxin from castor beans, cleaves an adenine base (N-glycosidic bond) from one rRNA of the 60S subunit. The sequence affected is required for binding elongation factors, therefore, protein synthesis stops.

Puromycin inhibits both prokaryotic and eukaryotic translation by binding to the A site. The molecule resembles the 3'-end of the aminoacyl tRNA. Peptidyl transferase attaches the peptide to puromycin, and the peptide with puromycin attached at the C-terminus is released, prematurely terminating chain growth.



Polysomes

Messenger RNA molecules are very long compared with the size of a ribosome, allowing room for several ribosomes to translate a message at the same time (polyribosome or polysome). Because ribosomes translate mRNA in the 5' to 3' direction, the ribosome closest to the 3'-end has the longest nascent peptide. Polysomes are found free in the cytoplasm or attached to the rough endoplasmic reticulum (RER), depending on the protein being translated.

Free ribosomes and rough endoplasmic reticulum

Although all translation of eukaryotic nuclear genes begins on ribosomes free in the cytoplasm, the proteins being translated may belong to other locations. Certain proteins are translated on ribosomes associated with the rough endoplasmic reticulum (RER). These include secreted proteins, proteins inserted into the cell membrane, and lysosomal enzymes. Proteins translated by free cytoplasmic ribosomes include cytoplasmic proteins, nuclear proteins and mitochondrial proteins that are encoded by nuclear genes.

Molecular Chaperones

Proteins translated on the RER generally fold and assemble into subunits in the ER before being transferred to the Golgi apparatus. Other proteins fold in the cytoplasm. Molecular chaperones assist in this process of protein folding (Lecture 2). Misfolded proteins are labeled by ubiquitin (a specialized small protein) and digested by cytoplasmic protein-digesting complexes called proteasomes.

N-Terminal hydrophobic signal sequence

This leader sequence is found in proteins destined to be secreted (e.g., insulin), placed in the cell membrane (e.g., Na^+-K^+-ATPase), or ultimately directed to the lysosomes (e.g., sphingomyelinase). These proteins require N-terminal hydrophobic signal sequences as part of their primary structure. Translation begins on free cytoplasmic ribosomes, but after translation of the signal sequence, the ribosome is positioned on the ER (now RER) with the help of signal recognition particle (SRP, a cytosolic ribonucleoprotein) and SRP receptor. During translation, the nascent protein is fed through the membrane of the RER and captured in the lumen. The signal sequence is cleaved off in the ER, then the protein passes into the Golgi apparatus for further modification and sorting. Glycosylation of the protein takes place in the ER and Golgi.

Lysosomal enzymes and phosphorylation of mannose

Lysosomal enzymes are glycosylated and modified in a characteristic way. Most importantly, when they arrive in the Golgi apparatus, specific mannose residues in their oligosaccharide chains are phosphorylated. This phosphorylation is the critical event that removes them from the secretion pathway and directs them to lysosomes. Genetic defects affecting this phosphorylation produce I-cell disease (mucolipidosis type II). This is a lysosomal storage disease in which lysosomal enzymes are released into the extracellular space, and inclusion bodies accumulate in the cell (I = inclusion bodies), compromising its function.

Mitochondrial proteins

Mitochondrial DNA genes are transcribed and translated inside the mitochondria. Mitochondrial proteins encoded by nuclear genes are translated by free cytosolic ribosomes. They have a targeting sequence and utilize cytosolic chaperones, mitochondrial membrane transporters, and mitochondrial matrix chaperones for their transport and proper folding. Their target sequence is cleaved inside the mitochondria.

Regulation of translation

Regulation of gene expression may be exerted at the translation level. An example of such regulation is the effect of intracellular iron concentration on the translation of transferrin receptors and ferritin. The mRNAs for both proteins contain an iron response element (IRE) that binds a specific protein (IRE-BP). At low iron concentration, this protein is free to bind the IRE of both mRNAs. The transferrin receptor mRNA is stabilized and is actively translated to produce more transferrin receptors. On the other hand, the translation of ferritin mRNA is inhibited. At high iron concentration, IRE-BP binds iron and leaves the mRNAs. The transferrin receptor mRNA is degraded, while the ferritin mRNA is actively translated. Another example is the regulation of globin synthesis by heme. Heme interacts directly with and inactivates the eIF2α kinase heme-regulated inhibitor (HRI). In the setting of heme deficiency, HRI is activated to attenuate globin translation in erythroid precursors.

Co- and post-translational covalent modifications

Proteins can be subject to modification while still being synthesized by the ribosomes or after the completion of translation of the message. Herein below is a list of the common co- and post-translational modifications:

- Proteolysis to remove the signal peptide.
- Disulfide bond formation for folding the polypeptide chain.
- Proteolysis to remodel the protein, e.g., processing of prohormones and the activation of pro-enzymes.
- Glycosylation in ER and Golgi, where oligosaccharide chains are N-linked (to asparagine) or O-linked (to serine or threonine).
- Phosphorylation of serine, and probably threonine residues as a mechanism of regulation of metabolism (activation or inhibition of key enzymes by phosphorylation/dephosphorylation). Tyrosine phosphorylation is important in signal transduction.
- Phosphorylation at carbon 6 of the terminal mannose of glycoprotein enzymes directed to the lysosomes.
- Hydroxylation of proline and lysine in procollagen, which can be followed by glycosylation.
- Vitamin K-dependent gamma-carboxylation of blood clotting factors.
- Ubiquitin-tagging of misfolded or other faulty proteins to be destroyed by proteasomes.

Selenocysteine

Selenium is incorporated in the form of selenocysteine (Sec) in a number of proteins including glutathione peroxidases, iodothyronine deiodinases, and thioredoxin reductases. Selenocysteine is a cysteine analog, containing selenium instead of sulfur. It is considered the 21st aminoacid sharing in protein synthesis. It is formed by the modification of an unusual serine-tRNA (Ser-tRNA^{Sec}) synthesized by the same seryl tRNA synthase. The selenocysteine-charged tRNA decodes a UGA codon (originally a stop codon). This process is controlled by a specific nucleotide sequence (selenocysteine insertion sequence, SECIS) and protein factors.

Study Questions

Choose one best answer for every question of the following:

20- The aminoacid tyrosine is specified by the codon UAC. What other codon may specify this aminoacid?
(A) AAC
(B) UCC
(C) UAU
(D) CAC

21- Which of the following is <u>not</u> true about the function of a tRNA molecule?
(A) It has an anticodon specific for one aminoacid.
(B) It carries only one specific aminoacid.
(C) It can make hydrogen bonds with mRNA.
(D) It carries a transcript of the gene for a specific protein.

22- Which enzyme acts as the dictionary in the process of translation?
(A) RNA polymerase I.
(B) RNA polymerase II.
(C) Aminoacyl-tRNA synthase.
(D) Peptidyl transferase.

23- A 12 base-pair deletion in the factor V gene impairs the secretion of this factor and leads to its accumulation in the cytoplasm. What region of the gene would this mutation most likely be located?
(A) 5' Untranslated region.
(B) First exon.
(C) Middle intron.
(D) Last exon.

24- Rifampin inhibits which of the following?
(A) Prokaryotic mRNA synthesis.
(B) Assembly of bacterial ribosomal subunits.
(C) Peptidyl transferase of bacterial ribosomes.
(D) Translocation step of the bacterial ribosome.

25- In I-cell disease (mucolipidosis type II), there is defective
(A) translation.
(B) glycosylation.
(C) phosphorylation.
(D) chaperone.

26- A new antibiotic was tested in an experimental system that translates a mRNA with the sequence: AUGUUUUUUUAG. The only product formed was the dipeptide fMet-Phe. What step of protein synthesis was most likely inhibited by this antibiotic?
(A) Initiation.
(B) Binding charged tRNA to A site.
(C) Peptidyl transferase activity.
(D) Ribosomal translocation.

27- Some antibiotics intended for inhibition of bacterial protein synthesis may also specifically inhibit the synthesis of the proteins of
(A) lysosomes.
(B) cell membrane.
(C) mitochondria.
(D) peroxisomes.

28- Thanks to the wobble hypothesis,
(A) less tRNAs than codons are needed.
(B) less than 20 tRNAs are needed.
(C) protein isoforms of varying length are produced.
(D) missense mutations have no effect.

29- Important post-translation modifications of some proteins are dependent on
(A) vitamin C.
(B) vitamin K.
(C) either (A) or (B).
(D) neither (A) nor (B).

30- Which strand of miRNA can inhibit translation of mRNA?
(A) Sense-strand.
(B) Antisense-strand.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

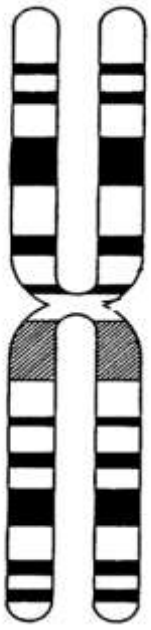
Genetics and Molecular Medicine Techniques

Karyotyping

Chromosomes are most easily visualized under the microscope during the metaphase stage of mitosis, when they are maximally condensed. They are photographed and ordered according to size, with the sex chromosomes (X and Y) placed at the end. This is called a karyotype. A typical somatic cell contains 23 pairs of chromosomes: 46,XX in a female, and 46,XY in males. This is a diploid cell. Gametes (sperms and ova) are haploid: 23X, and 23Y. All these are euploid cells.

Aneuploidy is seen when the number of chromosomes cannot be divided by 23. Two major types of aneuploidy are observed: trisomy (three copies of a specific chromosome) and monosomy (one copy of a specific chromosome). Monosomies and trisomies are usually caused by nondisjunction of the two members of the chromosome pair during meiosis, leading to a gamete with one extra or one less chromosome. All autosomal monosomies are lethal, but trisomies of three different autosomes (13, 18, and 21) are compatible with survival to term in at least some cases. This difference illustrates the fact that the body tolerates extra genetic material more successfully than a loss of genetic material. Trisomy 21 (47,XY,+21; 47,XX,+21) is the most common autosomal trisomy, which causes Down syndrome. Sex chromosome aneuploidy includes Klinefelter syndrome (47,XXY) and Turner syndrome (45,X).

To visualize chromosomes more accurately, various stains are applied so that the bands in chromosomes can be seen. The bands tend to reflect differences in chromosome structure or composition, e.g., regions rich in CG bases versus those rich in AT bases. A metaphase chromosome consists of two sister chromatids, each having a constricted area termed a centromere and an arm on either side. The centromere is the site of meeting of chromatids and the attachment site of spindle fibers during mitosis and meiosis.

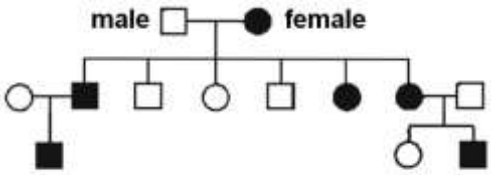


The short arm is labeled p, and the long arm is labeled q. Chromosome arms are subdivided further by regions and by bands within the regions. Thus, the designation 14q32 (fourteen que three two) refers to the second band within region 3 of the long arm of chromosome 14.

Modes of inheritance

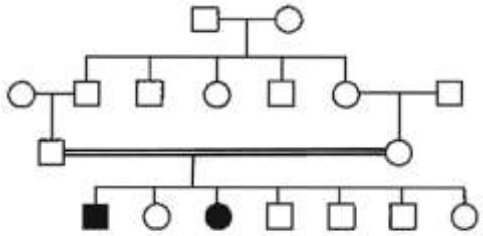
Pedigree analysis enables the identification of the mode of inheritance of a particular genetic disease. One can estimate the probability of the genotype and phenotype of an upcoming child.

In autosomal dominant mode, only one mutant allele is enough for the disease to be expressed (heterozygous). An affected person has at least one affected parent. Either sex can be affected. Male-to-male transmission of the mutant allele may be present.

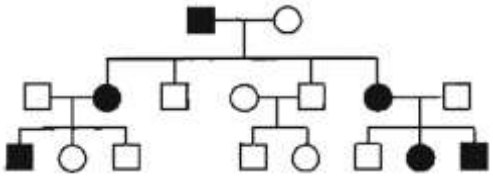


Autosomal dominant

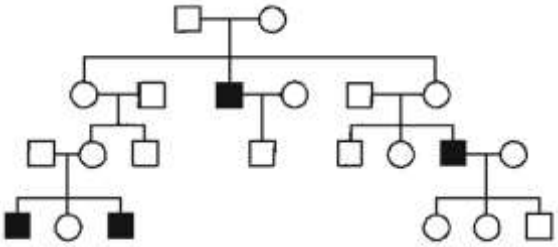
In autosomal recessive mode, two mutant alleles must be present for the disease to be expressed (homozygous). An affected person is usually born to unaffected parents. The disease appears more with consanguineous marriages. Either sex can be affected. Both parents of an affected child are obligate carriers. Male-to-male transmission of the mutant allele may be present.


Autosomal recessive

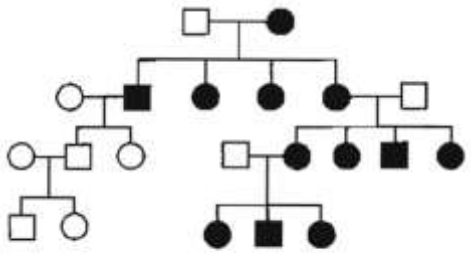
In X-linked dominant mode, only one mutant allele need be present for the disease to be expressed. Either sex can be affected. There is no male-to-male transmission. An affected male parent passes the trait to all of his daughters, but none of his sons. An affected female parent can pass the trait to both sons and daughters. Affected females often have more mild and variable symptoms than affected males. Very few diseases show X-linked dominant inheritance.


X-linked dominant

In X-linked recessive mode, usually males only are affected, not females (unless there has been unequal X chromosome inactivation during early embryogenesis). Usually, the mother is an unaffected carrier. There is no male-to-male transmission of the mutant allele.


X-linked recessive

Mitochondrial traits are inherited in a non-Mendelian fashion. The disease is inherited only maternally, since only the mother contributes mitochondrial DNA to the progeny. Both males and females can be affected by the disease. All offspring of an affected female are affected, whereas there is no inheritance of the disease from an affected male. Mitochondrial diseases are often expressed as neuropathies and myopathies because brain and muscle are highly dependent on oxidative phosphorylation. Mitochondrial genes encode some of the components of the electron transport chain and oxidative phosphorylation, as well as some mitochondrial tRNA molecules.


Mitochondrial inheritance

Complementary DNA (cDNA)

This is DNA synthesized on mRNA template by the enzyme reverse transcriptase (RNA-dependent DNA polymerase). The primer is oligo-dT, which anneals to the poly A tail of mRNA. Complementary DNA library differs from one tissue to another (and from one condition to another) according to specific gene expression. Complementary DNA has coding sequence only, with no introns, unlike genomic DNA. It also lacks the promoter and regulatory elements of the genes.

Synthetic Oligonucleotides

Oligonucleotides can be synthesized and used as probes (radio- or fluorescence-labeled) and as primers (for PCR and cDNA) or for gene arrays. Allele-specific oligonucleotide (ASO) probes may be used to detect mutations of a specific gene, e.g., sickle hemoglobin.

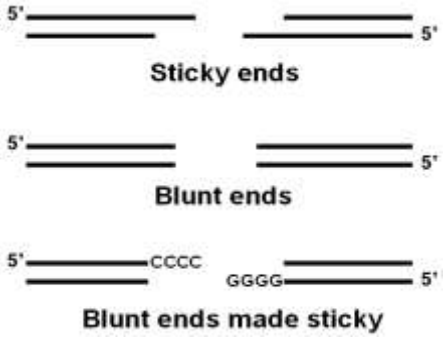
Restriction endonucleases

These are bacterial enzymes that cleave double stranded DNA at specific nucleotide sequences. They are called restriction enzymes because they cleave the DNA of invading viruses (bacteriophages) and restrict their replication. Restriction enzymes recognize short stretches of DNA (generally 4 or 6 base pairs) of specific sequences. A restriction sequence of the bacterial (host) DNA is protected by methylation. A restriction sequence is described as palindrome, meaning that it is read the same in both strands in 5' to 3' direction. Both strands are cut by the same specific enzyme.

The name of a restriction enzyme is derived from the name of the bacteria from which it is isolated. The first letter refers to the genus. The next two letters refer to the species. An additional subscript letter refers to the strain. A number indicates the order of discovery from that organism. For example, *Hae*III is the third restriction enzyme isolated from *Haemophilus aegyptius*. Hundreds of restriction enzymes are available as analytic reagents.

A restriction endonuclease can cleave the human DNA into many fragments. A restriction enzyme specific for four-nucleotide sequence produces more DNA pieces than a restriction enzyme specific for six-nucleotide sequence.

A restriction enzyme cleaves the two DNA strands producing blunt ends or sticky ends. Restriction endonucleases which produce sticky ends are particularly useful for the production of recombinant DNA molecules. Blunt end fragments can be made sticky by adding poly C to the 3'-end of one fragment and poly G to the 3'-end of the other fragment.



Recombinant DNA (genetic engineering)

Recombinant or chimeric DNA is the DNA formed from the DNA of more than one species (Chimera, according to Greek mythology, was a monstrous creature composed of the parts of more than one animal). It can be made by inserting a piece of human DNA in a vector's DNA. The vector is usually a bacterial plasmid (extra-chromosomal circular DNA) or a bacteriophage. Other vectors include cosmid (hybrid plasmid that contains a DNA sequence from bacteriophage) and bacterial artificial chromosome. The vector, carrying the piece of human DNA, is then introduced into (transform) the host cell, where it self-replicates.

Cloning genomic DNA

Recombinant DNA technology is useful for DNA cloning. Human DNA is fragmented using a restriction endonuclease. Plasmid DNA is split by the same enzyme. Restriction enzymes that produce sticky ends are preferred. The two type of DNA are allowed to hybridize to form the recombinant DNA. DNA ligase is used to bind the pieces. Bacterial cells are then transformed by the plasmids. Transformed colonies can be selected using the property of specific antibiotic resistance of the plasmid. Bacterial colonies grown contain the human DNA replicating with the plasmid DNA. The amplified pieces of human DNA are split off using the same restriction enzyme.

Cloning genomic DNA proved useful for establishing the genomic library and the human genome project. It is useful for constructing restriction maps that show sites of cutting by different restriction enzymes and their relation to disease mutation. In this sense, they have been useful for identifying genetic markers of some diseases. Cloning genomic DNA enables the studying of all DNA: coding and non-coding.

Cloning cDNA

Complementary DNA can be cloned the same way. Since cDNA represents the processed gene (no introns), it can be used to produce human proteins by bacterial colonies. In addition to antibiotic resistance gene, a bacterial promoter and a Shine-Dalgarno sequence (for identification by bacterial ribosomes) must be included in the cloning plasmid (expression vector) near the insertion site for the cDNA.

This method of human protein production has the advantage of production of the desired products at a large scale for use as medication, e.g., insulin, tissue plasminogen activator, erythropoietin, and anti-hemophilic globulin. These products have the advantage of being human, causing no immune reaction, and infection-free, carrying no risk of transmitting a human virus. Since the bacterial cell cannot process proinsulin, chains A and B of insulin are prepared separately, and then combined to form insulin. Recombinant HBsAg is now used in vaccination against hepatitis B, eliminating the risk of introducing viral particles during vaccination. Amplified cDNA can be used for gene therapy and production of transgenic animals. It can also be labeled and used as a probe to locate a gene in genomic DNA.

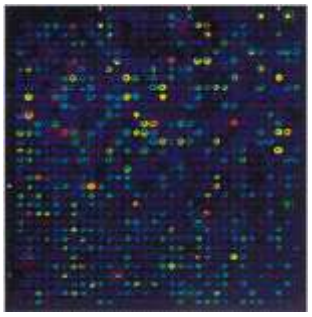
Blotting techniques

Blotting techniques have been developed to detect and visualize specific DNA, RNA, and protein among complex mixtures of contaminating molecules. In Southern blotting: DNA is subjected to agarose gel electrophoresis, denaturation by alkali, and transfer by blotting to nitrocellulose membrane. Radiolabeled oligonucleotide probes are applied, followed by washing and autoradiography. Southern blotting technique is named after its inventor. Northern blotting is a facetious name given to RNA analysis by the same technique. Western blotting is applied to proteins using a specific antibody as a probe.

The smaller molecules travel faster in electrophoresis and appear nearer to the bottom of the gel. Running molecular weight markers allows the determination of the molecular size of the band of interest. Dot blot follows the same technique but without electrophoresis. It helps identifying the presence of a specific DNA, RNA, or a protein in a sample.

Western blotting is used for confirmation of HIV infection, since the screening ELISA test is sensitive but of low specificity. Specific HIV proteins are separated by gel electrophoresis and blotted to a filter. The filter is incubated with the tested serum sample. If the sample contains antibodies to HIV, they will bind to the proteins on the filter. The filter is next washed and incubated with a labeled goat anti-human IgG to visualize any bound human antibodies (immunoblotting).

Gene array



In gene micro-array, a high number (thousands) of oligo-nucleotide probes representing different genes are fixed on a small slide. Tissue mRNA is isolated and fluorescence-labeled cDNA is prepared. This cDNA is allowed to hybridize with the microarray nucleotides. The slide is washed and scanned.

By this technique, the expression of different genes is studied simultaneously. By using a different color of fluorescence for another tissue or the same tissue under different conditions, different tissues or different conditions can be compared using the same micro-array slide. The computer software can analyze the different combinations of colors at each gene probe and make the comparison between the two samples. The pattern of gene expression in a sample may help tailoring the treatment for individual cancer patients.

This technique can be applied to genomic DNA, thus comparing a patient to a reference or a tumor to normal tissue. It is useful for detecting the ploidy state (copy number variation), a technique known as array comparative genomic hybridization (aCGH).

Fluorescence in situ hybridization (FISH)

In this technique, fluorescent probes are used to visualize genes using the fluorescence microscope. It was a valuable tool for gene mapping in the human genome project. It is useful for clinical diagnosis of various chromosomal abnormalities, including deletions, duplications, and translocations, e.g., Prader-Willi syndrome (deletion of cluster of genes on chromosome 15) can be confirmed using a probe specific for 15q11-13 region. It may be used for comparative genomic hybridization (CGH) and determining the copy number of cancer-related genes.

Restriction fragment length polymorphism (RFLP)

The human genome is about $3X10^9$ base pairs. Less than 2% of this genome encodes proteins (about 22,000 genes). Humans differ one from another in about 0.1% of their DNA sequence. These variations include polymorphism and mutation. Polymorphism is clinically harmless. Gene polymorphism is the presence of a variant of the gene in at least 1% of the population. A mutation is an infrequent variation that is potentially harmful, associated with disease.

The genetic variation may create or abolish a restriction site, cut by a specific restriction endonuclease, giving a restriction fragment shorter or longer. This is called restriction fragment length polymorphism (RFLP). It may be due to single nucleotide polymorphisms (SNPs), which is responsible for about 90% of genome variation. The RFLP may also be due to variable number of tandem repeats (VNTR) of a unit of nucleotides (usually 15-60 base pair sequences) in the non-coding DNA.

Some genetic diseases may be detected by being associated with RFLP. An example of point mutation leading to a disease and RFLP is sickle cell disease. Hemoglobin S gene has a point mutation: A→T, which abolishes a specific restriction site. Embryonic cells can be tested for the length of the specific restriction fragments and a prenatal diagnosis is established. An easier diagnosis can be reached by dot blot using allele-specific nucleotide probes for both the normal and sickle alleles. Embryonic cells can be obtained from the amniotic fluid, chorionic villi, or the pre-implantation embryo in cases of in vitro fertilization.

Restriction fragment length polymorphism was also used for DNA fingerprinting and family tracing, e.g., mixed newborns in a hospital or parenthood cases. Each individual carries two alleles for such a genetic marker: one inherited from the mother and one from the father.

DNA fingerprint (genetic fingerprint, DNA type, DNA profile)

This is the DNA pattern unique to an individual. It was originally based on RFLP, now replaced by short tandem repeats (STRs, microsatellites, 2-4 nucleotide sequences). Combined DNA Index System (CODIS) in the United States employs 20 loci (13 loci before 2017). For each locus, there are two or more alleles in the population. A locus length, dependent on the number of repeats, is inherited like any gene. Each individual has two alleles for every locus, one from each parent. The possibility of sharing identical DNA fingerprint by two individuals, other than identical twins, is practically nil. Biological gender can be determined using an X/Y marker gene, e.g., *amelogenin* (encoding a matrix protein of tooth enamel).

DNA fingerprinting is facilitated by polymerase chain reaction (PCR) and the use of computers. It is very useful in forensic medicine: identification of sample donors (assailants and victims), parenthood cases, and missing person cases. Using chromosome Y short tandem repeats (Y-STRs) in males and mitochondrial DNA (mtDNA) can also be useful.

Very rarely, a person can be chimeric, having two different DNA fingerprints. A blood sample for example would be different from a tissue sample. This occurs if one embryo dies in utero and is absorbed by its fraternal twin. It may also result from acquiring fetal cells by the mother.

Polymerase chain reaction (PCR)

This is a method for in vitro amplification of DNA. PCR involves repeated cycles of DNA replication. The reaction mixture for PCR includes: the piece of DNA containing the target gene, two primers 20-30 nucleotides long flanking the 3' ends of both strands of the gene (in excess), deoxy-nucleoside triphosphates (in excess), and *Taq* polymerase, in a suitable buffer. *Taq* polymerase is a DNA polymerase of the hot water spring bacteria *Thermus aquaticus*. It is used in PCR because it can stand high temperature.

The reaction mixture is placed in a thermal cycler. Each PCR cycle involves: heating to a high temperature (90-95^{o}C) in order to denature DNA (separate the two strands), cooling to a low temperature (50-60^{o}C) in order to anneal the primers to DNA, then heating to a medium temperature (68-72^{o}C) in order to synthesize new complementary DNA strands. It takes about 25-30 cycles, 2 minutes/cycle. By each cycle, the target DNA is doubled. DNA is amplified exponentially to produce millions of copies in a few hours. Different DNA pieces can be separated by electrophoresis. A particular gene can be identified using a labeled complementary oligonucleotide probe.

Amplification of DNA by PCR is much easier, faster, and more sensitive (even one cell is enough) compared to cloning. PCR is useful for prenatal diagnosis, tissue matching for transplantation, and for medico-legal purposes. It can be used for analysis of the smallest samples: blood, tissue, semen, saliva, etc. (even one hair follicle). It is helpful for DNA fingerprint, identification of assailants and victims and for parenthood cases. PCR can be applied for DNA from any source: human, bacterial, viral, etc. Therefore, it is useful for diagnosis of infections, e.g., viral hepatitis, HIV, and tuberculosis. This technique is not dependent on presence of antibodies, thus it is very useful for diagnosis of HIV in newborns from HIV-positive mothers, for early diagnosis after exposure to infection, and for determining the viral load. The use of PCR extends even more to the fields of anthropology and archeology.

To use PCR for RNA viruses or for mRNA, a reverse transcription (RT) is run first to produce cDNA, which is then subjected to PCR (RT-PCR). Real-time PCR is the technique of collecting data throughout the PCR process as it occurs, thus combining amplification and detection into a single step. This is achieved using fluorescent signals, thus correlating PCR product concentration to fluorescence intensity. Quantitative real-time PCR is abbreviated "qPCR", while "RT-qPCR" is reverse transcription quantitative PCR. The latter can be used for determination of viral load of RNA viruses like hepatitis C virus (HCV).

Measuring gene expression

Measuring the final product (protein) is achieved by specific protein targeting techniques. Specific antibodies are usually used for this purpose, with different detection methods according to the method of immunoassay used, e.g., radioimmunoassay, enzyme immunoassay, immuno-turbidimetry, etc.

Determination of mRNA production is achieved by gene array as explained before. Using real-time RT-PCR, a mRNA production under certain conditions can be compared to the production of mRNA of a house-keeping gene, a gene that is constantly expressed. Different fluorescence colors are used for the different genes to be compared in the same tube.

DNA sequencing

DNA sequencing means determining the order of the four nucleotides that make up the DNA molecule. DNA sequencing may be used to determine the sequence of individual genes, larger genetic regions (clusters of genes), full chromosomes, or entire genomes of any organism. DNA sequencing is also the most efficient way to indirectly sequence RNA or proteins (via their open reading frames). In fact, DNA sequencing has become a key technology in many areas of biology, medicine, forensics, anthropology and other sciences. Human genome sequencing helps better understanding, diagnosis and treatment of cancer, genetic diseases and various metabolic derangements.

Sanger sequencing

The Sanger technique for DNA sequencing (chain termination method) is based on incorporation of chain-terminating dideoxynucleotides, which stop the in-vitro DNA replication once incorporated in the new molecule. A new DNA is synthesized on an existing DNA template in the presence of a 2',3'-dideoxynucleotide. This produces variable pieces ending with this particular nucleotide. Four reaction mixtures are prepared for the four nucleotides. Electrophoresis then determines the relative lengths of the different pieces, which correspond to the order of nucleotides in the newly synthesized DNA.

The human genome project (HGP)

This was the international, collaborative research program (1990-2003) whose goal was the complete mapping and understanding of the genome (all the genes and non-coding DNA) of human beings. The project sequenced 92.1% of the genome, euchromatic regions, not including centromeres and telomeres. The number of coding genes was found to be about 22,300 genes.

The genome was broken into smaller pieces; approximately 150,000 base pairs in length. These were cloned using bacterial artificial chromosomes (BACs) as vectors. Each of these pieces was sequenced separately and then assembled and mapped to chromosomes.

The finished human reference genome is a mosaic of a small number of anonymous donors, all of the European origin. It provides a good approximation of the DNA of any single individual, keeping in mind the allelic diversity, e.g., blood groups and the major histocompatibility complex.

Subsequent projects have aimed at filling the gaps left by the HGP, sequencing the genomes of multiple distinct ethnic groups, and studying the variations linked to different diseases. Next-generation sequencing (NGS), also called high-throughput sequencing, techniques and advances in data analysis allow faster sequencing and at only a minor fraction of the old cost.

RNA sequencing (RNA-seq)

Next-generation sequencing of cDNA (RNA-seq) is now preferred to gene array in studying the differential gene expression and differential splicing of mRNAs. This RNA-seq can be used in studying the whole or selected populations of the transcriptome (all RNA). Direct RNA sequencing is evolving in order to avoid the complexity and artefacts of dealing with cDNA. Applications in medicine extend from diagnosis to designing new therapeutics.

Gene therapy

This may be the ultimate best treatment for genetic diseases, especially those attributed to a single defective gene. It is a logical alternative to the life-long enzyme replacement therapy. The normal gene is introduced into the cells of the affected tissue. A delivery vector is needed, which may be a retrovirus (RNA virus, e.g., lentivirus), an adenovirus, or a liposome. This is a treatment of somatic cells, and the transferred normal gene is not inherited by the offspring. The cells are treated either in vivo, or ex-vivo then re-introduced in the body.

A portion of the viral vector genome is replaced with the cloned gene (DNA or RNA) such that the virus can infect but becomes replication-defective. With a retrovirus vector, the transferred gene DNA, formed by reverse transcriptase, is integrated in the host cell DNA. The host cells should be replicating for successful therapy.

Random integration of DNA carries the risk of activating an oncogene. Some leukemias have already developed after delivering the interleukin receptor γ-chain gene into bone marrow stem cells of boys with X-linked SCID (severe combined immune deficiency). Adenovirus can infect non-dividing cells, but its DNA is not integrated in the host genome and is eventually lost. Targeting the appropriate tissue is a problem, and the transient expression of the therapeutic gene is still another problem. Host humoral and cellular immune responses occur to either the viral proteins or the therapeutic gene product itself since it may be considered a foreign protein.

The first approved gene therapy took place in the United States in 1990 for adenosine deaminase deficiency with severe combined immune deficiency (ADA-SCID). There have been several trials to overcome the aforementioned drawbacks, and successful gene therapies for several conditions have been produced. The high cost still remains a problem though it should be compared to the cost of the life-long enzyme replacement therapy.

In 2012, Glybera (trade name) was introduced in Europe for treatment of lipoprotein lipase deficiency; an intact copy of the gene was delivered by an adeno-associated virus. The drug was administered via a series of injections into the leg muscles, as many as 60, all in one session. It was abandoned in 2017 due to the high cost and rarity of the disease.

Strimvelis (trade name) was approved in Europe in 2016 to treat ADA-SCID. It is a personalized therapy; autologous hematopoietic stem cells ($CD34^+$ enriched cell fraction) are transduced with a retroviral vector that encodes the human ADA cDNA sequence. A similar treatment (Zynteglo) for β-thalassemia was approved in Europe in 2019. Luxturna, the first gene therapy for the eye to treat a genetic disease that causes blindness was approved in 2017 in the united states and 2018 in Europe. Other diseases treated by gene therapy include neuron disease, melanoma, and some blood and other malignancies.

Vaccination

The same principle of in-vivo gene therapy can be applied to vaccination. The aim is to produce the surface antigen of the pathogen inside the body to trigger the immune system. The Ebola vaccines, approved in 2019-2020, used recombinant viral vectors. In addition to the classical type of whole virus vaccine and the protein subunit vaccine used against COVID-19, a manufactured gene encoding the coronavirus spike protein was delivered to body cells using adenovirus. This technique has the advantage of mimicking natural infection, hence stimulating both B and T cells. This type of vaccine is relatively complex to manufacture, and a previous exposure to the vector may reduce its efficacy. RNA vaccines were also used for COVID-19 to direct the protein manufacturing machinery to the production of the viral protein.

Gene editing

Gene editing is intended as a more precise way to do gene therapy, to disable a bad gene or supply a good one that is missing. Trials on Hunter or Hurler syndrome (mucopoly-saccharidoses) started in 2017 with the corrective gene and an editing tool called zinc finger nucleases to insert it into the patient's DNA. Results showed a feeble but promising success.

CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) is a microbial immune system that confers resistance to invading phages. Cas9 is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. Combining it with a specifically designed guide RNA makes a gene editing tool that cuts DNA at specific sequences and alters genomes with relative ease and accuracy. Gene editing approaches can hopefully alter the DNA to precisely disrupt, delete, or repair the original diseased gene. The main disadvantage is the off-target changes made by this system.

In 2018, an announcement was made that the CRISPR tool had been used to produce gene edited babies in China. It was applied in IVF embryos to cripple production of an immune cell surface protein that HIV uses to establish an infection. This was denounced worldwide.

Preliminary results published in 2019 for CRISPR-Cas9 clinical trial showed a promise for the treatment of β-thalassemia and sickle cell disease by ex-vivo cleavage of the gene that represses the production of fetal hemoglobin. In-vivo CRISPR trials for treatment of the inherited blindness, Leber congenital amaurosis started in 2020. Other trials for other diseases including cancer are ongoing.

Many other applications are underway. For example, using CRISPR to modify the pig genome so that pig organs can be transplanted into humans without rejection. Another is the use of CRISPR as a diagnostics tool, e.g., to detect COVID-19. Combating coronavirus and other viruses using the CRISPR technique is another research area. New variants are developed, e.g., CRISPR-Cpf1 that makes it easier to replace one DNA sequence by another.

Silencing of gene translation

Anti-sense therapy depends on delivering a synthetic oligonucleotide strand complementary to a target mRNA. This strand pairs with and thus turns off the translation of the target mRNA. Mipomersen (approved in 2013 in the United States) targets ApoB-100 mRNA for treatment of homozygous familial hypercholesterolemia.

RNA interference by small interfering RNA (siRNA) has also been introduced in therapy. Patisiran has been approved in 2018 in the United States and Europe for the treatment of hereditary transthyretin-mediated amyloidosis with polyneuropathy. It targets an abnormal form of transthyretin (a transporter of thyroxine and retinol-binding protein bound to retinol). In 2019, the FDA-approved givosiran, an aminolevulinate synthase 1 (ALAS1)-directed siRNA for the treatment of adults with acute hepatic porphyria (AHP). Inclisiran has been approved in Europe in 2020 to silence the production of PCSK-9 in liver cells, thus inhibiting the catabolism of LDL receptors as a treatment of hypercholesterolemia.

Gene knockout, gene knock-in and transgenic animals

A mutation can be induced in a gene in the fertilized ovum or embryonic stem cells of an animal to produce a non-functional gene (gene knockout). A specific mutation, mimicking human disease, or a cloned cDNA gene can be inserted at a specific locus to produce gene knock-in. With traditional transgenic techniques, integration of the trans-gene is random, not targeted. The resulting heterozygous animals are mated to produce the genetically engineered homozygous knockout, knock-in, or transgenic animals. These animals are used as study models for various human diseases.

Animal cloning

Goats or cows could be genetically engineered to produce human proteins in their milk, and act as drug production lines. To have exact replicas of these animals, they should be cloned. Animal cloning is a reproduction method that avoids genetic alterations that may take place during meiosis. The genome is taken from a somatic cell of the animal to be cloned and introduced into a fertilized ovum from which the genome is removed. The embryo is implanted in the uterus of an animal to give birth to an offspring of the same genetic constitution as the genome donor animal. Many species have been cloned this way.

Mitochondrial transfer (three-parent babies)

Mutations of mitochondrial DNA (mtDNA), which represents about 1% of all cellular DNA, are maternally inherited. Even if the mother is a carrier and does not show the disease, her children may be diseased. Mitochondrial transfer aims at having a baby with normal mitochondria donated by another woman. A donor ovum is enucleated and loaded with the nucleus from the mother ovum before fertilization (spindle transfer) or from the zygote (pronuclear transfer). The resulting baby will have none or only a minor fraction of the mutated maternal mtDNA, and if a female, will pass on the normal mitochondria to her children. This technique was successful for cases of repeated miscarriages with no identified cause.

Measuring of viral load

Viral load (count) can be measured in a sample to put a treatment strategy or monitor the patient's condition. This can be achieved either by amplifying the target nucleic acid, e.g., by qPCR, or by amplifying the signal from the target, e.g., branched DNA (bDNA) technique (analogous to enzyme immunoassay for proteins). The same method should always be used.

Study Questions

Choose one best answer for every question of the following:

1- Restriction endonucleases are enzymes produced by
(A) bacteria.
(B) viruses.
(C) intestinal cells.
(D) cells of immune system.

2- Which of the following DNA sequences may be recognized by a restriction enzyme?
(A) GCGC
(B) GAGA
(C) GCCG
(D) GTTA

3- Recombinant or chimeric DNA is
(A) DNA resulting from fusion of two human cells or two bacterial cells.
(B) DNA synthesized by reverse transcriptase.
(C) DNA formed from the DNA of more than one species.
(D) DNA hybridized to its complementary RNA.

4- Recombinant DNA technology is used for which of the following?
(A) Diagnosis of viral hepatitis.
(B) Identification of the assailant in a rape case.
(C) Synthesis of human proteins to be used as drugs.
(D) Identification of the father of a child.

5- Electrophoresis may be used for analysis of:
(A) DNA by Western blotting.
(B) DNA by Northern blotting.
(C) RNA by Northern blotting.
(D) Proteins by Southern blotting.

6- Which of the following is used to detect which tissue expresses a certain gene?
(A) Southern blotting.
(B) Northern blotting.
(C) FISH.
(D) Karyotyping.

7- Complementary DNA (cDNA) is
(A) DNA synthesized on mRNA template.
(B) the complete cell genome.
(C) one of the two complementary DNA strands.
(D) one strand from the genomic DNA + one synthesized complementary strand.

8- What enzyme is used for synthesis of complementary DNA (cDNA)?
(A) DNA polymerase III.
(B) RNA polymerase II.
(C) DNA ligase.
(D) reverse transcriptase.

9- Complementary DNA is characterized by:
(A) Being synthesized by RNA polymerase
(B) Lacking introns present in genomic DNA.
(C) Lacking recognition sites for restriction enzymes.
(D) Being the same for all cell types.

10- The primer for complementary DNA synthesis is:
(A) Oligonucleotides complementary to the flanking regions of a gene.
(B) An RNA synthesized by primase enzyme.
(C) Synthetic poly dT oligonucleotide.
(D) Synthetic poly A oligonucleotide.

11- Complementary DNA not genomic DNA is used for genetic engineering to produce human proteins because
(A) genomic DNA cannot be merged with bacterial DNA.
(B) bacteria have no splicing mechanism for processing RNA product of genomic DNA.
(C) cDNA has introns required for protein synthesis.
(D) cDNA is easier to obtain.

12- *Taq* polymerase is used for PCR because:
(A) It is the only enzyme that can recognize the used primers.
(B) It stands high temperature without being denatured.
(C) It can catalyze the synthesis of both DNA and RNA.
(D) It is inhibited by the products of the PCR at the end of reaction.

13- The instrument used for PCR is:
(A) Fluorescence microscope.
(B) High speed centrifuge.
(C) Thermal cycler.
(D) Hot air oven.

14- The reaction mixture for PCR includes all the following except
(A) DNA containing the target sequence.
(B) Two primers flanking the 3'-ends of both strands of the gene.
(C) Deoxy-nucleoside triphosphates.
(D) Primase and a topoisomerase enzymes.

15- COVID-19 is diagnosed by
(A) PCR.
(B) RT-PCR.
(C) Western blotting.
(D) Southern blotting.

16- DNA sequencing means:
(A) Synthesis of DNA on mRNA template.
(B) Identification of DNA by a radio-labeled probe.
(C) Separation of DNA fragments by electrophoresis.
(D) Identification of number, types and order of DNA nucleotides.

17- A viral protein obtained by genetic engineering has which advantage as a vaccine?
(A) Eliminating the risk of introducing viral particles during vaccination.
(B) Multiplication inside the body of a vaccinated individual.
(C) Induction of a stronger immune response than the whole virus.
(D) Oral administration.

18- To compare the expression of multiple genes in two different tissues, we may use:
(A) Southern blotting.
(B) Gene microarray.
(C) Gene sequencing.
(D) Fluorescence in situ hybridization.

19- Gene therapy is preferred to enzyme replacement therapy because it
(A) costs less.
(B) gives a longer duration of action.
(C) is less complicated.
(D) has no side effects.

20- Translation of a specific protein is inhibited by
(A) monoclonal antibodies.
(B) siRNA.
(C) cDNA.
(D) puromycin.

21- Gene therapy should be directed to
(A) somatic cells.
(B) germ line cells.
(C) embryonic cells.
(D) the non-fertilized ovum.

22- If a patient with cystic fibrosis were to be treated by gene therapy, which type of cells should be targeted?
(A) Epithelial cells.
(B) Hemopoietic stem cells.
(C) Ova.
(D) Spermatozoa.

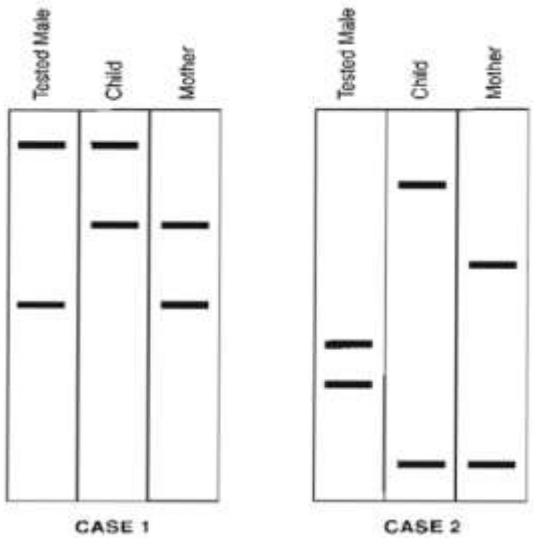
23- Antisense therapy inhibits
(A) replication.
(B) transcription.
(C) translation.
(D) post-translation modification.

24- A baby born after mitochondrial transfer may still carry some mutated mtDNA because of
(A) carry-over.
(B) mutation during the procedure.
(C) mtDNA from the father.
(D) faulty pickup of the embryo to implant.

25- CRISPR-Cas9 disruption of a gene depends on
(A) homologous repair.
(B) non-homologous repair.
(C) permanent cut of DNA.
(D) insertion of a mutated gene.

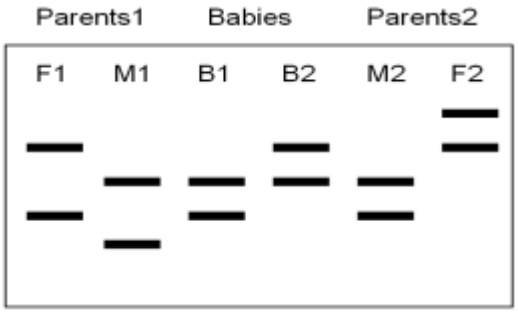
26- DNA fingerprint may be obtained by:
(A) Synthesis of cDNA on RNA from fingertips.
(B) Chromosomal analysis of the mother and father.
(C) Identification of the lengths of certain regions of DNA.
(D) Identification of the genes responsible for the thumb fingerprint

27- In a paternity test, DNA from one locus was amplified by PCR and subjected to electrophoresis as shown below:



(A) The tested male may be the father in case 1.
(B) The tested male may be the father in case 2.
(C) Both (a) and (b).
(D) Neither (a) nor (b).

28- In a mixed babies case, a DNA locus analysis is shown for fathers (F1,F2), mothers (M1,M2) and babies (B1,B2):



(A) B1 is the child of Parents1
(B) B2 is the child of Parents1
(C) B1 is the child of Parents2
(D) All the above is possible.

Match each item to the most proper technique:
(A) Gene microarray.
(B) FISH.
(C) Recombinant DNA.
(D) DNA fingerprint.

29- Identification of the assailant in a rape case.

30- Synthesis of human proteins.

31- Identification of a child's parents.

32- Comparing the expression of multiple genes in two different tissues.

33- Checking for presence of a certain gene.

34- Studying the effect of treatment on gene expression.

35- Diagnosis of a chromosomal deletion.

Match each item to the most proper technique:
(A) Southern blotting
(B) DNA sequencing.
(C) PCR.
(D) Western blotting.

36- Viral load.

37- Human genome.

38- One hair follicle as evidence.

39- Restriction fragment length polymorphism.

40- Labeled antibodies.

41- mRNA.

42- Diagnosis of neonatal HIV infection.

43- Aminoacid sequence.

44- Protein electrophoresis.

Molecular Biology of Cancer

Oncogenes and tumor suppressor genes

Cancer is a group of diseases that start in genes. Tumor suppressor genes are those that encode proteins whose normal function helps to prevent tumor formation by being involved in regulating the cell cycle or cell adhesion. Oncogenes are those that encode proteins that can lead to tumor formation, e.g., an overactive growth factor receptor. Proto-oncogenes are those that encode proteins involved in various aspects of cellular growth and proliferation and, when mutated, can become oncogenes. Proto-oncogenes include genes encoding: cellular growth factors, growth factor receptors, signal transduction factors, and nuclear transcription factors.

Activation of proto-oncogenes

Activation of proto-oncogenes takes place by different mechanisms. One of these mechanisms is insertion mutagenesis, as a result of retrovirus infection that causes random integration of cDNA of the viral genome, formed by reverse transcriptase, in the human cell genome. Random integration of a promoter (LTR, long terminal repeat of retrovirus) or an enhancer sequence in the host DNA may cause activation of an oncogene. An example is the activation of human *myc* gene (originally identified in the avian **my**elo**c**ytomatosis virus and produces a nuclear transcription factor) involved in numerous hematopoietic neoplasias.

Another mechanism of proto-oncogene activation is genome alteration leading to change of the control of the oncogene, e.g., translocation of the *myc* proto-oncogene in Burkitt lymphoma and Philadelphia chromosome associated with chronic myelogenous leukemia (CML). Chromosomal translocation between chromosomes 8 and 14 causes fusion of *myc* proto-oncogene with another gene forming an abnormal hybrid gene that leads to Burkitt Lymphoma. Philadelphia chromosome results from translocation between chromosomes 9 and 22, which creates bcr-abl protein, a fusion protein of two normal cell proteins. When the tyrosine kinase *abl* (Abelson murine leukemia virus) gene is moved from chromosome 9 to 22 and put under control of *bcr* (break-point cluster region) gene, it is mis-expressed and constitutively active. It is associated with abnormal growth of blood cells (chronic myelogenous leukemia, CML).

HER2/Neu (Human Epidermal growth factor Receptor-2/Neuroblastoma), also known as *c-ErbB-2* because of its similarity to avian erythroblastosis oncogene B (c=cellular in contrast to v=viral) is over-expressed in many cancers, especially aggressive breast cancer. It is used as a tumor marker and a target for monoclonal antibody therapy. The conversion of this proto-oncogene to an oncogene (present at a high concentration) requires only a single amino acid change in the transmembrane domain.

A point mutation in *ras* (rat sarcoma) genes can produce over-active ras proteins (monomeric G-proteins involved in signal transduction leading to cell growth and differentiation). *Ras* is the most common oncogene in human cancer, found in 20% to 30% of all human tumors and up to 90% in certain types of cancer, e.g., pancreatic cancer.

Inherited cancer syndrome

Inherited cancer syndromes may result from mutations in tumor suppressor genes or proto-oncogenes in the germ line. For an oncogene, a mutation in one allele can cause cancer. For tumor suppressor genes, mutation of the two alleles is usually needed for cancer to develop. A mutation of one allele is inherited as predisposition to hereditary cancer; the second mutation is experienced by the fetus in one somatic cell. This is called the two-hit model for cancer inheritance. Examples include retinoblastoma, familial breast cancer, familial colon cancer, familial melanoma, and neurofibromatosis.

Retinoblastoma

About one half of retinoblastoma cases are familial. The predisposition to retinoblastoma is caused by the inheritance of a mutation of *RB1*, a tumor suppressor gene on chromosome 13. For cancer development, the developing fetus must experience a second mutation in the other copy of *RB1* in a specific retinoblast (i.e., a somatic mutation). Normally, the *RB1* protein product regulates the cell cycle to cause cells to divide in a controlled manner. Once both copies of the tumor suppressor gene are mutated in a cell, cell cycle control is lost, and the cell can divide uncontrollably, leading to tumor formation. Those individuals who inherit a mutation and do not develop a retinoblastoma were fortunate enough to not experience a somatic mutation in any of their retinoblasts.

Breast cancer

About 5% of breast cancer cases are inherited in an autosomal dominant fashion. Most of these cases are the result of mutations in either the *BRCA1* gene (chromosome 17) or the *BRCA2* gene (chromosome 13). Women who inherit one of these mutations have an approximately 60% chance of developing a breast tumor. Women with a *BRCA1* mutation also have at least a 20% chance of developing ovarian cancer. Both *BRCA1* and *BRCA2* are involved in the DNA repair process. A small proportion of breast cancer cases are the result of mutations in the *p53* tumor suppressor gene.

Colon cancer

A small proportion of colon cancers results from inherited mutations in the *APC* (adenomatous polyposis coli) gene on chromosome 5. *APC* is a tumor suppressor gene that encodes a protein involved in regulating cell adhesion and signaling to the nucleus. Individuals who inherit an *APC* mutation develop hundreds of colonic polyps and have a very high risk of developing colon cancer. The two-hit model applies to this inherited cancer syndrome: a first hit is inherited, but a second hit in a colonic epithelial cell is required to produce a cell capable of tumor formation. In addition to its role in this inherited cancer syndrome, *APC* is an important part of the complex pathway to the common non-inherited colon cancer. About 85% of all colon tumor cells have somatic mutations in their *APC* gene.

A second type of inherited colon cancers is hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome). It is responsible for about 5% of cases of colon cancer. It can be caused by mutations in any of five different genes, all of which encode proteins involved in DNA mismatch repair.

Role of environmental factors in development of cancer

Somatic mutations in tumor suppressor genes and proto-oncogenes play a key role in the causation of common cancers such as most breast and colon tumors. Many of these somatic mutations can be caused by environmental factors, which include radiation, carcinogenic chemicals and infection with certain oncoviruses. DNA damage produced by environmental agents is usually corrected by DNA repair mechanisms. Inherited inability to repair DNA causes increased risk of developing a malignancy, e.g., in xeroderma pigmentosum.

Radiation

Ultraviolet (UV) rays, X-rays, and γ (gamma)-rays are mutagenic and carcinogenic. They can damage DNA by formation of pyrimidine dimers, elimination of bases thus forming apurinic or apyrimidinic sites, formation of single- or double-strand breaks or cross-linking of DNA strands. X-rays and γ-rays can cause the generation of reactive oxygen species, which can also be mutagenic and carcinogenic. Excess exposure to UV radiation is common due to exposure to sunlight and is linked to skin cancer, especially with low pigmentation (low skin melanin).

Chemicals

Certain chemicals, such as aromatic amines, alkylating agents, and cyclophosphamides are known carcinogenic. Nitrosamines in processed meat to which nitrite is added is linked to gastrointestinal cancers. Aflatoxin, the fungal product in beans and nuts is known to cause liver cancer. Asbestos was linked to lung cancer. Tobacco is the source of a lot of documented carcinogens. Recognized carcinogens include benzene, formaldehyde, and alcohol products among others.

Development of cancer depends on the volume, duration and route of exposure in addition to personal factors. Carcinogenic chemicals can cause base deamination, other covalent modifications, and formation of free radicals. Cigarette smoke can cause somatic mutations in the *p53* gene, which protects against several cancers including lung cancer.

Oncogenic viruses (oncoviruses)

About 15% of all human cancers worldwide may be attributed to viruses. Both DNA and RNA viruses are capable of causing cancer in humans. Epstein-Barr virus, human papilloma virus, hepatitis B virus, and human herpes virus-8 are four DNA oncoviruses. Human T lymphotropic virus type 1 (a retrovirus) and hepatitis C virus are two RNA oncoviruses. Only a minority of infected individuals progress to cancer, usually years or even decades after primary infection. Additional events and host factors, such as immunosuppression, somatic mutations, genetic predisposition, and exposure to carcinogens may also play a role.

The genetic material of the virus uses the host cell machinery to produce new viral genome and proteins, i.e., new viral particles. Doing so, the oncovirus causes deregulation of the cell cycle, inhibition of apoptosis, and abnormalities of cell signaling pathways leading to abnormal growth and metastasis.

The DNA oncoviruses often act by inhibiting the tumor suppressor genes: *p53* and retinoblastoma (*RB*). Oncoviruses may encode homologs of Bcl-2, which inhibits apoptosis. RNA oncoviruses often carry oncogenes in their genomes. Retroviruses may cause insertion mutagenesis by activation of an oncogene as explained above.

Role of *p53* gene

This gene is called the guarding angel gene, the guardian of the genome, or the policeman of the cell. It normally encodes a 53 kDa protein that plays a key role in G_1 and G_2 checkpoints for monitoring and repairing DNA. The encoded protein is an important transcription factor that helps in arresting cell division and providing enzymes needed for DNA repair. If DNA damage is beyond repair, the affected cell undergoes apoptosis (programmed cell death).

A disabled *p53* results in the persistence of damaged cells, which can lead to the formation of tumors. Over 50% of human tumors have developed an inactivating mutation in p53 activity. The autosomal dominant Li-Fraumeni syndrome characterized by development of multiple cancers is attributed to mutation of *p53*.

Apoptosis

Apoptosis is a gene-directed active form of cell death, programmed cell death, or cell suicide, which leads to elimination of billions of unwanted cells every day. (Greek: apo = off, ptosis = falling). It is different from passive necrosis resulting from trauma and associated with inflammation.

Apoptosis is essential for proper embryonic morphogenesis, e.g., having separate fingers and toes. It is also important for involution of the uterus after delivery and regression of mammary glands after weaning. One important function of apoptosis is getting rid of harmful cells, e.g., cells with damaged DNA or aged cells.

Apoptosis is initiated by extrinsic inducers, e.g., nitric oxide (NO), cytokines, and hormones or intrinsic inducers, which arise from within the cell in response to severe cell stress, e.g., DNA damage by radiation or chemotherapy, nutrient deprivation, viral infection, and hypoxia. The *p53* gene product initiates apoptosis by activating the transcription of the pro-apoptotic protein, BAX.

The apoptosis pathways activate proteolytic enzymes, caspases (contain cysteine in the catalytic site and cleave the substrate at aspartyl residues), which degrade the cellular organelles. the cells become apoptotic bodies, which are eliminated by phagocytosis. Phagocytes recognize phosphatidyl serine, originally in the inner leaflet of the cell membrane, appearing on the surface.

Apoptosis is impaired in some conditions like inactivating mutation of *p53* gene and infection with viruses that encode homologs of the apoptosis inhibitor, Bcl-2 (first discovered in B cell lymphoma). Apoptosis is absent in immortal tumor cells, which contain telomerase that prevents chromosome shortening with division.

Tumor Markers

Tumor markers are substances that are produced by cancer cells or by other cells of the body and increase in cases of cancer. Most tumor markers are made by normal cells as well as by cancer cells; however, they are produced at much higher levels in cancerous conditions. These substances may be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of cancer patients.

Several tumor markers have been characterized and are in clinical use. Some are associated with only one type of cancer, whereas others are associated with two or more cancer types. The choice of a tumor marker for a patient depends on the type of tumor, not the organ to which it has metastasized.

Major types of tumor markers:

- Protein antigens.
- Enzymes.
- Hormones and hormone catabolites.
- Hormone receptors.
- Patterns of gene expression and changes to DNA. These are assessed in tumor tissue specifically.

Limitations of tumor markers:

- There is no “universal” tumor marker that can detect any type of cancer.
- Tumor markers have not been identified for every type of cancer.
- Sensitivity and specificity of tumor markers are not 100%.
- No tumor marker is sufficiently sensitive or specific to be used on its own to screen for cancer. For example, the prostate-specific antigen (PSA), in the blood, was often used to screen men for prostate cancer. However, an increased PSA level can be caused by benign prostate conditions, and most men with an elevated PSA level do not have prostate cancer. PSA routine testing may lead to overdiagnosis and overtreatment, and at best leads to only a small reduction in the number of prostate cancer deaths.

Usefulness of tumor markers:

- They help detect and diagnose some types of cancer. Tumor markers are usually combined with other tests, such as biopsies and imaging.
- In some types of cancer, the level of a tumor marker reflects the stage of the disease and/or the patient’s prognosis.
- Tumor marker levels may help in planning the appropriate therapy.
- They may be used to monitor response to therapy. Therefore, it is important to know the baseline level of a tumor marker before starting treatment. Sampling should ideally be repeated after 5-6 half-lives of the marker in question; but if found elevated, the next sampling after 2-4 weeks, for additional evidence, may be justified.
- After treatment has ended, tumor markers are used to check for recurrence. Tumor markers are more sensitive than other methods for detection of recurrence.

Some tumor markers in common use

Marker	Cancer types	Sample	Usage
Alpha-fetoprotein (AFP)	Liver cancer and germ cell tumors	Blood	to help diagnose liver cancer and follow response to treatment; to assess stage, prognosis, and response to treatment of germ cell tumors
Beta-2-microglobulin (B2M)	Multiple myeloma, chronic lymphocytic leukemia, and some lymphomas	Blood, urine, cerebro-spinal fluid	to determine prognosis and follow response to treatment
Beta-human chorionic gonado-tropin (Beta-hCG)	Choriocarcinoma and testicular cancer	Urine, blood	to assess stage, prognosis, and response to treatment
BCR-ABL fusion gene	Chronic myeloid leukemia	Blood, bone marrow	to confirm diagnosis and monitor disease status
CA19-9 (cancer antigen, carbohydrate associated antigen 19-9)	Pancreatic cancer, gallbladder cancer, bile duct cancer, and gastric cancer	Blood	to assess whether treatment is working
CA15-3/CA27.29	Breast cancer	Blood	response to treatment /recurrence
CA-125	Ovarian cancer	Blood	diagnosis, response to treatment, recurrence
Calcitonin	Medullary thyroid cancer	Blood	diagnosis, response to treatment, recurrence
Carcinoembryonic antigen (CEA)	Colorectal cancer and breast cancer	Blood	spread of colorectal cancer; breast cancer recurrence / response to treatment
CD20	Non-Hodgkin lymphoma	Blood	whether treatment with a targeted therapy is appropriate
Chromogranin A (CgA)	Neuroendocrine tumors	Blood	diagnosis, assessment of treatment response, and evaluation of recurrence
Cytokeratin fragments 21-1	Lung cancer	Blood	recurrence
EGFR mutation analysis	Non-small cell lung cancer	Tumor	to help determine treatment and prognosis
Estrogen receptor (ER)/progesterone receptor (PR)	Breast cancer	Tumor	to determine whetehr hormonal therapy (such as tamoxifen) is appropriate
Fibrin/fibrinogen	Bladder cancer	Urine	to monitor progression and response to treatment
HER2/Neu	Breast cancer, gastric cancer, and esophageal cancer	Tumor	to determine whether treatment with trastuzumab is appropriate
Gastrin	Gastrinoma	Blood	diagnosis / response to treatment / recurrence
Immunoglobulins	Multiple myeloma and Waldenström macroglobulinemia	Blood, urine	diagnosis / response to treatment / recurrence
Prostate-specific antigen (PSA)	Prostate cancer	Blood	diagnosis / response to treatment / recurrence
Thyroglobulin	Thyroid cancer	Tumor	response to treatment / recurrence

Study Questions

Choose one best answer for every question of the following:

1- A tumor suppressor gene may encode
(A) an enzyme of DNA repair.
(B) a growth factor.
(C) a growth factor receptor.
(D) an inhibitor of apoptosis.

2- Insertion mutagenesis may be caused by
(A) chromosomal rearrangement.
(B) epigenetic changes of the genome.
(C) retrovirus infection.
(D) gene inducers.

3- The product of *p53* gene may function by inhibiting
(A) telomerase.
(B) apoptosis.
(C) mitosis.
(D) DNA repair.

4- Two-hit model for cancer inheritance entails that
(A) two mutated alleles are inherited.
(B) two oncogenes are present.
(C) somatic mutations occur in two proto-oncogenes.
(D) one mutation is inherited and one is acquired.

5- Which of the following meets the definition of tumor markers?
(A) produced exclusively by cancer cells.
(B) high levels in cancerous conditions.
(C) non- protein.
(D) only in blood.

6- Hormone receptors may be measured as tumor markers in the
(A) blood.
(B) urine.
(C) stool.
(D) tumor tissue.

7- The choice of a tumor marker for a patient usually depends on the
(A) organ of origin of the tumor.
(B) organ to which the tumor has metastasized.
(C) treatment received for the tumor.
(D) severity of the disease.

8- To screen a population for a certain tumor, you prefer a marker with
(A) high sensitivity.
(B) high specificity.
(C) low sensitivity.
(D) low specificity.

9- The wise use of tumor markers in diagnosis of cancer dictates
(A) using the most expensive markers.
(B) combining with other tests, such as biopsies.
(C) repetition of the analysis.
(D) doing it only after surgery.

10- Which of the following is true about the prostate-specific antigen (PSA)?
(A) It is a sensitive and specific marker used on its own to diagnose prostate cancer.
(B) It may be measured in the blood to screen high-risk men for prostate cancer.
(C) Its level is not raised by benign prostate conditions.
(D) Most men with an elevated PSA level do have prostate cancer.

11- Which of the following is true about the use of tumor markers for diagnosis?
(A) No single tumor marker is used on its own for diagnosis of a tumor.
(B) The use of a combination of tumor markers can guarantee 100% sensitivity and 100% specificity.
(C) Both (A) and (B).
(D) Neither (A) nor (B).

12- Which of the following is true about total and free PSA?
(A) A high total PSA level and a high free PSA level generally indicate prostate cancer.
(B) Free PSA may be useful when total PSA level is borderline (4-10 ng/mL).
(C) Men with a total PSA in the “gray area” and a free PSA greater than 25% are more likely to have cancer.
(D) Men with a total PSA in the “gray area” and a free PSA below 10% are more likely to have a benign condition, needing no biopsy.

13- Which of the following would you measure in a case of multiple and atypical peptic ulcers?
(A) CEA.
(B) AFP.
(C) Gastrin.
(D) Epinephrine.

Answers of MCQs

L 1		L 6	L 9	L 12	L 13		L 18	L 22	L 24	L 27	L 30
1-D	11-C	1-D	1-B	1-D	1-C	41-B	1-A	1-C	1-A	1-D	1-A
2-C	12-D	2-B	2-A	2-A	2-C	42-B	2-C	2-D	2-C	2-C	2-A
3-C	13-D	3-D	3-A	3-B	3-B	43-D	3-B	3-C	3-B	3-C	3-C
4-A	14-A	4-A	4-B	4-B	4-A	44-A	4-A	4-A	4-D		4-C
5-C	15-C	5-B	5-C	5-B	5-C	45-C	5-C	5-A	5-A	**L 28**	5-C
6-B	16-C	6-D	6-C	6-B	6-A	46-B	6-C	6-B	6-B	1-D	6-B
7-B	17-D	7-C	7-B	7-A	7-A	47-A	7-A	7-C	7-D	2-D	7-A
8-C	18-D	8-B	8-A	8-D	8-C		8-A	8-A	8-D	3-D	8-D
9-A	19-C	9-C	9-C	9-D	9-A	**L 15**	9-D	9-C	9-C	4-D	9-B
10-B	20-C	10-A	10-D	10-C	10-B	1-C	10-A	10-D		5-D	10-C
11-D		11-D	11-A	11-C	11-C	2-B	11-B	11-B	**L 25**	6-A	11-B
12-C	**L 3**	12-D	12-D	12-C	12-D	3-A	12-C	12-A	1-D	7-B	12-B
13-A	1-A	13-D	13-A	13-D	13-B	4-C	13-B	13-A	2-A	8-D	13-C
14-B	2-B		14-A	14-B	14-A	5-B	14-C	14-A	3-A	9-D	14-D
15-D	3-A	**L 7**	15-A	15-C		6-A	15-C	15-C	4-B	10-C	15-B
16-A	4-C	1-A	16-A	16-B	**L 14**	7-A	16-B	16-C	5-D	11-A	16-D
17-C	5-B	2-D	17-B	17-C	1-A	8-A		17-D	6-C		17-A
18-B	6-C	3-C	18-C	18-C	2-C	9-A	**L 19**	18-A	7-D	**L 29**	18-B
19-B	7-D	4-D	19-C	19-D	3-B	10-B	1-C		8-D	1-C	19-B
20-C	8-D	5-D	20-C	20-A	4-C	11-A	2-B	**L 23**	9-A	2-D	20-B
21-C	9-A	6-D	21-D	21-C	5-A		3-A	1-B	10-A	3-D	21-A
22-C	10-C	7-A	22-D	22-B	6-D	**L 16**	4-A	2-B		4-A	22-A
23-C	11-D	8-C	23-B	23-B	7-D	1-A	5-D	3-A	**L 26**	5-B	23-C
24-C	12-D	9-C	24-A	24-C	8-C	2-C	6-C	4-D	1-B	6-C	24-A
25-C	13-B	10-D	25-D	25-A	9-C	3-D	7-B	5-A	2-A	7-B	25-B
26-C	14-B	11-C	26-D	26-A	10-C	4-D	8-A	6-A	3-A	8-A	26-C
27-C	15-B	12-B	27-D	27-B	11-B	5-C	9-D	7-A	4-D	9-B	27-A
28-C	16-C	13-A	28-C	28-A	12-C	6-A	10-C	8-B	5-C	10-A	28-A
29-D	17-C	14-C	29-A	29-C	13-A	7-A		9-D	6-D	11-B	29-D
30-D	18-D	15-B	30-B	30-D	14-C		**L 20**	10-D	7-D	12-C	30-C
31-D	19-C	16-D	31-D	31-C	15-C	**L 17**	1-A	11-D	8-B	13-C	31-D
32-D		17-D	32-C	32-D	16-B	1-B	2-C	12-B	9-A	14-C	32-A
33-D	**L 4**		33-D	33-A	17-D	2-A	3-B	13-D	10-D	15-D	33-B
34-D	1-D	**L 8**	34-A	34-D	18-C	3-D	4-C	14-D	11-D	16-A	34-A
35-D	2-D	1-B		35-B	19-D	4-B	5-A	15-B	12-B	17-B	35-B
36-D	3-B	2-C	**L 10**	36-C	20-D	5-D		16-A	13-C	18-D	36-C
37-D	4-C	3-D	1-C	37-B	21-C	6-C	**L 21**	17-D	14-D	19-C	37-B
38-D	5-A	4-C	2-D	38-C	22-C	7-B	1-D	18-A	15-A	20-C	38-C
39-D	6-C	5-A	3-C	39-C	23-B	8-B	2-D	19-A	16-A	21-D	39-A
40-D	7-B	6-B	4-B	40-C	24-A	9-D	3-D	20-D	17-D	22-C	40-D
41-D	8-D	7-D	5-C	41-C	25-A	10-B	4-B	21-D	18-D	23-B	41-C
42-D	9-B	8-A	6-A	42-B	26-B	11-A	5-D	22-D	19-D	24-A	42-C
43-D	10-A	9-A	7-B	43-A	27-D	12-D	6-C		20-D	25-C	43-B
44-D	11-A	10-B	8-B	44-B	28-C	13-C	7-B		21-B	26-D	44-D
45-D	12-C	11-C	9-D	45-B	29-A	14-A	8-A		22-C	27-C	
46-D	13-A	12-C	10-D	46-B	30-C	15-D	9-D		23-D	28-A	**L 31**
47-D	14-B	13-D		47-D	31-D	16-C	10-D		24-D	29-C	1-A
	15-C	14-C	**L 11**	48-C	32-C	17-C	11-B		25-A	30-B	2-C
L 2		15-A	1-C	49-A	33-C	18-B	12-A		26-D		3-C
1-C	**L 5**	16-C	2-C	50-D	34-C	19-B	13-A		27-C		4-D
2-D	1-B	17-D	3-D	51-D	35-B	20-A	14-B		28-C		5-B
3-D	2-B	18-B	4-D	52-A	36-B	21-D	15-D		29-A		6-D
4-B	3-D	19-B	5-A	53-C	37-B	22-C	16-A		30-B		7-A
5-A	4-D	20-C	6-C	54-B	38-D	23-C	17-A		31-D		8-A
6-A	5-A	21-C	7-A	55-A	39-C	24-A	18-B				9-B
7-C	6-A	22-A	8-C	56-A	40-B	25-C	19-C				10-B
8-D		23-B	9-C	57-B	→		20-C				11-A
9-A		24-C	10-A	58-B			21-B				12-B
10-D		25-B	11-B	59-B			22-D				13-C
→		26-D	12-B	60-C							