

BIOLOGY SHORT NOTE AND RELATED QUESTIONS ON :
ENZYME
CELL BIOLOGY
ENERGY TRANSFORMATION

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3. UNIT THREE

ENZYMES

At the end of the unit students will be able to:

- Define enzymes and explain the properties of enzymes.
- Appreciate the importance of enzymes in industries and local products.
- Explain how enzymes lower activation energy.
- Discuss the action of apoenzyme and coenzymes.
- Explain factors that affect enzyme activity.
- Explain allosteric regulation and feedback control mechanism of enzyme activity.

What is enzyme ?

- In 1877, German physiologist Wilhe kuhne first used the term **enzyme**, which comes from Greek "leavened" or "in yeast".
- Eduard Buchner submitted his first paper on the study of yeast extracts in 1897.
- He found that sugar was fermented by yeast extracts even when there were no living yeast cells in the mixture.
- He named the enzyme that brought about the fermentation of sucrose **zymase**.

Structure and properties of enzyme

- Enzymes are life's great facilitators.
- They create the conditions needed for biochemical reactions to happen fast.
- The general name that chemists use for a chemical entity that increases the speed of a reaction is a "catalyst."
- Enzymes are biological catalysts as they catalyze the chemical reactions inside living things.
- A catalyst speeds up a chemical reaction with no effect on:
 - The products formed
 - The energy change
 - The nature of the catalyst itself
 - Course (equilibrium) of the reaction.
- Nearly all biological catalysts are enzymes. They are globular proteins with a specific tertiary shape, part of which forms an active site.
- Enzymes are globular proteins with a complex 3-D structure. The shape and the chemical environment inside the active site permits a chemical reaction to proceed more easily.

- The substrates of enzymes are the reactants that are activated by the enzymes. A substrate molecule binds with the active site to form an enzyme–substrate complex. This then forms the products.
- An enzyme's activity decreases markedly outside its optimal temperature and pH, and many enzymes are (permanently) denatured when exposed to excessive heat, losing their structure and catalytic properties
- Enzyme activity can be affected by: inhibitors molecules that decrease enzyme activity, and activators molecules that increase activity.

Examples: therapeutic drugs and poisons are enzyme inhibitors

- Enzyme can be used over and over, because it is unaltered by the reaction.
- Enzyme are effective in small amount, of enzyme can affect a large amount of substrate.

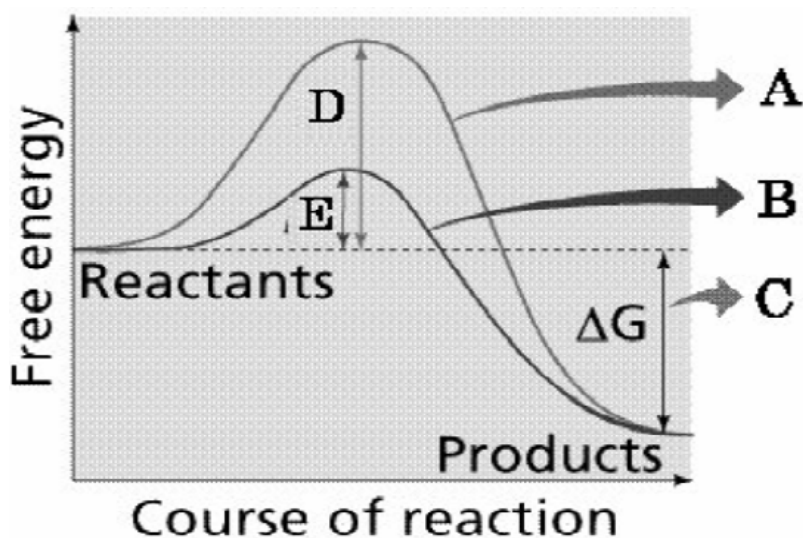
Functions of Enzyme

- Enzymes are known to catalyze more than 5,000 biochemical reaction types.
- Other biocatalysts are catalytic RNA molecules, called ribozymes. Enzymes' specificity comes from their unique three-dimensional structures.
- Like all catalysts, enzymes increase the reaction rate by lowering its activation energy. Example: orotidine 5'-phosphate decarboxylase, which allows a reaction millions of years to occur in milliseconds.
- Some enzymes are used commercially, for example, in the synthesis of antibiotics.
- Some household products use enzymes to speed up chemical reactions:
- **Example:** In biological washing powders break down protein, starch or fat stains on clothes, and enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.
- In order for molecules to react, they must have sufficient energy.
- **Activation energy (or E_a)**, is minimum energy needed to start off the reaction.
- The reactant must 'climb an activation energy hill' before anything happens.
- Under normal conditions, very few molecules have sufficient kinetic energy to 'climb the activation energy hill', so the reaction proceeds slowly.
- More reactant molecules can meet this lower energy requirement and so the reaction proceeds more quickly.

Answer the following multiple choice questions.

1. It is more useful for biochemists to know the specific activity of an enzyme than the activity of the enzyme because:
 - A. High levels of activity indicate very busy enzymes
 - B. Enzyme activation is brought about only under specialized conditions
 - C. Biochemistry is a very specialized field
 - D. Specific activity tells us exactly which reaction is being catalyzed and results in more positive identification of enzymes**

For the following questions, match the letters with the appropriate description of the graph of the energy flow of a chemical reaction shown below.



- A. Energy path of an uncatalyzed reaction _____
- B. Energy released from this reaction _____
- C. Activation energy of a catalyzed reaction _____
- D. Activation energy of an uncatalyzed reaction _____
- E. Energy path of a catalyzed reaction _____

Enzyme Nomenclature and Classification

- Different enzymes are named in different ways. This includes:
 - By adding 'ase' to part of the name of the substrate.
Example: Lipase (lipid hydrolyzing enzyme), *Sucrase* (sucrose hydrolyzing enzyme).
 - On the basis of the reaction that they catalyze.
Example: polymerase (aids in polymerization – joining similar units together), *dehydrogenase* (removal of hydrogen atoms or ions).

- On the source from which they were first identified. E.g. *papayin* from papaya.
- According to their site of action. E.g. *intestinal* protease acts on proteins in the intestine.
- Some enzymes end with ‘**in**’, indicating that they are basically proteins.
Example: *pepsin*, *trypsin*, etc.

Because of the varied ways in which enzymes had been named, biologists at the **Enzyme Commission** decided to produce a systematic way of naming enzymes, based on the ways in which the **enzymes act**.

The enzyme commissions (EC) numbers divide enzymes into six main groups according to the type of reaction catalyzed

1. Oxidoreductases

- Catalyses redox reactions, in which hydrogen or oxygen atoms or electrons are transferred between molecules.

Example: Dehydrogenases (hydride transfer), oxidizes (electron transfer to molecular oxygen), oxygenase (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide).

2. Transferees

- Catalyze the transfer of an atom or group of atoms (e.g. acyl-, alkyl- and glycosyl-), between two molecules, but excluding such transfers as are classified in the other groups (e.g. oxidoreductases and Hydrolases).
For example: aspartate aminotransferase, L-aspartate: 2-oxoglutarate aminotransferase.

3. Hydrolases

- Catalyses hydrolytic reactions, is commonly present within the field of enzyme technology and digestive process. Example: esterases, glycosidase, lipases and proteases.

4. Isomerases

Catalyze molecular izomerization and includes the epimerases, racemases and intermolecular transferases.

- Example: xylose isomerase, D-xylose ketol-isomerase; commonly called glucose isomerase).

5. Lyases

- Catalyses elimination reactions in which a group of atoms is removed from the substrate.
Example: aldolases, decarboxylase, dehydratases and some pectinases.
Histidine ammonia-lyases, L-histidine ammonia-lyases; also called histidase).

6. Ligases or synthetase

- Catalyses formation of a covalent bond joining two molecules together, coupled with the hydrolysis of a nucleoside triphosphate.
Example: glutathione synthase (g-L-glutamyl-L-cysteine: glycine ligase (ADP-forming); also called glutathione synthetase).

International Union of Biochemistry (1984) classified Enzyme. This enzyme commission assigned each enzyme name and four distinguishing number.

Example: • in Enzyme **EC 3.4.11.1** is:

EC stands for Enzyme Commission

- The first number shows to which of the six main classes the enzyme belongs
- The second figure indicates a subclass
- The third figure gives a sub-subclass
- The fourth figure is the serial number of the enzyme in its sub subclass.
- **A Hydrolase** – all the enzymes in class 3 hydrolyse some kind of bond.
- **A peptidase** – all the enzymes in subclass 4 of class 3 are peptidases and hydrolyse peptide bonds
- **An amino-peptidase** – all the enzymes in sub-subclass 11 of subclass 4 are amino-peptidases; they hydrolyse peptide bonds at the amino end of a polypeptide chain
- **Leucyl-amino-peptidase** – this particular amino-peptidase is number 1 of this sub subclass

Some industrial uses of enzyme technology

Sector	Application area	Benefits
Dairy	• Biochymosin to produce cheese. • Lactase to produce lactose-free milk	• Supplies of natural rennet from calves livers are limited. • Lactose-intolerant people suffer fewer cramps
Detergents	• Use of proteases, lipases and amylases in biological washing powders. • Use of proteases and amylases in dishwasher detergents	• Many biological stains are removed efficiently at low temperatures (saving energy). • Remove food particles at lower temperatures and require fewer bleaching products to be added
Textiles	• Proteases to remove hair and lipases to degrease animal hides. • Use of cellulase to 'bio-polish' cotton fabrics. • Use of cellulase to 'bio-stone' denim	• Process is carried out much quicker than by traditional methods. • Produces a smoother and glossier finish. • The enzyme gives the 'stone-washed' effect much more easily
Food processing	• Pectinase to process fruit juice. • Invertase to produce liquid-centre chocolates	• Clarifies fruit juice • Sucrose paste in the chocolate is made liquid by injection of the enzyme

Pulp and paper	• Amylases used in starch conversion • Use of xylanase enzymes in pre bleaching the pulp. • Use of esterases in control of 'stickies' (glues introduced during paper recycling)	• Reduces the quantity of starch in the paper and improves quality. • Produces a whiter paper. • Stickies would otherwise clog the machinery and reduce the quality of the paper
Medicine	• Glucose oxidase in clinistix strips, tests for glucose. • Liver enzymes. • Pulmozyme to treat cystic fibrosis	• Allows easy diagnosis of diabetes by testing urine. • Testing for high levels of these in the blood confirms liver damage. • Reduces viscosity (stickiness) of mucus
Pharmaceutical	• Streptokinase to dissolve clots of heart-attack patients. • Production of abacavir sulphate is controlled by enzymes	• Restores blood supply to area of heart muscle. • Abacavir sulphate is an important anti-AIDS drug

Match the following enzyme under column "A" with their application under column "B".

<u>A</u>	<u>B</u>
1. Biochemosin	A. produce lactose-free milk
2. proteases, lipases and amylases	B. biological washing powders
3. Cellulase	C. 'bio-stone' denim
4. Pectinases	D. to process fruit juice
5. Inverase	E. to produce liquid-centre chocolates
6. Lactase	F. Enzymes in pre bleaching the pulp.
7. Xylanase	G. Use of in control of 'stickies'
8. esterases	
9. Glucose oxidize	H. In clinistix strips, tests for glucose.
10. Pulmozyme	I. to treat cystic fibrosis
11. Streptokinase	J. to dissolve clots of heart-attack patients
	K. to produce cheese

THE TWO MODELS OF ENZYME ACTION

- *Both of these models suggest that the enzyme catalyses the reaction by lowering the activation energy. However, they differ in explaining how the substrate binds to the active site of the enzyme.*

A. THE LOCK-AND-KEY MODEL

- Proposed in 1894 by a German biochemist named Fischer.
- This model proposes that the shapes of the substrate molecules are complementary to that of the active site, rather like the shape of a key is complementary to that of the lock it fits.
- Fit between the substrate and the active site of the enzyme is exact.
- Like a Key fits into a lock very precisely Enzyme-substrate complex formed.
- Products have a different shape from the substrate and Products are released from the active site leaving it free for another substrate molecule.
- The complementary substrate molecule binds with the active site of the enzyme to form the **enzyme–substrate complex**.



- The complex causes the reactants to enter a transition state in which the activation energy of the reaction is lowered.
- The reaction takes place and the products formed are released.
- The lock-and-key model of enzyme action suggests that the enzyme lowers the activation energy by providing an alternative pathway for the reaction.
- This model sees the enzyme–substrate complex as the intermediate, which is part of a pathway that requires less energy than the normal pathway. However, a weakness of this model is that it does not explain how the intermediate reduces activation energy.

B. THE INDUCED-FIT MODEL

- Proposed in 1958 by Koshland. This model suggests that the active site and the substrate aren't naturally complementary in shape, but the binding of substrate molecules produces a conformational change in the active site. This allows the substrate and active site to bind fully.
- The conformational change also puts the substrate molecules under tension, so they enter a 'transition state' and are able to react because of the lowered activation energy.

- In the transition state, bonds in the reactants are put under strain and break more easily and rejoin with other bonds to form the products.
 - Most biologists now prefer the induced-fit model over the lock and-key model:
 - As it explains other properties of enzymes, such as enzyme inhibition,
 - **The rate of a chemical reaction** is the rate at which reactants are converted into products.
 - In the case of an enzyme-controlled reaction, this is determined by how many molecules of substrate bind with enzyme molecules to form enzyme–substrate complexes.
 - **The turnover rate of the enzyme**, the number of molecules of reactants that form enzyme–substrate complexes with each molecule of an enzyme, per second.
2. A molecule of an enzyme that has a high turnover number:
- A. Can easily be denatured
 - B. Can easily be replaced with another enzyme
 - C. Converts substrate to product very rapidly**
 - D. Needs a constant supply of cofactors

COFACTORS

- An **active enzyme** made from **two molecules**, neither of which has enzyme activity without the other. The two parts are the apoenzyme and the cofactor.
 - **Apoenzyme** – a protein that combines with a cofactor, to form an active enzyme.
 - The protein (apoenzyme) is inactive on its own.
 - **Cofactor** – a small non-protein particle essential for the activity of some enzymes.
 - **Holoenzyme** - Where an active enzyme molecule comprises an apoenzyme and a cofactor, the whole is sometimes referred to as the **Holoenzyme**.
 - Cofactors include: **coenzymes** and **mineral ions**.
 - Coenzymes are organic molecules and many are derived from vitamins.
 - They bind with the enzyme to give catalytic activity
3. Most synthetic reactions in which carbon dioxide is used require the vitamin biotin. The biotin becomes covalently bound to a lysine molecule that is part of an enzyme, and the biotin can then attach to and carry the carbon dioxide molecule. Biotin is best referred to as an:
- A. Coenzyme**
 - B. Enzyme inhibitor
 - C. Organic acid
 - D. Enzyme

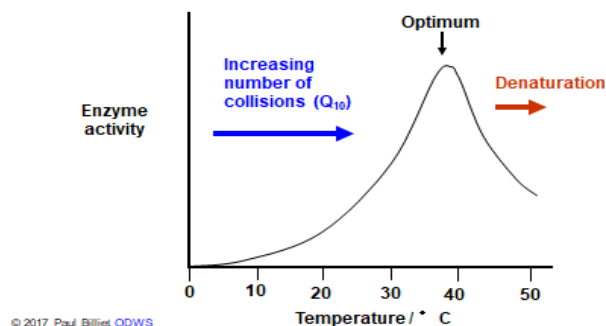
Factors affecting the functions of enzymes

The turnover rate and, therefore, the activity of the enzyme are influenced by a number of external factors

B. Temperature

- ❖ Usually, the reaction rate increases with temperature, but with enzyme reactions, a point is reached when the reaction rate decreases with increasing temperature.
- ❖ **When the temperature is raised, particles are given more kinetic energy.**
 - This has two main effects: ‘Free’ particles move around more quickly.
 - This increases the probability that a substrate particle will collide with an enzyme molecule.
- ❖ **Particles within a molecule vibrate more energetically.**
 - This puts strain on the bonds that hold the atoms in place.
 - Bonds begin to break and, in the case of an enzyme, the shape of the molecule, and the active site in particular, begin to change. The enzyme begins to lose its tertiary structure and denature.
 - The activity of an enzyme at a given temperature is a balance between these two effects.
 - If the raised temperature results in little denaturation but a greatly increased number of collisions, the activity of the enzyme will increase.
 - If the higher temperature causes significant denaturation then, despite the extra collisions, the activity of the enzyme will probably decrease.
- Above the optimum temperature, the enzyme denatures very quickly to the point at which the shape of the active site has changed so much that an enzyme–substrate complex cannot form. At this point the reaction rate is zero.

The effect of temperature



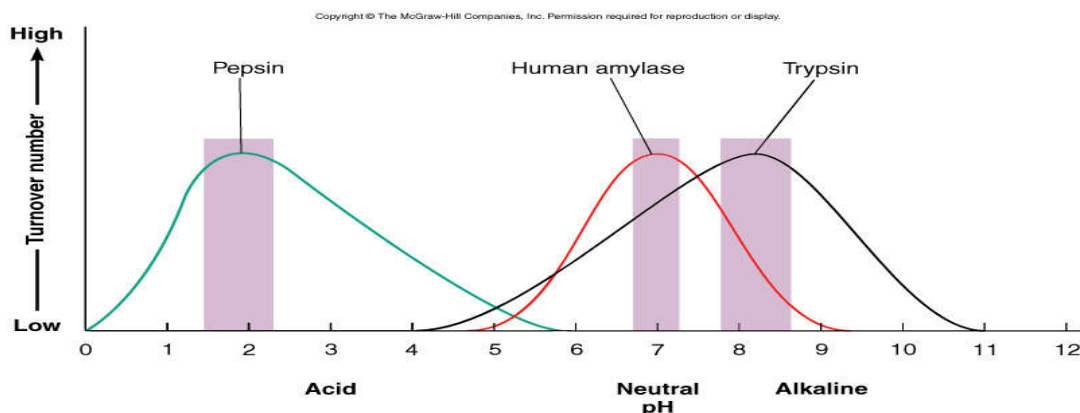
- For most enzymes the optimum temperature is about 30-38°C
- Many are a lot lower, cold water fish will die at 30°C because their enzymes denature
- A few bacteria have enzymes that can withstand very high temperatures up to 100°C.

4. The optimum temperature of an enzyme is the temperature at which:

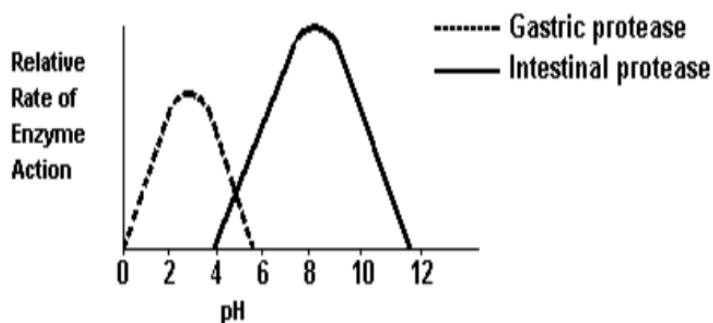
- A. There is no denaturation.
- B. The maximum number of enzyme-substrate complexes is formed.**
- C. There is the maximum number of collisions between enzyme and substrate
- D. the particles have the most kinetic energy

B. The pH

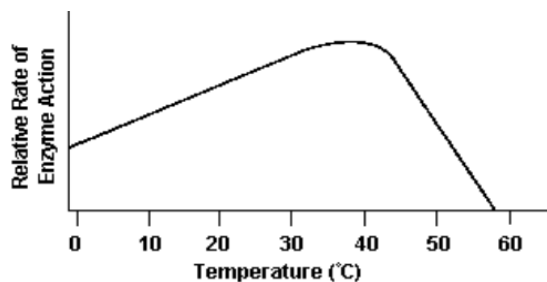
- pH also affects the rate of enzyme-substrate complexes.
- As the pH is decreased or increased, the nature of the various acid and amine groups on side chains is altered with resulting changes in the overall shape structure of the enzyme.
- The majority of enzymes in most mammals function most efficiently within the pH range 6.0 – 8.0, although the optimum pH of pepsin (an enzyme found in the stomach) is between pH 1.0 and pH 3.0.
- Significant changes in pH can affect an enzyme molecule by:
 - Breaking ionic bonds that hold the tertiary structure in place; this leads to denaturation of the enzyme molecule.
 - Altering the charge on some of the amino acids that form the active site; this makes it more difficult for substrate molecules to bind. Most enzymes have an optimum pH of around 7 (neutral). However, some prefer acidic or basic conditions



5. Enzyme A digests proteins in the stomach (environment with a pH of 2). Enzyme B digests proteins in the small intestine (environment with a pH of 8). Which of the following is **NOT** true?
- Enzyme A would be denatured in the small intestine.
 - Enzyme A works best in acidic conditions.
 - Enzyme A can also work in the small intestine.**
 - Enzyme A helps in the hydrolysis of proteins.
6. Base your answer on the graph and on your knowledge of biology. The most likely result of mixing both enzymes with their substrates in a single test tube is that:

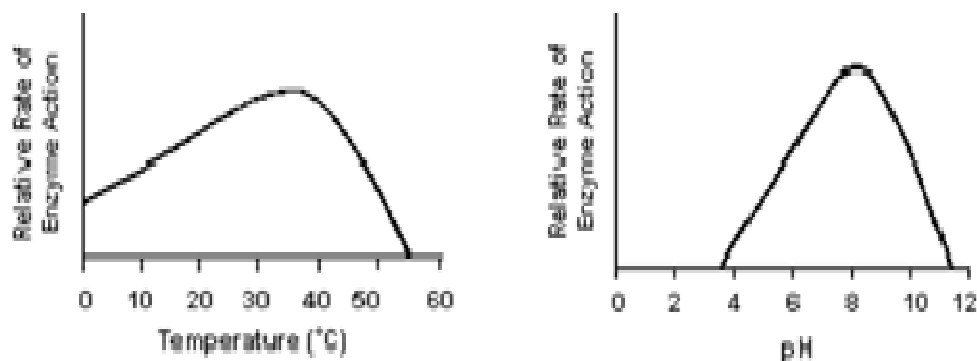


- Only gastric protease would be active if the pH of the mixture was basic.
 - Intestinal protease would be more active than gastric protease at pH 4.
 - Both enzymes would exhibit some activity at pH 5.**
 - Gastric protease would be more active than intestinal protease at pH 6
7. Extreme pHs can inactivate enzymes because they:
- Alter the charge on the amino acids in the active site**
 - Alter the charge on the amino acids in the allosteric site.
 - Alter the charge on amino acids away from the active site and allosteric site
 - All of the above**
8. The optimum temperature for the action of this enzyme is approximately

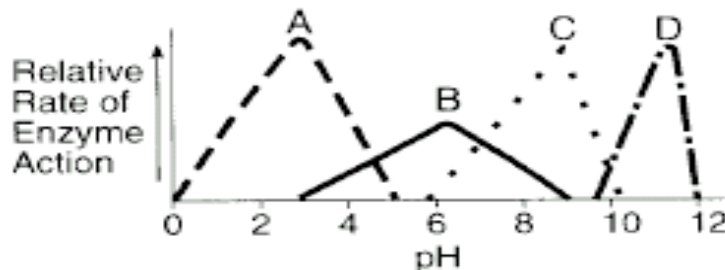


- 15°C
- 22°C
- 37°C**
- 50°C

9. Which statement best describes the enzyme represented in the graphs?



- A. This enzyme works best at a temperature of 35°C and a pH of 8.
 B. This enzyme works best at a temperature of 50°C and a pH of 12.
 C. Temperature and pH have no effect on the action of this enzyme.
 D. This enzyme works best at a temperature above 50°C and a pH above 12.
10. The graph shows the relative rates of action of four enzymes, A, B, C, and D. Which enzyme shows the greatest change in its rate of action with the least change in pH?



- A. Enzyme A B. Enzyme B C. Enzyme C D. Enzyme D
11. In the above graph on question 11. Which two enzymes would function in a region of the human body having a neutral pH?
- A. Enzymes A and B C. Enzymes B and C
 B. Enzymes C and D D. Enzymes B and D

Base your answers to questions 10 and 11 below on the following passage and your knowledge in Biology Laboratory class.

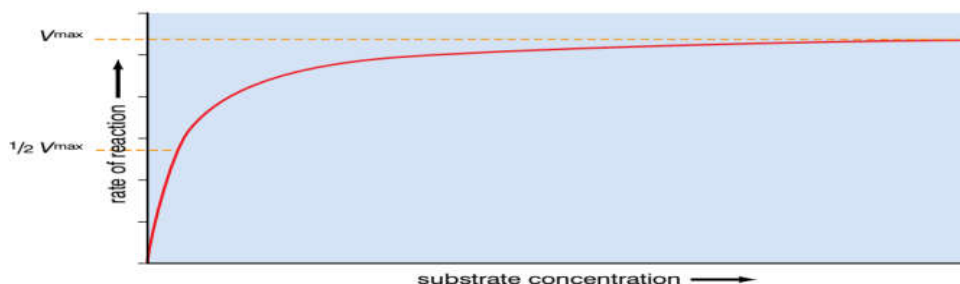
A student ground 1 g of fresh liver or potato in a mortar and pestle, placed the ground liver or potato in a test tube, and added 1 cm³ of hydrogen peroxide. The gas that was generated was collected A glowing splint burst into flames when placed in the gas. The student then repeated the procedure, using 1g of boiled liver and 1g of liver or potato treated with a strong acid. When hydrogen peroxide was added to each sample of liver or potato, no gas was generated

12. The gas that was generated was probably
- A. Oxygen B. Nitrogen C. Carbon dioxide D. Hydrogen

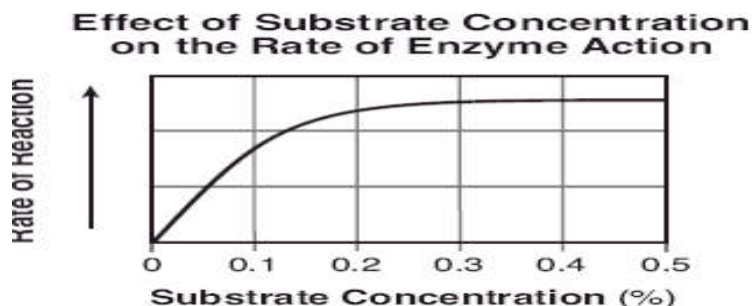
13. If the substance in the liver that acted on the hydrogen peroxide was an enzyme, it could
- A. **Be recovered from the living tissue that had not been boiled or treated with acid after the reaction ceased.**
 - B. Not be recovered because it was consumed while engaging in its catalytic reaction activities
 - C. Not be recovered because there is no enzyme in liver that catalyzes the breakdown of peroxide
 - D. Not be recovered because grinding would break up the molecule

C. SUBSTRATE CONCENTRATION

- The activity of an enzyme depends on the number of substrate molecules per second that bind to form enzyme–substrate complexes (Turn over rate).
- A small number of substrate molecules mean few collisions and so only a few enzyme–substrate complexes form.
- Increasing the concentration of the substrate means more collisions and more enzyme–substrate complexes. So, the overall rate of reaction is increased.
- Eventually, because of the high substrate concentration, each enzyme molecule could be working at maximum turnover – that is, each active site is binding with substrate molecules all the time and there is no ‘spare capacity’ in the system.
- Increasing the substrate concentration beyond this point will have no effect on the activity of the enzyme because all the active sites are occupied all the time.



14. The graph below shows the effect of substrate concentration on the action of enzyme X. This enzyme is functioning at its optimal temperature, 36°C, and at its optimal pH, 5.5.



When the substrate concentration increases from 0.4% to 0.5%, the rate of the reaction

- A. decreases
- C. remains the same**
- B. increases
- D. increases, then decreases

15. According to the induced-fit model of enzyme action, reacting molecules enter a transition state in which:

- A. Reacting molecules assume a complementary shape
- B. Apoenzyme and cofactor assume a complementary shape
- C. Bonds in the apoenzyme and coenzyme are put under tension
- D. Bonds in reacting molecules are put under tension.**

D. Enzyme concentration

- ❖ Assuming a constant large supply of substrate molecules, each enzyme molecule will work at maximum turnover. Therefore, the reaction rate will be directly proportional to the number of enzyme molecules – the concentration of the enzyme.
- ❖ Increasing the concentration will increase the reaction rate. *However*, increasing the concentration of the enzyme will not increase the activity of the enzyme. Each enzyme molecule will be working at maximum turnover, so the activity of the enzyme is likely to remain constant.

E. Enzyme inhibitors

- Enzyme inhibitors are molecules that interact in certain ways with the enzyme to prevent it from functioning in a normal manner.
- They can alter the catalytic action of the enzyme and consequently slow down, or even stop catalysis. Poisons and drugs are examples of enzyme inhibitors.

1. Irreversible Inhibitors

- Form strong (permanent) covalent bonds with an enzyme. The structure of the enzyme is modified to the degree that it ceases to work.
- These inhibitors may act at, near, or remote from the active site. Consequently, they may not be displaced by the addition of excess substrate. S
- Since many enzymes contain sulfhydryl (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor.
- Heavy metals such as Ag^+ , Hg^{2+} , Pb^{2+} have strong affinities for -SH groups.
- Nerve gases such as di isopropylfluorophosphate (DFP) inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester.
- Oxalic and citric acid inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

2. REVERSIBLE INHIBITORS

- Reversible inhibitors bind to enzymes only weakly (temporarily) and the bond that holds them breaks easily releasing the inhibitor and allows the enzyme to become active again.
- There are two main kinds of reversible inhibitors:
 - **competitive inhibitors** and • **non-competitive inhibitors**

A. Competitive inhibitors

- A competitive inhibitor could be any compound that closely resembles the chemical structure and molecular geometry of the substrate.
- The inhibitor competes for the same active site with the substrate molecule. A competitive inhibition is usually temporary and reversible.
- Therefore, the level of inhibition depends upon the relative concentrations of the substrate and inhibitor.

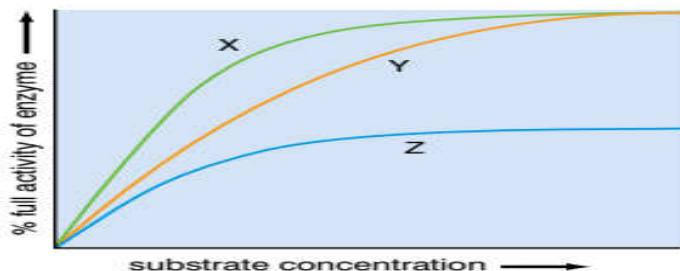
Methanol poisoning occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.

B. Non competitive Inhibitors:

Non-competitive (allosteric) inhibitors bind to a region away from the active site, producing a conformational change in the enzyme that prevents the substrate from binding; the extent of the inhibition is independent of the substrate concentration.

16. If the ratio of competitive inhibitor molecules to substrate molecules is **5:10**, the enzyme controlling the reaction will be:

- A. **50% inhibited** C. 100% activated
- B. 25% inhibited D. In no way affected by the concentration of the substrate

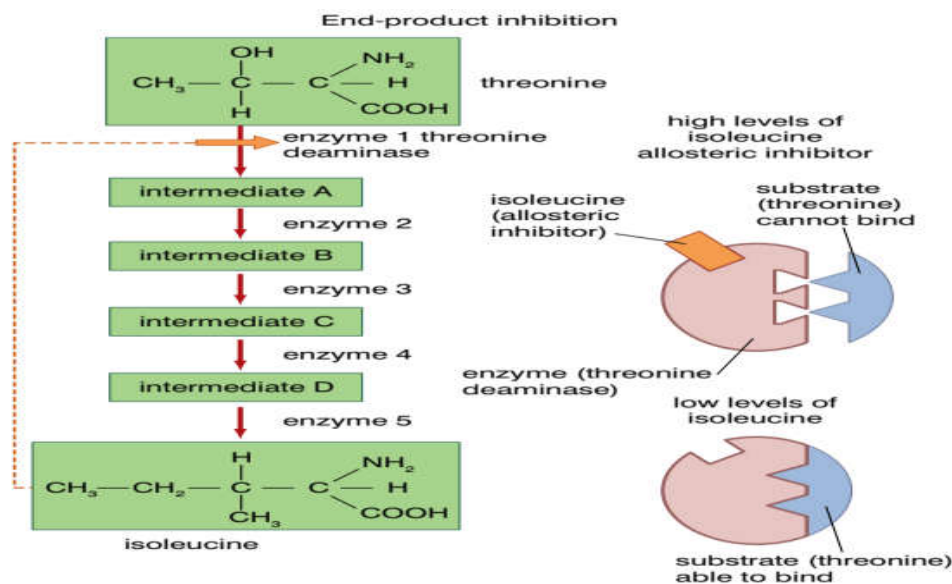


17. Which of the following represents the correct interpretation of the graph

- A. . X is with the competitive inhibitor, Y with the noncompetitive inhibitor and Z with no inhibitor
- B. X is with the non-competitive inhibitor, Y with the competitive inhibitor and Z with no inhibitor.
- C. **X is with no inhibitor, Y with the competitive inhibitor and Z with the non-competitive inhibitor**
- D. X is with no inhibitor, Y with the non-competitive inhibitor and Z with the competitive inhibitor.

Allosteric inhibition can control metabolic pathways.

The final product of a series of reactions inhibits the enzyme controlling the first reaction in the series; this is also known as **end-product inhibition**.



- Non competitive inhibitors are usually reversible, but are not influenced by concentrations of the substrate as is the case for a reversible competitive inhibitor.

18. End-product inhibition or negative feedback control of a metabolic pathway occurs when:

- A. **The last product of the pathway inhibits the enzyme controlling the first reaction.**
- B. The last product of the pathway inhibits the enzyme controlling the last reaction.
- C. The first product of the pathway inhibits the enzyme controlling the last reaction.
- D. The last product of the pathway inhibits the enzyme controlling the 2nd reaction.

19. Melanin, the dark pigment in our hair and skin, is produced by a series of enzymatic reactions that start with phenylalanine. The phenylalanine is converted to tyrosine, then to α -DOPA, and finally through a series of steps to melanin and other substances. If the conversion of phenylalanine to tyrosine were blocked, the individual affected would probably:-

- A. Have dark-colored hair
- B. Produce pleasant body odors
- C. **Have light-colored hair**
- D. Be able to function in environments having low O₂

Application of the Enzyme Inhibitors

- Enzyme inhibitors play important roles in pharmaceutical and biochemical industries.
- They can be widely used in metabolic control, as metabolic poisons and medicines.
- For example, many poisons work by inhibiting the action of enzymes involved in metabolic processes, which defends a plant or animal against predators.
- In addition, some enzyme inhibitors can be used as drugs in the treatment of various diseases.
- Some antimicrobial drugs are enzyme inhibitors that deactivate the enzymes that are needed for the survival of pathogens.

Answer the following multiple choice questions. Don't visit answer key before you try each question on your own.

1. What is the function of enzymes within living systems?
A. structural elements C. catalysts
B. neurotransmitters D. hormones
2. In which of the following is the pairing between enzyme type and enzyme function incorrect?
A. nucleases - hydrolysis of sugar-phosphate ester bonds in nucleic acids
B. synthetase - formation of new bond between two substrates
C. kinase - transfer of amino groups between substrates
D. carboxylase - removal of carbon dioxide from substrate
3. Which of the following is always present in both conjugated enzymes and simple enzymes?
A. Protein B. A vitamin C. a cofactor D. a coenzyme
4. The protein portion of a conjugated enzyme is called a(n)
A. Apoenzyme. B. coenzyme. C. Holoenzyme. D. cofactor
5. Which of the following enzyme properties is explained by the lock-and-key model for enzyme action?
A. high turnover rate C. susceptibility to denaturation
B. absolute specificity D. susceptibility to deactivation
6. An enzyme active site is the location in the enzyme where:
A. Protein side groups folded together to form a site for interactions with substrates
B. The catalyst interactions with the enzyme
C. Catalyst molecules are generated
D. The substrate creates the catalyst molecules
7. Which of the following statements concerning a competitive enzyme inhibitor is correct?
A. it competes with substrate for occupancy of the enzyme's active site
B. binds at the active site simultaneously with the substrate
C. breaks the enzyme down to its constituent amino acids
D. its effect can be overcome by increasing the temperature

8. Which of the following binds to an enzyme at a location other than the active site?
- A. Substrate
 - C. reversible competitive inhibitor
 - B. Irreversible inhibitor
 - D. reversible noncompetitive inhibitor
9. The number of substrate molecules acted upon per minute by one molecule of enzyme is called the:
- A. Active site number.
 - C. **turnover number.**
 - B. Enzyme activity number.
 - D. optimum number.
10. The final product of a series of enzyme-catalyzed reactions causes the enzyme that catalyzes the first reaction of the series to be inhibited. This is an example of:
- A. Positive regulator control.
 - C. Substrate control.
 - B. Feedback control.
 - D. Competitive control.
11. The major function for B vitamins within the human body is as
- A. Antioxidants.
 - B. Components of coenzymes.
 - C. Regulators of cell differentiation.
 - D. Regulators of calcium ion and phosphate ion concentrations in blood.
12. In which of the following pairs of terms do the two terms have the same meaning?
- A. Holoenzyme and conjugated enzyme
 - C. coenzyme and simple enzyme
 - B. apoenzyme and cofactor
 - D. no correct response
13. An enzyme active site is the location in an enzyme where substrate molecules
- A. Is generated
 - C. undergoing change.
 - B. Become catalysts
 - D. no correct response.
14. Which of the following statements concerning enzyme active sites is incorrect?
- A. They generally involve only a small portion of the enzyme.
 - B. Noncompetitive inhibitors can change active site shape.
 - C. The lock-and-key model of enzyme activity assumes that an active site has a fixed, rigid geometrical conformation.
 - D. None of the above
15. Which of the following statements about a competitive inhibitor is correct?
- A. It must resemble the substrate in general shape.
 - B. Its effect can be diminished by increasing substrate concentration.
 - C. It and the normal substrate simultaneously occupy the active site.
 - D. A & B are correct answers

ANSWER KEY

1.C	9.C
2.D	10.B
3. A	11.B
4.A	12.A
5.B	13.C
6.A	14.D
7.A	15.D
8.D	

UNIT 4

CELL BIOLOGY

At the end of the unit students will be able to:

- Tell the history of cell biology.
- Describe cell theory and investigate the size, structure and shape of cells.
- Explain the difference between prokaryotic and eukaryotic cells.
- Construct and show the arrangement of the phospholipids and proteins in Davson-Daniel and fluid mosaic model.
- Explain the role of glycoprotein and other components in the cell membrane.
- Name the different parts of the cell and explain their functions.
- State and explain the mechanisms of substance transport across a cell membrane.

Cell theory

- It may seem obvious now that we, and other living things, are made up of cells.
- Prior to the 1600s, however, it wasn't obvious at all, for the simple reason that no one had ever seen a cell up close and personal.
- To distinguish individual cells in a piece of tissue or individual bacteria in a sample of liquid required the development of relatively high-powered **microscopes**, instruments used for magnifying objects otherwise too small to be seen.
- The first person to observe cells as microscopic structures was the British scientist Robert Hooke. In fact, he was the person who gave cells their name.
- In his book *Micrographia*, he used the term cell to refer to the box-like structures he saw when he looked at dead cork tissue through a simple microscope.
- He chose cell as the name because these boxes reminded him of the cells of a monastery, the simple rooms in which monks slept.
- The cells that Hooke observed, however, were in dead tissue, and were in fact cell walls left behind after the death of the real cells.
- The first person to observe living, moving cells was Anton van Leeuwenhoek, a Dutch shopkeeper and crafter of lenses.
- In the 1670s, inspired by Hooke's book, he began to build his own, more powerful microscopes with these; he was able to observe living single-celled organisms—such as bacteria—and sperm cells, which he collectively called animalcules.

A timeline for the development of the cell theory

Robert Hooke (1665)

- ❖ He makes drawings of cork and sees tiny structures that he calls 'cells'. Also, Hooke saw only dead cells.

Anton van Leeuwenhoek (1674)

- ❖ Sees living, moving unicellular organisms (protoctistans) in a drop of water.
- ❖ He is using a simple microscope with only one lens.
- ❖ However, van Leeuwenhoek is very skilled at grinding lenses and so his microscope can achieve magnifications of 300×. He calls the moving organisms 'animalcules'.
- ❖ He also sees bacteria (from his teeth), which he also calls 'tiny animalcules'.

Rene Dutrochet (1824)

- ❖ The French biologist Dutrochet states the cell theory by recognizing that all organisms are made of cells.

Matthias Schleiden and Theodor Schwann(1839)

- ❖ Matthias Schleiden and Theodor Schwann put forward the first clearly stated cell theory.
- ❖ the cell is the unit of structure, physiology and organization in living things
- ❖ the cell retains a dual existence as a distinct entity, and a 'building block' in the formation of organisms
- ❖ cells form by free-cell formation (spontaneous generation)
- ❖ Although we still accept the first two ideas, the final idea of spontaneous generation has now been proved false.

Rudolf Virchow (1858)

- Rudolf Virchow, a German doctor who develops many surgical techniques and promotes several fields of modern medicine, declares that: 'Omnis cellula e cellula', which means that a cell can only arise from another cell like it.(cell come from preexisting cell)

With this **Virchow** completes the first accepted version of the cell theory:

- All organisms are made up of one or more cells
- All cells come from pre-existing cells
- The cell is the unit of structure, physiology and organization in living things
- The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

Modern cell theory

All known living things are made up of cells

- The cell is a structural and functional unit of all living thing
 - All cells come from pre-existing cells by division (no spontaneous generation of cells).
 - Cells contain hereditary information which is passed from cell to cell at cell division.
 - All cells have basically the same chemical composition
 - All energy flow (the metabolism and biochemistry of life) occurs within cells
1. The word 'cell' was first coined by Robert Hooke when he examined:
 - A. Living cells in cork tissue
 - B. **Dead cells in cork tissue**
 - C. dead cells in skin
 - D. living cells in skin
 2. Anton van Leeuwenhoek saw what he called 'animalcules' and 'tiny animalcules'. These were, respectively:
 - A. Bacteria and viruses
 - B. Bacteria and protoctistans
 - C. protoctistans and viruses
 - D. **proctotistans and bacteria**
 3. The first cell theory stated by Schleiden and Schwann was not completely accurate because it held that:
 - A. All living things are made of cells
 - B. The cell is the basic unit of living things
 - C. the cell retains a dual existence
 - D. **cells form by cell-free formation**
 4. Which of the following is incorrect pairing of biologists and their contribution in the development of cell theories?
 - A. Robert hook- Coined the word cell
 - B. Anton Van Leeuwenhoek – Animalcules and tiny animalcules
 - C. Rudolf Virchow – Cell come from preexisting living cell
 - D. **Rene Dutrochet- Cell is structural and functional unit of life**

Cell size

- ❖ *Most cells fall within a much narrower range of sizes than the unfertilized ostrich's egg cell and the smallest bacterium.*
- ❖ The length of most animal and plant cells fall within a range of **10 μm to 100 μm .**
- ❖ Most bacteria are about *one-tenth* of this length.
- ❖ The animal cell may be just ten times as long – but it is also ten times as wide and ten times as deep. This makes it 1000 times bigger than the bacterium!

What units shall we measure cells in?

- It all depends on which cells, but first we should understand which units are available and which ones would be convenient to use.
- Example: to size of RBC measure cells in metres, it would be approximately **0.000007m.**

Counting all these zeros is very confusing, so we use other, smaller units to measure the size of cells and molecules.

- There are three smaller units commonly used:
 - Millimeters (mm) – 1/1000 of m
 - micrometres (μm) – 1/1000 of a millimeter, and 1/1 000 000 m
 - Nanometres (nm) – 1/1000 of a micrometer, 1/1 000 000 of a millimeter, and 1/1000 000 000 of a meter.

We can convert the units from one to another as shown below:

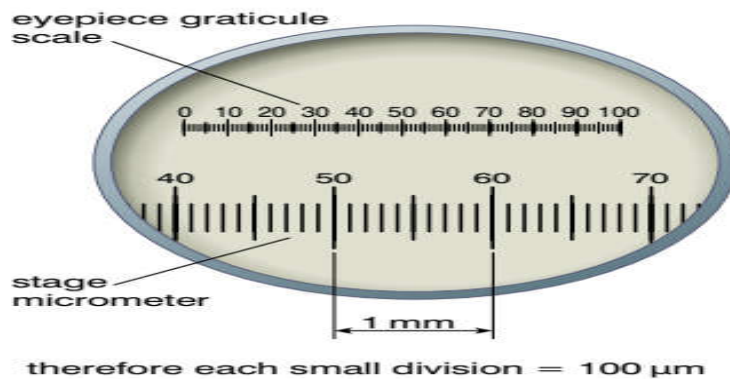
$$\begin{array}{ccccccc} & \times 1000 & & \times 1000 & & \times 1000 & \\ \text{m} & \xleftarrow{\hspace{1cm}} & \text{mm} & \xleftarrow{\hspace{1cm}} & \mu\text{m} & \xleftarrow{\hspace{1cm}} & \text{nm} \\ & \div 1000 & & \div 1000 & & \div 1000 & \end{array}$$

5. If an object having a size of 0.005 millimeter is magnified by an eye piece of 10x and objective lens of 10x. What is the magnified size of an object in micrometer?
- A. 5 micrometer B. 50 micrometer **C. 0.5 micrometer** D. 500 micrometer

Calibrating the eyepiece graticule

A stage micrometer – this is really a microscope slide with a very precise scale etched onto it.

An eyepiece graticule – this is a piece of plastic with a less accurate scale than the graticule that fits inside the eyepiece of the microscope. When you put the micrometer onto the slide and look at it through the eyepiece containing the graticule, you see something like the figure below.



therefore each small division = 100 μm

The smallest divisions on the stage micrometer slide are 100 μm . So each large division on the stage micrometer is 10 times that – 1 mm.

If we look at the two scales, we can see that the range 50–60 on the stage micrometer corresponds with the range 35–72 on the graticule.

Ten divisions on the micrometer scale correspond to 37 divisions on the graticule scale and 1 micrometer division therefore corresponds to 3.7 graticule divisions. But we know that 1 micrometer division = 100 μm (or 0.1 mm).

So 1 division of the eyepiece graticule = $100\text{ }\mu\text{m} \div 3.7 = 27\text{ }\mu\text{m}$ (or 0.027 mm). This is called **calibrating** the eyepiece graticule.

If we now put an object on an ordinary slide (having removed the stage micrometer) and view it at the same magnification, we can calculate its size.

If it takes up 26 graticule divisions, then this length is: $26 \times 27 = 702\text{ }\mu\text{m}$ (or 0.702 mm). However, the graticule will need recalibrating if it is to be used at a different magnification.

6. 40 divisions on the scale of an eyepiece graticule correspond to 16 small divisions on the stage micrometer. Each small division on the stage micrometer = $10\text{ }\mu\text{m}$. 8 cells fit across 40 divisions of the eyepiece graticule. The length of each cell is:

A. $10\text{ }\mu\text{m}$ B. $40\text{ }\mu\text{m}$ C. $30\text{ }\mu\text{m}$ **D. $20\text{ }\mu\text{m}$**

As cells increase in size, the surface-area-to-volume ratio decreases; this affects their ability to obtain the resources they need to carry out their metabolism.

7. The surface-area-to-volume ratio of a cell is important because it is a measure of:
- A. How efficiently the cell releases energy in respiration
 - B. How efficient the cell is in conserving energy
 - C. How efficient the cell is in obtaining the oxygen it needs for respiration**
 - D. How efficiently the cell uses the energy it releases in respiration

Types of cell

- There are two main types of cells: prokaryotic cells and eukaryotic cells; the table shows the differences between them

Feature	Prokaryotic cells	Eukaryotic cells
Size	1–10 μm	10–100 μm
Nucleus	No membrane-bound nucleus	Nucleus surrounded by nuclear envelope
DNA	In a continuous loop, not associated with protein to form chromosomes	Linear DNA associated with histone proteins in chromosomes
Mitochondria	Absent	Present
Chloroplasts	Absent (but some prokaryotic cells contain a kind of chlorophyll and can photosynthesise)	Present in some cells (some plant cells and some algal cells)
Ribosome	Present, but smaller than in eukaryotic cells (70S)	Present, but larger than in prokaryotic cells (80S)
Cell wall	• Always present Not made from cellulose (often made from peptidoglycan)	• Present in plant cells, algal cells and fungal cells • Cellulose in plant cells, various materials in other cells

8. Differences between prokaryotic and eukaryotic cells are:
- A. prokaryotic cells are smaller but contain more organelles
 - B. prokaryotic cells are larger and contain more organelles
 - C. prokaryotic cells are larger and contain fewer organelles
 - D. prokaryotic cells are smaller and contain fewer organelles**
9. The DNA in prokaryotic cells is:
- A. Linear and bound with proteins
 - C. circular and not bound with proteins**
 - B. Linear and not bound with proteins
 - D. circular and bound with proteins
10. The cell wall of prokaryotic cells is made from:
- A. protein
 - B. cellulose
 - C. peptidoglycan**
 - D. another substance
11. It is thought that eukaryotic cells originated by several prokaryotic cells becoming associated. This theory is the:
- A. **Endosymbiont theory**
 - C. both of the above
 - B. Membrane association theory
 - D. neither of the above

Parts of the cell and their functions

Cell membrane /plasma membrane/

The membrane that surrounds and encloses a cell is sometimes called the **cell surface membrane**, but most biologists now refer to it as the **plasma membrane**.

Cell membrane plays a crucial role in:

- To enclose organelles and other contents in cytoplasm.
- To protect the cell.
- To allow substances into and out of the cell.
- To have metabolic reactions on its surface.
- Cell signaling; various molecules in the membrane allow the cell to be recognized by hormones and the immune system (in animals) and (in plants) growth regulator substances, such as auxins.
- The plasma membrane clearly has a vital role in isolating the cell from its environment, whilst allowing necessary exchanges with that environment.

Models of plasma membrane

A. The Davson–Danielli model / sandwich model of cell membrane/

In 1935, Davson and Danielli knew that both proteins and phospholipids were involved in the structure of plasma membranes. Without any direct observational evidence to assist them Davson

and Danielli suggested a kind of ‘sandwich’ of protein and phospholipid. The protein was to form the ‘bread’ of the sandwich with the phospholipid forming the ‘filling’.

In 1954 they proposed a revised model in which they included protein-lined pores.

B. The fluid mosaic model of cell membrane

- In 1972, Singer and Nicholson suggested that the arrangement of proteins and phospholipid bilayer was not static, but was fluid and constantly changing.
- Our current idea of membrane structure still assumes this fluid-mosaic nature.

The key features of the model as we currently understand it are:

- The phospholipid bilayer as the basis for the membrane
 - **Integral proteins** (also known as intrinsic proteins and transmembrane proteins) that span the membrane.
- Some of these proteins play an important role in moving substances across the membrane. There are two main types of these transport proteins:
- **Channel proteins** – these proteins have a channel through them along which a specific ion can pass; there are different channel proteins for different ions.
 - **Carrier proteins** – these proteins act in a more sophisticated way to move larger molecules through the membrane by facilitated diffusion or active transport; the ones involved in active transport are often referred to as pumps.
- **Peripheral proteins** (also known as extrinsic proteins) that span only one layer (or sometimes less) of the membrane. They have a range of functions; some are enzymes, others anchor integral proteins to the cytoskeleton.
- **Glycoproteins and Glycolipid** – protein and lipid molecules that have carbohydrate chains attached to them and often serve as signals to other cells. They also act as receptor sites for hormones and drugs. The carbohydrate component of each can be cell specific and so allow identification of the cell by the immune system.
- **Cholesterol** – reduces the fluidity of the membrane.
12. In the fluid mosaic model of membrane structure, intrinsic proteins can be:
- | | |
|-------------------------|----------------------------|
| A. Glycoproteins | C. Carrier proteins |
| B. Ion channel proteins | D. All of the above |
13. The fluid mosaic model suggests that
- A. Cholesterol molecules in this bilayer reduce the fluidity of the membrane
 - B. The plasma membrane has protein molecules ‘studded’ in the bilayer
 - C. Some proteins are intrinsic, whilst others are extrinsic like peripheral proteins.
 - D. All of the above**

14. Which of the following is not the function of membrane proteins?

- A. Play catalytic role as enzyme. C. cell signaling and cell recognition
 B. Transport materials. D. **hormone secretion**

Transport across cell membrane

➤ We can group the processes by which substances cross plasma membranes into two main types:

- **passive processes** – these processes rely only on the kinetic energy of the particles of the substances and on concentration gradients; they need no extra energy from the cell's metabolism
- **Active processes** – these require energy from the cell's metabolism in the form of ATP to drive the transport.

Passive processes		Active processes	
Process	Brief description	Process	Brief description
Simple diffusion	Particles move from a high concentration to a low concentration.	Active transport	Particles move from a low concentration to a higher concentration using a carrier protein (pump).
Facilitated diffusion	Particles move from a high concentration to a low concentration through an ion pore or carrier protein.	Endocytosis	Large particles are engulfed by the plasma membrane invaginating and forming a vesicle.
osmosis	Water molecules move from a high water potential to a lower one.	Exocytosis	Large particles are secreted by a vesicle in the cell merging with the plasma membrane to release the substance.

Passive process includes:

A. Simple diffusion

- To pass through the plasma membrane by simple diffusion particles must be:
 - **Small sized Lipid soluble Non-charged**
- Small, hydrophobic or **fat-soluble** molecules, such as oxygen, cross the cell membrane quite readily because of "fat dissolving fat" interaction.
- Small, uncharged, hydrophilic or **water-soluble** molecules, such as water and carbon dioxide, would also be able to cross the cell membrane although there is no "fat dissolving fat" interaction.
- Large, hydrophilic molecules are usually impermeable to cell membrane.

- Any molecules carrying strong electrical charges (i.e. ions) are always impermeable to cell membrane, unless transported by special mechanisms.
- When particles diffuse across a plasma membrane, there must be a concentration difference between the two sides of the membrane (a concentration gradient) to drive the process.
- As diffusion proceeds, the high concentration will decrease and the low concentration will increase until the two concentrations are the same.
- At this point there will be no further net diffusion.

This means that although particles will still move across the membrane, they will move equally in both directions, so there will be no overall effect.

The rate at which diffusion across a membrane takes place is influenced by:

- **The concentration gradient** – a bigger difference in concentration results in faster diffusion than a smaller gradient
- **The thickness of the membrane** – a shorter distance results in faster diffusion
- **The surface area of the membrane** – clearly if there is more membrane where diffusion can take place, diffusion will happen faster
- **Temperature**. Diffusion occurs faster at higher temperatures because the particles have more kinetic energy and so move faster.

B. Facilitated diffusion

- Facilitated diffusion is essentially the same process as diffusion but it allows large, hydrophilic molecules to cross the cell membrane.
- Note in both cases that the particles are moving from a high concentration to a low concentration (as with simple diffusion).
- However, also note that whilst the ions can simply move straight through the ion pore of a channel protein, but the carrier protein must undergo a conformational change (change in shape) to move particles through the membrane.
- The rate of facilitated diffusion depends largely on the number of protein carriers available; when all proteins carriers are bound "saturation" occurs and the diffusion rate stabilizes.

C. Osmosis

- Osmosis is the movement of water from a system with a high (less negative) water potential to one with a lower (more negative) water potential, across a partially permeable membrane.

- Water moves across a partially permeable membrane. It is the diffusion of water. The symbol for water potential is the Greek letter Ψ (psi). Water potential is measured in units of pressure: Pa, kPa or MPa.
- Pure, liquid water has a higher water potential than any other system. Ψ (pure water) = 0 Pa
- All other systems (cells, solutions and suspensions) have a water potential that is lower than that of water which is negative value.
- The rate at which osmosis proceeds is influenced by the same factors as simple diffusion
 - surface area of the membrane
 - difference in water potential
 - distance the molecules must travel
- When comparing the water potential of a solution to that of a cell, we could describe it as:
 - **Isotonic** – having the same water potential as the cell.
 - **Hypertonic** – having a lower (more negative) water potential than the cell.
 - **Hypotonic** – having a higher (less negative) water potential than the cell.

Animal cells

- In the hypertonic solution, the cells lose water by osmosis and shrink.
- In the hypotonic solution, the cells gain water by osmosis and swell.
- The pressure will eventually burst the weak plasma membrane: this is called **haemolysis**.
- There is no change in the isotonic solution.

Plant cells

- **In the hypertonic solution,**
 - The cytoplasm of the cells loses water by osmosis and shrinks. Because of this, there is no pressure from the cytoplasm on the cell wall.
 - The cell is said to be flaccid. If the cytoplasm shrinks too much, it loses contact with the cell wall and we say the cell has been plasmolysed.
- **In the hypotonic solution,**
 - The cells gain water by osmosis and swell. However, because of the cell wall, the cell cannot become much larger. Plant cells in this condition are turgid.
- **Isotonic solution;** There is no change as same amount enter and leave the cell.
 - **Turgidity** is important in supporting young, non-woody plant stems. If the plant is kept well watered, the cells will remain turgid.
 - The turgid cells will press against each other and this pressure will keep the plant upright. If the plant is not watered, the cells will be **plasmolysed** and become **flaccid**.
 - They will no longer press against each other and the support will be lost. The plant will **wilt**.

15. During diffusion, substances move from an area of ____ concentration to an area of ____
Concentration.
A. **Higher, lower** B. Lower, higher C. higher, equal D. lower, equal
16. A channel protein does which of the following?
A. Carries ions or molecules across the membrane.
B. Forms tiny holes in the membrane.
C. Changes shape as it transports molecules.
D. All of the above
17. The sodium-potassium pump
A. Uses energy to move sodium ions out of the cell and potassium ions into the cell.
B. Uses energy to move potassium ions out of the cell and sodium ions into the cell.
C. Moves sodium out of the cell and potassium ions into the cell without using energy.
D. Moves potassium out of the cell and sodium ions into the cell without using energy.
18. Osmosis
A. Is the diffusion of water.
B. Is the diffusion of water and other small molecules.
C. Is the diffusion of water and small ions.
D. Is the diffusion of small molecules and ions.
19. Endocytosis and exocytosis
A. Is both a type of vesicle transport.
B. Move very large molecules either in or out of the cell.
C. Is both a form of active transport.
D. all of the above
20. Intravenous saline injection is often given as a treatment for severe dehydration. The concentration of saline (0.9%NaCl) in these injections is the same as that present in human cells. What would happen if pure water was introduced into the body instead of saline?
A. The cell would gain water and swell
B. The cell would lose water and shrivel
C. The cell would become impermeable to Sodium ion.
D. The cell would become impermeable to Chloride ion.
21. Which of the following does not use energy in the form of ATP
A. Active transport B. Endocytosis **C. Facilitated diffusion** D. Phagocytosis
22. If red blood cells are immersed in a hypotonic solution, they will:
A. Take in water by osmosis, swell and become turgid .
B. Lose water by osmosis and shrink
C. Lose water by osmosis and become flaccid
D. Take in water by osmosis, swell and burst
23. Suppose three potato cylinders are kept for sometimes in 15%, 8% and 4% sucrose solutions respectively and the 4th cylinder is kept in distilled water, which one of the cylinders will be more flaccid?
A. The cylinder in 4 %solution **C. The cylinder in 15%solution**
B. The cylinder in distilled water D. The cylinder in 8%solution

24. Which of the following will happen if a plant cell is kept in a solution that is stronger than its protoplasm?
- A. The cell will become turgid C. The cell will swell and burst
 B. The central vacuole will expand **D. The protoplasm will get plasmolysed**
25. Identify the incorrect statement about the process of simple diffusion?
- A. The process happens spontaneously.
 B. The greater the difference in concentration the faster the diffusion.
 C. The diffusion of one molecule is independent of the diffusion of the other molecule.
D. It is assisted by carrier and channel proteins toward concentration gradients.

3. Active processes

A. Active transport

- Substances move against a concentration gradient – from a low concentration to a higher one.
 It can only happen if metabolic energy is used to drive the process.
 - In living organisms, this energy is released from the ATP produced in respiration.
 - The proteins used to actively transport substances across plasma membranes are called pumps.
26. A sodium potassium pump within a cell membrane requires energy to move sodium and potassium ions into or out of a cell. The movement of glucose into or out of a cell does not require energy. Which statement best describes the movement of these materials across a cell membrane?
- A. Sodium and potassium ions move by active transport, and glucose moves by osmosis.
B. Sodium and potassium ions move by active transport, and glucose moves by facilitated diffusion.
 C. Sodium and potassium ions move by facilitated diffusion, and glucose moves by osmosis.
 D. Sodium and potassium ions move by facilitated diffusion, and glucose moves by active transport.
27. In order for nerve cells and muscle cells to function properly, they require a high concentration of potassium ions inside the cells and a high concentration of sodium ions outside the cells. To maintain this condition, cells utilize sodium□potassium pumps embedded within their cellular membranes to move the ions against their concentration gradients. Since sodium□potassium pumps require an input of energy to operate, they are an example of...
- A. Passive transport. B. filtration. C. facilitated diffusion. **D. active transport.**

B. Endocytosis

- In this process, large particles are engulfed by a cell. There are several ways in which it can happen, but, essentially, part of the plasma membrane surrounds the particles to form a vesicle which is then processed by the cell and require ATP.

There are three types of endocytosis.

a. Phagocytosis (cell eating)

Involves the creation of pseudopodia to enclose large particles or even whole organisms outside the cell. Once enclosed by the pseudopodia, they form an internal vesicle which is then moved further inside the cell.

b. Pinocytosis (cell drinking)

It involves the ingestion of smaller particles and does not require the formation of large pseudopodia to engulf the particles.

c. Receptor-mediated endocytosis

The membrane infolds to form vesicles only in regions where particles have bound to specific receptors. The binding stimulates the infolding.

C. Exocytosis

- In this process, substances are moved from the inside to the outside of the cell. It is the reverse of endocytosis.
- It is the process by which enzymes and hormones are secreted. Again, ATP is used to alter the configuration of the membrane.

Cell organelles

- Animal cells contain a nucleus, mitochondria, Lysosome, ribosome, ER (rough and smooth) as well as Golgi apparatus, all enclosed within a plasma membrane.
- Plant cells contain all the same organelles but also contain chloroplasts, a cellulose cell wall and a permanent vacuole.
- The organelles of cells have specific functions:
 - **Nucleus** contains DNA which controls the metabolism of the cell.

- **Mitochondria** carry out aerobic respiration to release energy from organic molecules and store it in the ATP molecule.
- **Ribosome** synthesizes proteins from amino acids.
- **Golgi body** modifies proteins and distributes them to the appropriate part of the cell
- **Lysosome** contains hydrolytic enzymes that digest worn out or damaged organelles as well as engulfed bacteria.
- **Chloroplasts** in plant cells carry out the reactions of photosynthesis.
- **Cell wall** supports and protects the contents of the cell; it is freely permeable to all molecules.
- **Vacuole** in plant cells contains a solution of mineral ions and sugars; it is important in maintaining the turgor of the cell.

28. In many eukaryotic cells, DNA stored in the nucleus is transcribed into messenger RNA. The mRNA is then transported into the cytoplasm where ribosomes assist in their translation into proteins. Finally, these proteins are packaged and sorted in the Golgi apparatus for use in other parts of the cell or in preparation for secretion into other cells. Which of the following statements is supported by this description?

- A. **Various organelles interact with each other to carry out life processes.**
- B. Organelles within a cell act independently of each other at all times.
- C. Some organelles are more important than other organelles within a cell.
- D. Only up to three organelles may interact with each other at any given moment in time.

29. Which of the following statements concerning mitochondria and chloroplasts is correct?

- A. Only mitochondria are surrounded by two membranes.
- B. The inner membrane of chloroplasts is folded into cristae.
- C. **Both organelles have a fluid interior.**
- D. Mitochondria contain stacks of membranes called thylakoid.

30. The functions of the rough endoplasmic reticulum and the Golgi body are related because:

- A. **Proteins synthesized by the rough endoplasmic reticulum are modified by the Golgi body**
- B. Proteins synthesized by the Golgi body are modified by the rough endoplasmic reticulum
- C. Lipids synthesized by the Golgi body are modified by the rough endoplasmic reticulum
- D. Lipids synthesized by the rough endoplasmic reticulum are modified by the Golgi body

31. Which of the following is not part of the structure of mitochondria?

- A. double membrane surrounding the organelle
- B. A fluid matrix inside the organelle
- C. **Stacks of membranes know as thylakoid**

32. In which of the following organelles is a cell's ATP produced?
 A. **Mitochondrion** B. Golgi apparatus C. endoplasmic reticulum D. Lysosome
33. Proteins are made on the
 A. Mitochondria. B. Nucleus **C. Ribosomes.** D. plasma membrane.
34. The packaging and distribution center of the cell is the
 A. Nucleus. B. central vacuole. **C. Golgi apparatus.** D. nuclear envelope.
35. The double membrane surrounding the nucleus is called the
 A. Nucleolus. B. nucleolus. C. nuclear wall. **D. nuclear envelope.**
36. All of the following are found in both plant and animal cells, except
A. A cell wall. C. mitochondria.
 B. A plasma membrane. D. the endoplasmic reticulum.
37. How are chloroplasts like mitochondria?
 A. They can both use energy from sunlight. **C. They both contain DNA.**
 B. They look alike. D. They are both found in animal cells.
38. The organelles associated with photosynthesis are the
 A. Mitochondria. B. Golgi apparatus. **C. Chloroplasts.** D. Vacuoles.
39. Plant cells have a large membrane-bound space in which water, waste products, and nutrients are stored. This place is known as a _____
 A. Mitochondrion. B. Golgi apparatus. C. Chloroplast **D. Central vacuole.**
40. Plant cells
 A. Do not contain mitochondria.
 B. Have a large central vacuole instead of a Golgi apparatus.
 C. Have a cell wall instead of a plasma membrane.
D. Have chloroplasts and cell wall.
41. Which of the following groups are believed to be the first photosynthetic organisms to evolve on earth?
 A. Green plants B. Green algae **C. Blue Green Algae** D. lichens
42. Which of the following is NOT true about cells:
 A. All cells have a plasma membrane
 B. Animal cells are on average smaller than plant cells
 C. All living things are composed of cells
D. Plant cells usually have a cell wall just inside the plasma membrane.
43. The cell's plasma membrane is best described as:
 A. **A lipid bilayer with imbedded proteins**
 B. A carbohydrate bilayer with imbedded lipids
 C. A protein bilayer with imbedded proteins
 D. A protein monolayer with an imbedded lipid bilayer
44. Which of the following is NOT true of proteins:
 A. They can act as hormones
 B. Their activity is altered by temperature changes
C. They store genetic information and pass it from generation to generation.
 D. Their activity is altered by pH changes

45. All cells have the following:
- Plasma membrane, cytoplasm, DNA & ribosome.**
 - DNA, ribosome, and cell wall.
 - Plasma membrane, nucleus, and DNA.
 - plasma membrane, cytoplasm, and nucleus
46. Controlling what enters and leaves the cell in an important function of the
- Nucleus.
 - plasma membrane.**
 - Vesicle.
 - Golgi apparatus.
47. Cytoskeleton elements are responsible for giving cells their shape and provide a basis for cell movement, migration, and cell divisions in eukaryotic cells are
- Microtubules (composed of tubulin)
 - Actin filaments (made up of actin);
 - intermediate filaments
 - All of the above**
48. Eukaryotic cells have extensive intracellular membranes, termed the endomembrane system, which divide the cell into structurally and functionally distinct compartments, or organelles. These include the inner cell membrane, nuclear envelope, endoplasmic reticulum, the Golgi apparatus, and Lysosome. Name a cellular component whose membrane is not part of the endomembrane system.
- Mitochondria and chloroplasts**
 - Mitochondria and nucleus
 - Chloroplast and chlorophyll
 - all of the above
49. Name the organelle that contains high levels of Hydrolases and is responsible for digestion of proteins, sugars, nucleic acids and lipids.
- Ribosome
 - mitochondria
 - Lysosome**
 - Golgi body
50. What common cell types are regulated by ion channels and are commonly referred to as excitable cells?
- Neurons
 - muscle cells
 - sensory receptor cells
 - All of the above**

Cell fractionation

- The technique is based on the fact that the masses of organelles vary and depend on their size.
- The large nucleus requires a relatively low centrifuge speed to make it settle out and much smaller ribosome's require a much higher speed. The technique is carried out as follows

- The cell sample is stored in a suspension that is:

Buffered – the neutral pH to prevents damage to the structure of proteins and enzymes.

Isotonic – this prevents osmotic water gain or loss by the organelles; gaining too much water could rupture the organelles

Cool – this reduces the overall activity of enzymes released later in the procedure.

- The cells are homogenized in a blender and filtered to remove debris.
- The homogenized sample is placed in an ultracentrifuge and spun at low speed. The nuclei settle out, forming a pellet.

4. The supernatant (the suspension containing the remaining organelles) is spun at a higher speed – chloroplasts settle out (if plant tissue is used).
 5. The supernatant is spun at a higher speed still – mitochondria settle out.
- The process is repeated at ever higher speeds until all the organelles have been separated.

Answer the following multiple choice questions. Doesn't visit answer keys before you try it on your own?

1. The principle behind separating cell organelles by ultracentrifugation is that:
 - A. The various organelles have different masses
 - B. The various organelles have different volumes
 - C. The various organelles have different Shapes
 - D. The various organelles have different widths
2. Which of the following statements is false?
 - A. Prokaryotic cells are smaller than eukaryotic cells
 - B. Eukaryotic organisms can be multicellular
 - C. Prokaryotic lack a nucleus
 - D. Plant cells are not eukaryotic
3. Which of the following clues would tell you whether a cell is prokaryotic or eukaryotic?
 - A. The presence or absence of a rigid cell wall
 - B. Whether or not the cell has membrane bounded organelles
 - C. The presence or absence of ribosome
 - D. Whether or not the cell contains DNA
4. The electron microscope has been particularly useful in studying bacteria, because
 - A. Electrons can penetrate tough bacterial cell walls.
 - B. Bacteria are so small.
 - C. Bacteria move so quickly they are hard to photograph.
 - D. Few organelles present, bacteria distinguished by differences in individual molecules.
5. A cell has mitochondria, ribosome, smooth and rough ER, and other parts. Based on this information, it could not be
 - A. A cell from a pine tree.
 - B. A bacterium.
 - C. A grasshopper cell.
 - D. Actually, it could be any of the above.
6. Which of the following is **NOT** an example of a eukaryotic cell?
 - A. Red blood cell
 - B. Bacteria
 - C. Cheek epithelial cell
 - D. Onion root cell
7. The organelle found in both animal and plant cells that is clear but contains dissolved nutrients and ions is the:
 - A. Vacuole
 - B. cytoplasm
 - C. rough ER
 - D. Golgi apparatus
8. Which organelles are responsible for digesting cell waste and foreign bacteria?
 - A. Golgi apparatus
 - B. cytoskeleton
 - C. nucleus
 - D. Lysosome
9. Which two structures are **NOT** present in animal cells?
 - A. A vacuole and a nucleus
 - B. A cell wall and chloroplasts
 - C. A Golgi apparatus and mitochondria
 - D. An endoplasmic reticulum and cytoplasm.

10. A researcher made an interesting observation about a protein made by the rough ER and eventually used to build a cell's plasma membrane. The protein in the membrane was actually slightly different from the protein made in the ER. The protein was probably changed in the:
 A. Golgi apparatus. B. smooth ER. C. mitochondrion. D. nucleus.
11. Which of these organelles modifies cell products and then packages them for distribution?
 A. The nucleus C. The cell membrane
 B. The mitochondrion D. The Golgi apparatus
12. Which of the following correctly matches an organelle with its function?
 A. mitochondrion -photosynthesis
 B. Nucleus -creation of energy (ATP) from sugars.
 C. Lysosome - movement
 D. central vacuole - storage
13. After you add a chemical to cells growing in a test tube, proteins accumulate in the cell's rough endoplasmic reticulum (ER). The chemical added prevents movement of proteins from the ER to which organelle?
 A. Golgi apparatus B. Lysosome C. Mitochondria D. Ribosome
14. Which organelle(s) is/are involved in the process of producing and exporting of proteins?
 A. Ribosome C. Endoplasmic reticulum
 B. Golgi apparatus D. All of the above
15. Which of the following statements is true about mitochondria?
 A. They are the site of energy (ATP) production in plant cells.
 B. They are the site of energy (ATP) production in animal cells.
 C. They provide photosynthesis in plants
 D. They are the site of energy (ATP) production in both animal and plant cells
16. To enter or leave a cell, substances must pass through
 A. A microtubule. B. the Golgi apparatus. C. a ribosome. D. the cell membrane.

Answer Key

1.A	7. A	13.A
2. D	8. D	14.B
3.B	9.B	15.D
4.B	10. A	16.D
5.B	11.D	
6.B	12.D	

UNIT FIVE

ENERGY TRANSFORMATION

At the end of the unit students will be able to:

- Describe the structure of ATP and its role in cellular metabolism.
- Explain how ATP is adapted to its role as an energy transfer molecule within a cell.
- Describe in detail each stage of aerobic respiration.
- Explain the processes of alcoholic fermentation and lactate production.
- Appreciate the importance of lactate production during running and other sports.

How energy is transformed?

First law of thermodynamics states as energy neither created nor destroyed, but transform (converted) from one form to another. Where, respiration and photosynthesis are two key processes to transform energy between cell and their environment.

Respiration

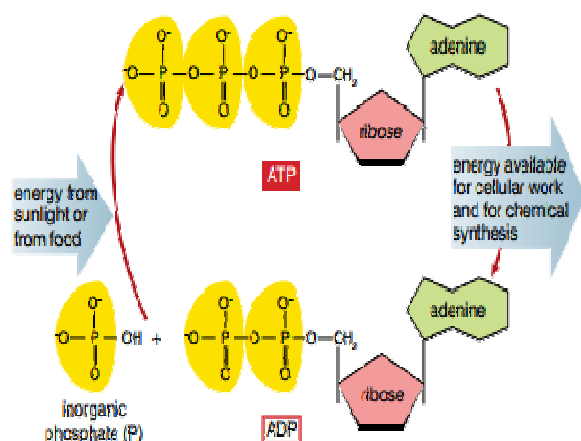
What is respiration?

Is the metabolic process by which all living cell breakdown (combust) organic molecules into smaller molecules to releases energy stored in form of ATP. Living cells respire all the time to produce ATP without exceptions.

What is the ATP molecule like?

- ATP stands for Adenosine Tri-phosphate. It a nucleic acid (polymer) made from smaller monomer called Nucleotides. All nucleotide contains:
 - ✚ Nitrogenous bases (**Adenine**)
 - ✚ Pentose sugar (**Ribose**)
 - ✚ Phosphate group (**PO₄⁻**)
- ATP is considered as an energy currency and storing molecule for the cell that is based on its nucleotides.
- Is described as phosphorylated nucleotides by adding one or two phosphate group the molecule.
- Is also an adenine nucleotide with two extra phosphates added on it.

The figure below indicates ADP and ATP interconversion

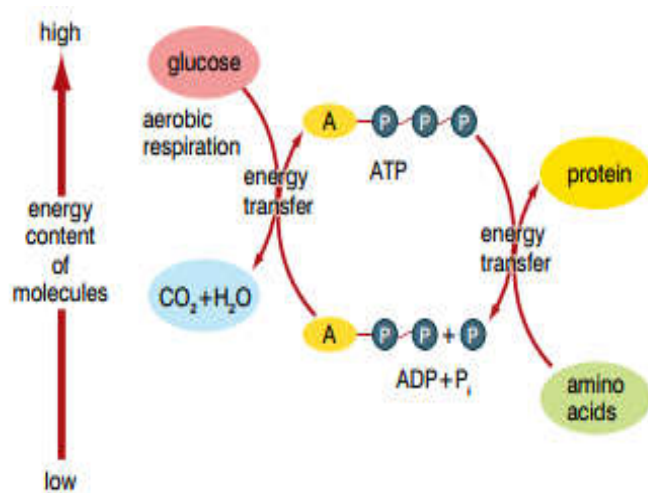


Hint:

- Adding the extra phosphates (third) requires energy, as energy is stored in the ATP molecule and when the bonds that hold third phosphate are broken energy is released again.

How ATP adapted as an energy transferring molecule in the cell?

- The energy from sunlight or glucose cannot directly used to drive available cellular process rather used to produce ATP. We say that it is coupled processes as the process takes place simultaneously at the same time.



ATP is adapted to its role because of the following:-

- It releases energy in small amount that matches biological process of the cell.
- It releases energy only in single step only, **Hydrolysis**.
- It able to move around the cell easily, but not escape from the cell.

The following major process requires energy from ATP

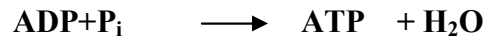
- Synthesis of macromolecules E.g Protein, polysaccharides
- Active transport across plasma membrane

- Muscle contraction
- Conduction of nerve impulses
- Initial step of respiration, Glycolysis

How ATP is produced in the cell?

Almost all ATP produced in the cell is the same way.i.e.

- Involving ADP and Pi joining to form ATP that requires input of energy.
- It involves enzyme ATP synthase (in chloroplast and mitochondrial membrane)

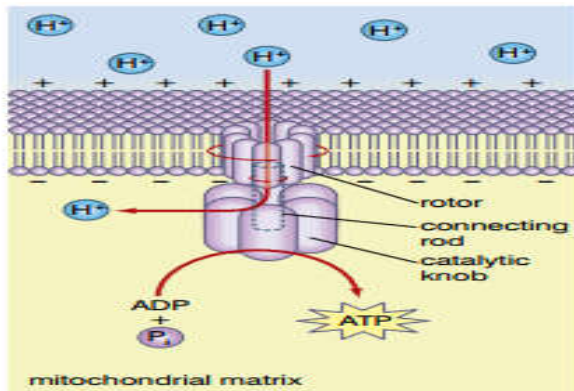


How ATP synthase work?

1. When rotor spin by hydrogen ion passing through it.
2. Energy of spinning activate catalytic knob that converts ADP and Pi to ATP.

Compare and contrast aerobic and anaerobic respiration.

This figure indicates how ATP synthase works



How ATP produced in respiration?

Compare and contrast aerobic and anaerobic respiration.

Aerobic pathway

- Usually takes place in higher animals
- Occur in mitochondria and involves oxygen
- Involves Glycolysis, Krebs cycle and Electron transport chain.
- Generate more ATP per oxidation of single glucose molecule
- End with water and carbodioxide

Anaerobic pathway

- ✚ Usually takes place in microorganisms.
- ✚ Occur in cytoplasm and no oxygen requirement.
- ✚ Only involves Glycolysis
- ✚ Generate two ATP oxidation of glucose molecule
- ✚ Ends with different end products E.g. alcohol, CO₂ and lactic acid.

How is ATP produced in aerobic respiration?

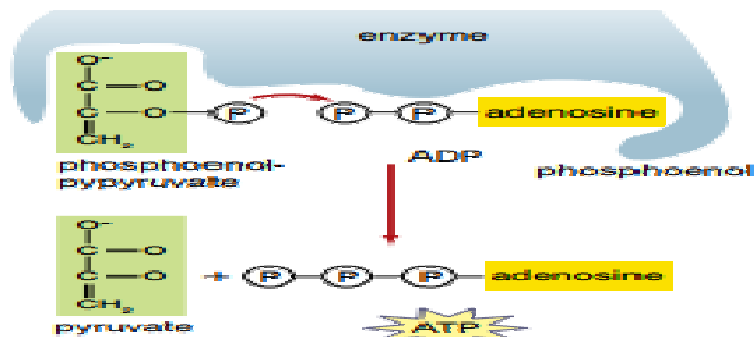
Compare and contrast substrate and oxidative level phosphorylation

A. Substrate level phosphorylation (SLP)

- ✚ Is catalyzed by enzyme, but not ATP synthase.
- ✚ It produces small amount of ATP produced by aerobic respiration (10%).
- ✚ It can take place in cytoplasm and mitochondrial matrix

Example

Phosphoenol pyruvate (PEP) is a substrate that able to transfer attached phosphate group directly to ADP, **Phosphorylation**.



E.g. ATP produced in glycolysis and Krebs cycle

B. Oxidative phosphorylation (OLP)

- Is an oxygen dependent production of ATP, **phosphorylation**.
- It produces about 90% of ATP produced by aerobic respiration.
- The process is catalyzed by enzyme that is based on proton spinning through rotor spin of ATP synthase.
- Occur in mitochondrial inner membrane

E.g. **Electron transport chain** and **chemiosmosis**

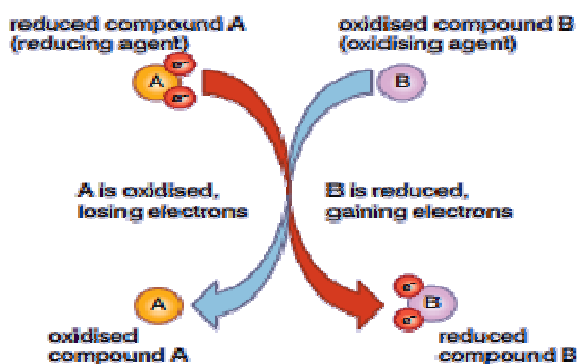
How are hydrogen ions transferred from glucose to ATP synthase?

Glucose $\longrightarrow$ ATP synthase

Two molecules are important in this transfer process

- **NAD** (nicotine Adenine Dinucleotide)
 - **FAD** (Flavin Adenine Dinucleotide)
- They are coenzymes and capable of accepting hydrogen ions and get reduced. That is written as **NADH** (red.NAD) and **FADH₂** (red.FAD.)
- They can release their hydrogen ion and oxidized where hydrogen ion used in rotor spin of ATP synthase.

Redox (coupled) reaction; is state when both oxidation and reduction reaction happen together simultaneously. This means when one compound loss an electron another compound accepts.



- A compound is get oxidized when it loses hydrogen, loss electron, gain oxygen and increases in oxidation number and reduced when compound gain hydrogen, gain electron, loss oxygen and decreases in oxidation number

Answer the following questions accordingly and refer relative sources where necessary. Don't key given before you tried it on your own.

1. Which one of the following equation bestly represents when cell prefer to obtain energy from ATP to synthesis protein from amino acid molecules?
A. Glucose + heat $\rightarrow$ Pyruvate + 2ATP + CO₂ C. ADP + Pi $\rightarrow$ ATP + H₂O + heat
B. Glucose + heat $\rightarrow$ Lactic acid + 2ATP + CO₂ D. ATP + H₂O $\rightarrow$ ADP + Pi + heat

What are the stages of aerobic respiration of glucose?

List and explain each step of aerobic respiration.

There are four stages in the aerobic respiration of glucose. These are:

- ✚ Glycolysis
- ✚ Link reaction
- ✚ Krebs cycle
- ✚ Electron transport and Chemiosmosis

Glycolysis (means glucose splitting)

- It is the first stage of cellular respiration.
- It takes place in cytoplasm (Cytosol) of a cell.
- It is a process of anaerobically breakdown of carbon six glucose molecules into two carbon three molecules.



Glycolysis has two phases with ten steps.

- a. ATP utilized stage and glucose converted to **2G3P**.
- b. ATP production phase and **2G3P** converted to pyruvate.

Glucose doesn't enter into mitochondrial membrane because of:-

- It is medium sized and not lipid soluble.
- There is no carrier protein to transport glucose across membrane.
- But pyruvate and coenzyme can enter mitochondrial membrane.

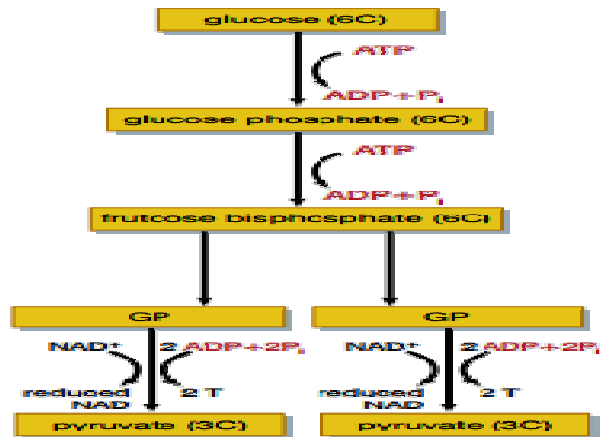
The following reactions take place in glycolysis?

- There are ten steps in glycolysis that are catalyzed by different enzymes.
- In phosphorylation process, glucose converted to another carbon six sugar (Fructose, 1-6, biphosphate).
- Fructose, 1-6, biphosphate split into two carbon three sugar glyceraldehyde –phosphate (G3P)
- Each **G3P** converted to pyruvate
- Two ATP molecules used up to make glucose more active.

End product of glycolysis per glucose molecule

- ✚ Four total ATP molecule by SLP {two ATP used to activate glucose and two net ATP gain}.
- ✚ Two pyruvate molecule
- ✚ Two reduced NAD molecule
- ✚ Then pyruvate and coenzyme enter into mitochondrial membrane

Main stages of Glycolysis

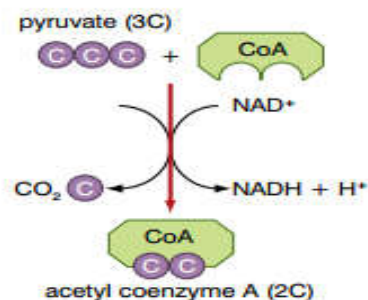


Summary of overall reaction of Glycolysis:-



Link reaction

- Is act as a transition or link step between glycolysis and kreb cycle.
- It takes place in fluid matrix of mitochondria and involves aerobic process.
- Then pyruvate reacts with coenzyme A (**CoA**) to form carbon two molecule acetyl-coA (**acetyl CoA**). **Hint.** CoA is derived from **Vit.B** panthonic acid which is needed for respiration.



Two main reactions takes place in link reaction are:-

- **Dehydrogenation** -hydrogen is lost from a molecule and red.NAD formed.
- **Decarboxylation** - a carbon atom is lost in form carbon dioxide.

End product of this reaction

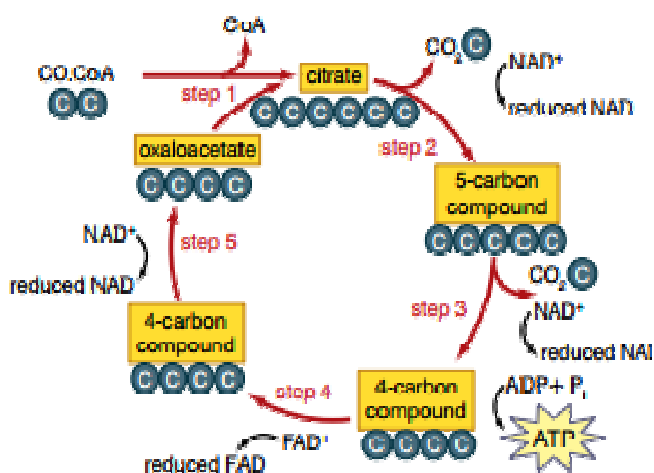
2CO₂, 2acetyl CoA, and 2NADH

KREB CYCLE (Citric acid cycle) / Tricarboxylic acid

- It is takes place in fluid matrix of mitochondria and aerobic process.
- In all glycolysis, link reaction and kreb cycle hydrogen atom transferred to NAD and FAD molecules.

What happens in the Krebs cycle?

- Two-carbon group acetyl-coA reacts with the four-carbon compound **oxaloacetate** to form a six-carbon compound called **citrate**.
- Citrate decarboxylated to form a five-carbon compound (**Isocitrate**) and CO₂.
- Five-carbon compound further decarboxylated to form a four-carbon compound and CO₂, ATP molecule by **SLP**.
- Four-carbon compound regenerate the original four-carbon compound (oxaloacetate) and begin reacting with another molecule of acetyl CoA.



➤ *What is end product of kreb cycle after regeneration?*

End of Kreb cycle as regeneration is:

- Six reduced NAD
- Four carbodioxide
- Two ATP molecules produced by SLP
- Two reduced FAD

A summary of the overall reaction of the Krebs cycle is:-



Electron Transport Chain (ETC) and Chemiosmosis

Define and Explain how these two reactions takes place?

- Together makes up oxidative level phosphorylation (**OLP**).
- It is an aerobic process involving ATP synthase enzyme.
- It takes place in mitochondrial inner membrane, **Cristae**.

The following events take place on Cristae:

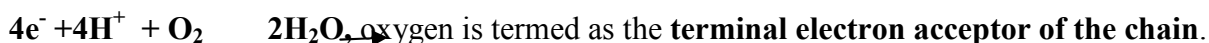
- Hydrogen atoms carried by red.NAD and red.FAD are released and split into protons and electrons.
- The electrons pass along electron carriers from one carrier to next that form, ETC.
- They loss energy as they pass from one carrier to next. Where energy lost is used to empower the pump (makes the process more energetic) from mitochondrial matrix into the inter-membrane space.

Three molecules that act as electron carriers in the ETC are:

- Red. NAD dehydrogenase (also a proton pump)
- Ubiquinone (also a proton pump), and
- Cytochrome (proteins that contain iron).

Where reduced NAD is dehydrogenated by the NAD dehydrogenase complex, reduced FAD is dehydrogenated by Ubiquinone.

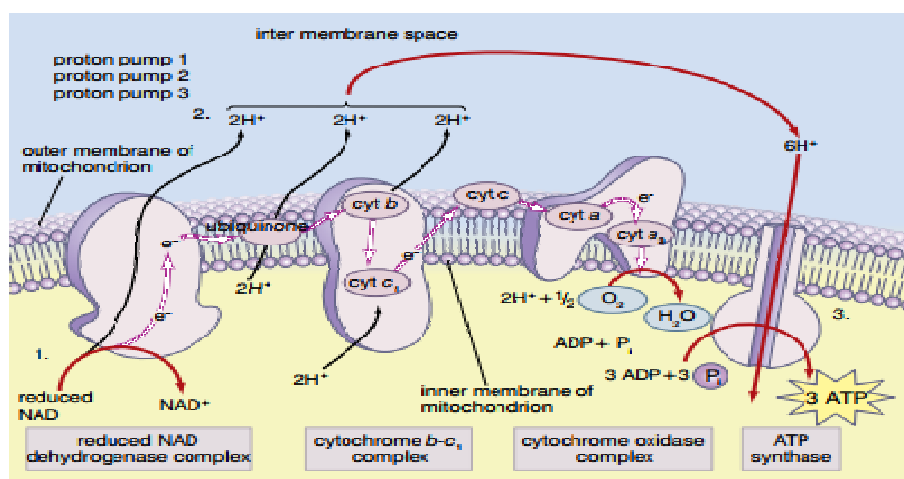
At the end of ETC, electrons combine with protons and with oxygen to form molecules of water.



Because of the action of the proton pumps,

- ✚ Protons accumulate in the inter-membrane space creating a higher concentration there than in the matrix.
- ✚ This proton gradient results in protons diffusing through the ATP synthase.
- ✚ This makes rotor 'spin' of synthase and produce ATP from ADP and P_i ,
phosphorylation

Chemiosmosis is diffusing of hydrogen ions through the ATP synthase



- ✚ The oxidation of one red. NAD molecule results in six protons passing through ATP synthase and so leads to the synthesis of 3ATP molecules.

1red. NAD → pump six proton → three ATP

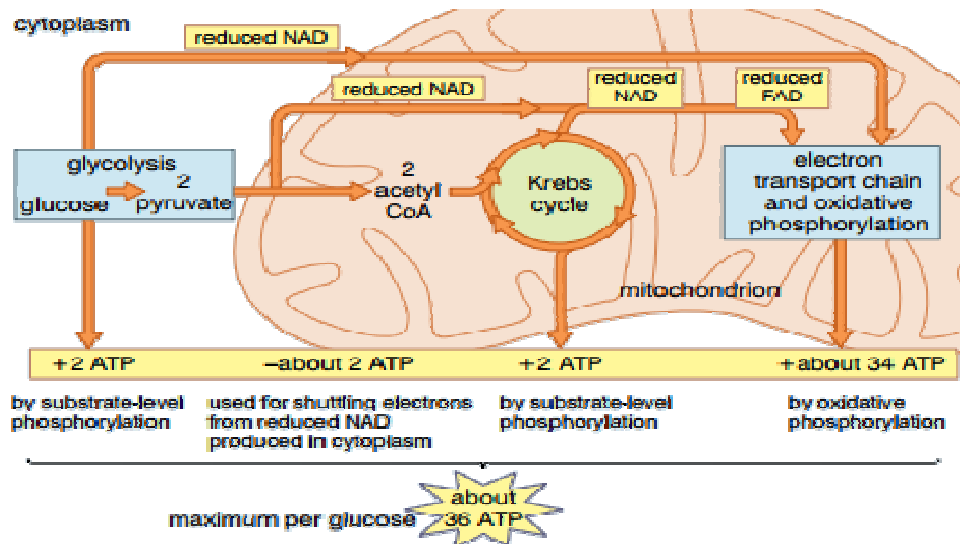
- ✚ The oxidation of one of red. FAD molecule results in four protons passing through ATP synthase and leads to the synthesis 2ATP molecules.

1red. FAD → pump four proton → two ATP

A summary of overall reaction of ETC is:

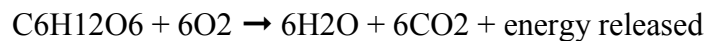
6 reduced NAD (from Krebs' cycle) + 2 reduced NAD (from glycolysis) + 2 reduced FAD (from Krebs' cycle) + 30 ADP + 30 P_i – 2ATP (used in proton pumps) → 36 ATP + 8 NAD + 2FAD

- The actual yield is about **36** molecules of ATP per molecule of glucose.



The production of ATP during the aerobic respiration of glucose

The summary equation for aerobic respiration is:



- During which stage of aerobic respiration carbon dioxide is produced?
 - Link reaction and Krebs cycle
 - Citric acid cycle and glycolysis
 - Link reaction and electron transport chain
 - Glycolysis and electron transport system
- The oxidation of glucose molecule into two molecules of pyruvate, ATP, NADH is known as _____ and takes place in _____.
 - Glycolysis and mitochondria
 - Glycolysis and cytoplasm**
 - link reaction and cytoplasm
 - Link reaction and mitochondria
- Which of the following is **not true** concerning Adenosine tri phosphate?
 - It is formed in the same amount during aerobic & an aerobic pathway**
 - It releases energy in a single step of hydrolysis reaction
 - Used to couples energy releasing and energy requiring reaction
 - It is said to be nucleotide with two extra phosphate group
- What is role of oxygen in the aerobic respiration?
 - It combines with CO_2 to form citrate
 - Act as terminal electron acceptor in the electron transport chain**
 - It combines with acetyl coenzyme A to form oxalo acetate
 - It combines with ADP in the formation of ATP

6. Which of the following is true about the site where kreb cycle?

- A. It occur in the matrix of mitochondria
- B. It takes place in inner membrane of mitochondria
- C. It occur in the intermembrane space of mitochondria
- D. It occur in the Cytosol of cytoplasm

Respirometer

What is Respirometer?

- Is a device used to measure the rate of respiration in living organism by measuring the rate of CO₂ and O₂ exchange per minute. As, an organism respire they take oxygen from air and give up carbodioxide.
- Have different forms, but all works on principle that predicts the volume of oxygen used is equal to volume of carbodioxide produced.

Basic Respirometer over leaves:-

- For every volume of oxygen an organism uses, the same molecule of carbodioxide produced that is absorbed by KOH. So, over a time volume inside Respirometer reduced. Equationally indicated as:

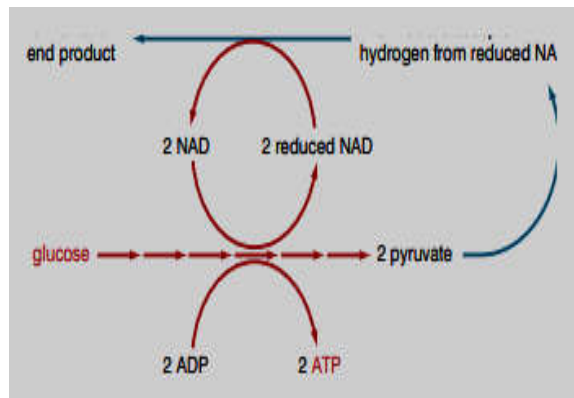


$$V [\text{6O}_2] \text{ used up} = V [\text{6CO}_2] \text{ produced}$$

What happens in the anaerobic pathway?

If there is no oxygen:-

- ✚ The final reaction of oxidative phosphorylation, where electrons and protons react with oxygen to form water stops.
- ✚ Link reaction, kreb cycle and electron transport chain comes to a halt.
- ✚ No protons are pumped (chemiosmosis).
- ✚ The action of ATP synthase also stops. There is a further 'knock-on' effect (stop).
 - However, glycolysis *can* continue.
 - As red.NAD formed during glycolysis is regenerate anaerobically by converting the pyruvate into another product in a reduction form.
 - Reduced NAD supplies the hydrogen for both reduction and oxidized itself.



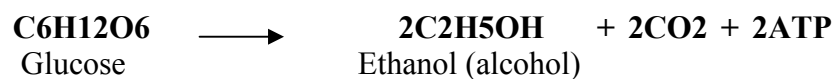
Different organisms produce different fermentation end products.

Anaerobic fermentation named differently on basis of their product.

Example.

Yeast cells

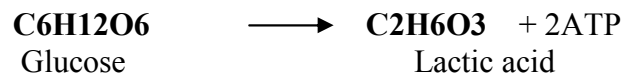
- Produce **ethanol** (ethyl alcohol) by fermenting glucose.



- termed as alcoholic fermentation

Animal cells

- Produce **lactate (lactic acid)** when ferment glucose.



- is termed as Lactic fermentation

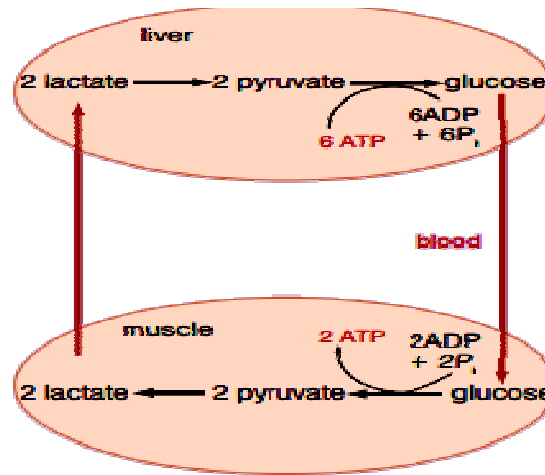
Lactate fermentation during exercise

- During exercise energy demand increases.
- Where fermentation of glucose supply extra energy to meet this demand.

Oxygen debt

- After exercise lactic acid accumulated in the muscle that may be toxic to the cell, which can result muscle fatigue /cramp.
- Is extra oxygen needed after exercise finished to break accumulated lactate that is transported to the liver and converted into pyruvate.

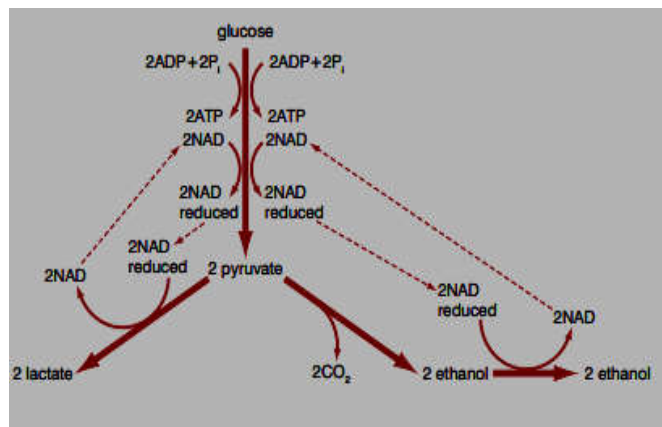
Cori cycle



The advantage of this cycle in liver is:-

- ✚ To prevent lactic accumulation in muscle
 - ✚ To regenerate glucose as energy source for muscle in low oxygen level.
- Commonly both alcoholic and lactic fermentation
- ✚ Uses glucose as starting substrate.
 - ✚ Produces two ATP and two red.NAD per cycle.

The fermentation of glucose in animal cells and yeast



Briefly compare and contrast aerobic and anaerobic respiration.

Aerobic respiration	Anaerobic respiration
Yield 36ATP slowly from oxidation of glucose molecule E.g. Long race run slowly and respire aerobically	Yields two net ATP oxidation glucose molecule E.g. Short race and sprint run quickly and respire anaerobically
Produce sufficient energy to sustain cell metabolism	Produces insufficient energy
No lactate accumulation after exercises as sufficient oxygen	Accumulates lactate after exercise as no oxygen leads to cramp (tiredness)

List the advantage of anaerobic fermentation product in different industries.

What substances can be used as energy sources?

- Not only glucose as respiratory substrate but also protein and lipid can enter into aerobic pathway by hydrolyzing into their building blocks to releases energy.
- The degraded products enter kreb cycle where they converge.

Give your correct answer for the following multiple choice questions. Refer relative sources where necessary.

- Which of the following reactions represents cellular respiration?
 - $6 \text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6 \text{H}_{12} \text{O}_6 + 6 \text{O}_2$
 - $\text{CO}_2 + \text{RUBP} \rightarrow 2\text{G3P}$
 - $\text{O}_2 + \text{RUBP} \rightarrow 1\text{G3P} + \text{phosphoglycolate}$
 - $\text{C}_6 \text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow \text{C}_6 \text{H}_{12} \text{O}_6 + 6 \text{O}_2$
- During which of the following pairs of respiration stage carbon dioxide is produced as an end product?
 - Electron transport chain and Krebs cycle
 - kreb cycle and Link reaction
 - Link reaction and chemiosmosis
 - Glycolysis and Link reaction
- Among the following identify one that bestly explains respiration?
 - Reductive ,catabolic and exergonic
 - Reductive ,endergonic and anabolic
 - Oxidative, exergonic and catabolic
 - Oxidative ,exergonic and catabolic
- Which phosphate group bond of ATP is broken when energy it contains needed for cellular process?
 - The second bond
 - C-C bond
 - The first bond
 - The third bond

5. As electron passes down the electron transport chain of the mitochondria:-
 - A. They produce reduced NAD to the next process
 - B. Carbon dioxide is produced & FAD^+ is reduced
 - C. They lose energy as they pass from one electron carrier to next.
 - D. They gain energy as they pass from one electron carrier to the next.
6. In which of the following pair's cellular respiration does substrate level phosphorylation occur?
 - A. Glycolysis and Chemiosmosis
 - B. Electron transport chain and Chemiosmosis
 - C. Krebs cycle and Glycolysis
 - D. Krebs cycle and Chemiosmosis
7. What does the fermentation of glucose by yeast normally yields:-
 - A. 2CO_2 , 2ATP & lactic acid
 - B. 2CO_2 , 36ATP & ethanol
 - C. 2CO_2 , 2ATP & ethyl alcohol
 - D. 2ATP and lactate
8. Which one of the following is NOT component of nucleotide during phosphorylation process?
 - A. Phosphate group
 - B. Pentose sugar unit
 - C. Nitrogenous bases
 - D. Amino acid sequence
9. How many ATP molecules is produced per complete oxidation of single glucose molecule during cellular respiration?
 - A. 4ATP
 - B. 24ATP
 - C. 30ATP
 - D. 36ATP
10. Starting with two molecules of acetyl CoA and oxaloacetate, kreb cycle after regeneration ends with:
 - A. 1ATP , 2CO_2 , 1FADH₂ and 3NADH
 - B. 2pyruvate, 2ATP and 2CO_2
 - C. 10NADH, 34ATP and 2FADH₂
 - D. 2ATP, 4CO_2 , 2FADH₂ and 6 FADH₂
11. During kreb cycle, to which one of the following coenzyme does most of energy released from glucose molecule is transferred?
 - A. NAD
 - B. NADP
 - C. FAD
 - D. AMP
12. What is role of oxygen in the aerobic respiration? It:-
 - A. Combines with CoA to form acetyl CoA
 - B. Used as final electron acceptor in the electron transport chain.
 - C. Combines with pyruvate to form acetyl CoA
 - D. Transfer proton from one carrier to next
13. During which one of the following processes of cellular respiration is most of ATP produced?
 - A. Glycolysis
 - B. Krebs cycle
 - C. Link reaction
 - D. Chemiosmosis
14. What chemiosmosis represent during aerobic respiration pathway?
 - A. Passing of electron from one carrier to next
 - B. Splitting of glucose anaerobically
 - C. Diffusing of proton down concentration gradient
 - D. Regeneration of coenzymes under glycolysis
15. Among the following cellular process one releases carbon dioxide to the surrounding atmosphere:
 - A. Assimilation
 - B. Photosynthesis
 - C. Respiration
 - D. Feeding

16. Which one of the following is **NOT** true about oxidative level phosphorylation?
- It comprises ETC and chemiosmosis
 - It is catalyzed by ATP synthases enzyme
 - It depends on proton passing through rotor spin of ATP synthases
 - It produces ATP by substrate level phosphorylation
17. When athletes take part in short distance race, how does the cell generate energy needed? By:
- Aerobic respiration in muscle cell
 - anaerobic respiration in muscle cell
 - alcoholic fermentation in cytoplasm
 - aerobic respiration in inner membrane
18. When the muscle cells are in short supply of oxygen, which of the following compounds would be accumulate in them?
- Ethanol
 - Acetic acid
 - Pyruvic acid
 - Lactic acid
19. One of the following molecule acts as a final/terminal electron acceptor of a respiration?
- NADP
 - FADH₂
 - Water
 - Oxygen
20. The first molecule produced when Acetyl COA combines with oxaloacetate in fluid matrix of mitochondria?
- Citrate
 - Malate
 - Isocitrate
 - Succinate
21. Starting with two molecules of pyruvate and two coenzymeA, link reaction results which of the following end products?
- 2Acetyl COA , 2CO₂ and 2NADH
 - 2Acetyl COA, 2ATP and 2CO₂
 - 2Acetyl COA, 2ATP and 2NADH
 - 2Acetyl COA, 2CO₂ and 2FADH₂
22. From the oxidation of **10NAD** and **10FADH₂** molecule, how many proton pumped through ATP synthase and how many ATP synthesized in rotor spin respectively?
- 54H⁺ and 27ATP
 - 100H⁺ and 50 ATP
 - 60H⁺ and 30 ATP
 - 70 H⁺ and 35 ATP

5. 2. Photosynthesis

At the end of this section students should be able to:

- Draw, label and describe a chloroplast.
- Describe light-dependent and -independent stages of photosynthesis
- Explain how the structure of a photosystem is related to its role.
- Describe the factors that affect the rate of photosynthesis
- Distinguish between C3 and C4 plants and give at least two examples for each.
- Appreciate the importance of C4 plants in Ethiopia cereals.
- Explain the CAM photosynthetic pathway and its benefits to desert plants.

How do plants harness light energy in photosynthesis?

What is photosynthesis?

Defination: Derived from photo- light synthesis – manufacture

- Is a process that nourishes almost all life in the biosphere by providing organic compound to eat and oxygen to breath.
- Is process of transducing light energy into chemical energy of organic molecules.
- Is process of converting inorganic molecules (water and carbodioxide) into range of organic molecule.
- Is process by which green plants, chlorophyll pigment, that absorbs light energy to transform into chemical energy stored in glucose.



List and explain phase of photosynthesis

Light dependent stage	Light Independent stage
• Based on light energy for phosphorylation and photolysis of water.	• Based on product of light energy ,not need light
• Takes place in thylakoid(grana) membrane of chloroplast	• Takes place in fluid stroma of chloroplast

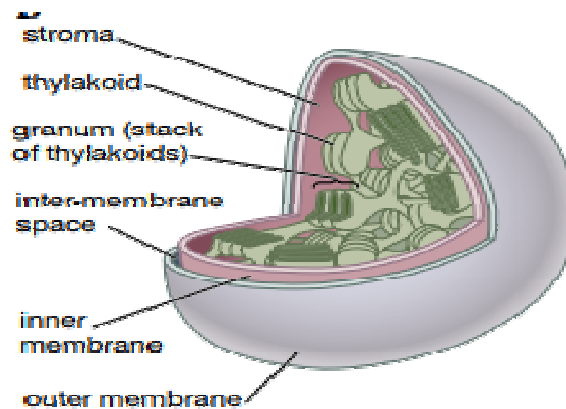
How structure of chloroplast suited to its function?

Is adapted for photosynthesis due to:-

- ✚ Presence of chlorophyll and photosensitive pigment arranged in photosystem that linked with ETC.
- ✚ Each photosystem and ETC is fixed in thylakoid membrane.
- ✚ Is disc-shaped with large surface area volume ratio to absorb more light.
- ✚ Has fluid –filled stroma that contains enzymes.

Structure of Chloroplast

Label each structure of chloroplast indicated below.



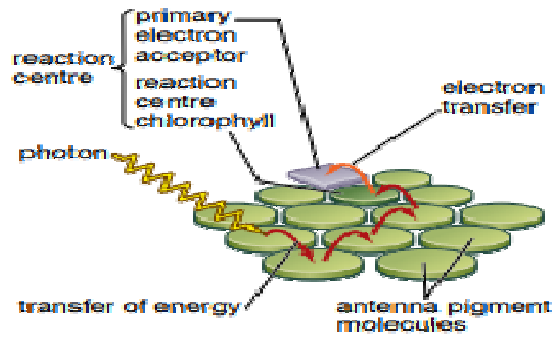
Photosystem

- ✚ Is a biochemical mechanism by which chlorophyll absorbs light energy.
- ✚ Sensitive to different light wavelength PsI (700λ) and PsII (680λ) and linked to different ETC.
- ✚ Is a site where light reaction begun.

What is the structure of a photosystem?

- ✚ A photosystem is complex proteins (pigment emended) in thylakoid membrane.
- ✚ It consists a number of pigment molecules clustered around one chlorophyll molecule called **Reaction centre molecule** (positioned next to ETC).
- ✚ This cluster of pigment molecules is called an **Antenna complex**.
- ✚ **Antenna complex** is an array of protein and chlorophyll light harvesting molecule in thylakoid membrane.
- ✚ Energy absorbed by other molecules in the photosystem is transferred to the **reaction centre molecule**, where the light-dependent reactions begin.
- ✚ Different pigment in the **antenna complex** can absorb different wavelengths of light, making the whole system more efficient.
- ✚ The pigments in the antenna complex include chlorophyll **a**, chlorophyll **b** and Carotenoids.
- ✚ Where **chlorophyll a** is always reaction centre molecule.

Structure of photosystem

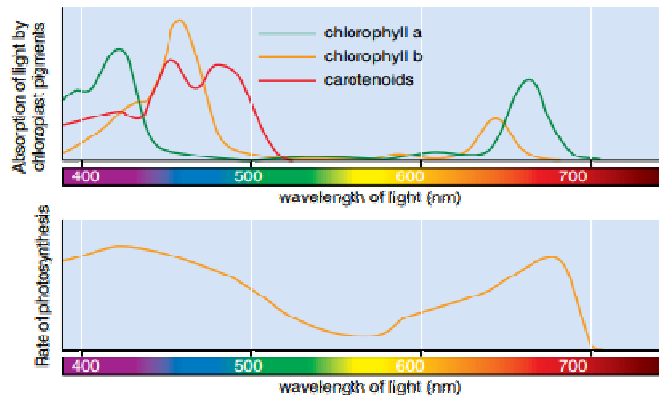


Absorption spectrum to indicate which pigments absorb what wavelength of light.

+ Is measured by **spectrophotometer**.

+ **Action spectrum** shows maximum peak at which photosynthetic effectiveness at each wavelength.

Absorptive spectrum of chlorophyll a, chlorophyll b and Carotenoids.



Why plants appear green?

- Because they absorb blue and red light wavelengths, but reflect green light wavelengths.
- There are two photosystems in the light-dependent reaction which have enormous pigments (200-300) associated with proteins and enzymes.

Differentiate both types of photosystem

Photosystem I(PsI)	PhotosystemII(PsII)
Located at outer surface of thylakoid membrane	Located at inner surface of thylakoid membrane
Has 700 λ absorptive wavelength	Has 680 λ absorptive wavelength
Involved both cyclical and non-cyclical phosphorylation	Only involved in non-cyclical phosphorylation
Not associated with photolysis of water	Associated with photolysis of water

What happens in the light-dependent reactions?

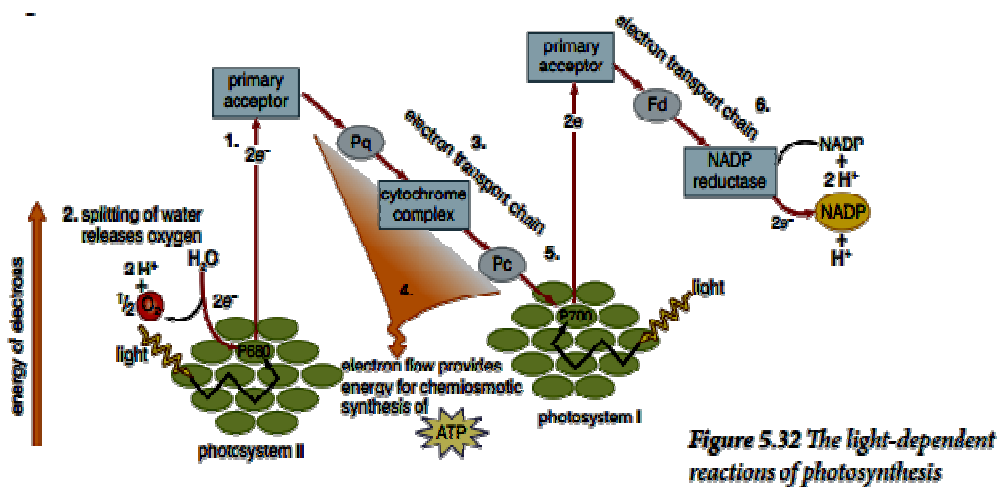
- Light-dependent reactions use energy from light (photon) to drive synthesis of two molecules that drive the light independent reactions.

These reactions are:-

1. photolysis of water (light dependent splitting of water)

$$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$$
2. Phosphorylation reaction to synthesis ATP that provides the energy for the later reactions, and
3. Produces reduced NADP which is similarly used to transporting hydrogen ions as NAD in respiration. It provides the hydrogen ions for later reduction reaction.

Z-scheme representation of light dependent reactions.



Photosystem I and photosystem II

The following major reactions take place inside PsI and PsII respectively

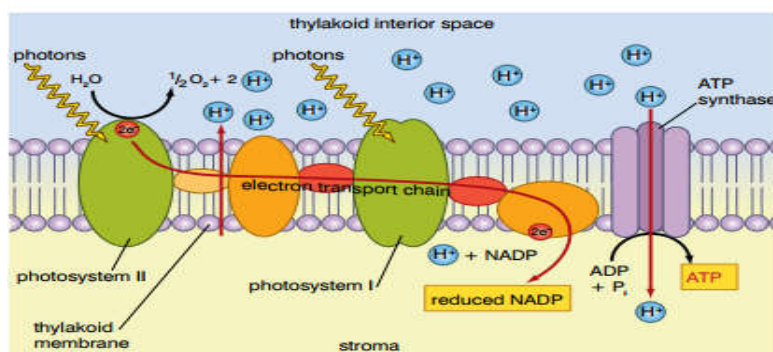
1. Electrons in photosystem **II** are excited by the energy in photons and become more energetic as extra energy.
 - They escape from the chlorophyll and pass to **primary electron acceptor**.
 2. Energy from sunlight split water molecule in chloroplast, **photolysis**.

$$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$$
 - Where electrons replace those lost from the chlorophyll molecule.
 3. Primary electron acceptor passes electrons to the next first **ETC₁** (plastoquinone or '**Pq**'), Cytochrome and to last carrier plastocyanin (**Pc**) respectively.
 - Electrons lose energy as they are pass from one carrier to the next, this empower the pump from outer stroma to inner thylakoid membrane.
 4. In the cytochrome complex of ETC there is a proton pump between stroma and inner thylakoid.
 - This creates protons accumulation inside the thylakoid, which drives the chemiosmotic synthesis of ATP.
 5. Electrons in photosystem **I** excited as absorbs photons and escape from chlorophyll molecule. They are replaced by the electrons that have passed down ETC from photosystem **II**.
 6. Electrons then pass along a second ETC₂ involving ferredoxin (**Fd**) and NADP reductases.
- Finally, electron reacts with protons and NADP in the stroma of the chloroplast to form reduced NADP.

Photosynthetic unit-

- Is an arrangement of pigment molecule, electron carrier molecules and ATP synthase capable of carrying out all reactions in the light-dependent stage of photosynthesis.
- Arrangement of all molecules to carryout light dependent reaction of photosynthesis.

How photosynthetic unit arranged in thylakoid membrane



Phosphorylation

There are two types phosphorylation in light dependent reaction.

These are **cyclical** and **Non-cyclical** phosphorylation.

Both process produces ATP because of:-

- ✚ There is an accumulation of protons in the interior of a thylakoid membrane.
- ✚ This creates a concentration gradient between the thylakoid and the stroma of the chloroplast
- ✚ Protons move through ATP synthase causing the rotor to spin for phosphorylation.

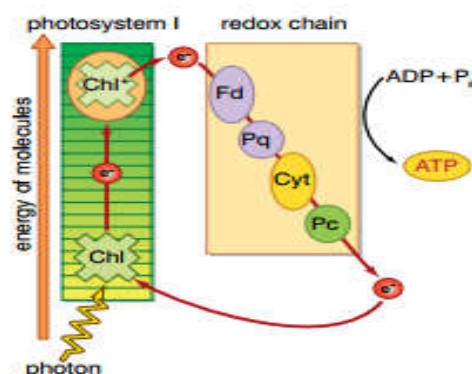
Non-cyclical phosphorylation

- Said non-cyclical as electron travel in non-cyclical manner.
- Involves one way of electron flow from water to NADP.
- First electron donor is water and final electron acceptor is NADP
- It end with net product of ATP,NADP and Oxygen
- Involves both PsII and PsI

Cyclical phosphorylation E.g. Bacteria

- ✚ Said cyclical as electron travel cyclical manner(Back-back)
- ✚ Is simple and rare process of phosphorylation.
- ✚ Involves only PsI
- ✚ Initial donor and final electron acceptor is chlorophyll molecule.
- ✚ The net product is only ATP molecules.

Structure of cyclical phosphorylation



Plants rarely synthesis ATP by cyclical phosphorylation when:-

- No oxygen and reduced NADP availability.
- Sugar cannot synthesize as lack of carbodioxide.

Brief summary of the light-dependent reactions

Light energy is used to excite electrons. This cause:-

- Protons transfer to inside of the thylakoid membrane as they pass along ETC that leads to ATP synthesis.
- React with hydrogen ions and NADP at the end of the second ETC to form reduced NADP.
- Generally, ATP and reduced NADP are used to drive the synthesis of carbohydrates in the light-independent reactions of photosynthesis

1. All of the following are describes why chloroplast is suited to its function “**Except**”
 - A. The chlorophyll & other photosensitive pigment molecules are arranged in photosystem.
 - B. The molecules of photosystem & the ETC are fixed in the membrane of thylakoid.
 - C. It’s composed of stroma provides fluid medium for the reaction of dark reaction.
 - D. **The inner membrane is folded to speed up reaction of light dependent stage.**
2. Which of the following is **true** regarding cyclic photophosphorylation?
 - A. Only photosystem II is involved in the process.
 - B. **It produces oxygen & reduced NADP.**
 - C. The flow of electron is from PSI to PSI.
 - D. Is the most common type of photophosphorylation
3. Which of the following is **NOT true** about photosystem II?
 - A. It contains the reaction center chlorophyll **a**
 - B. It absorbs maximum light at 680nm wave length
 - C. **It absorbs maximum light at 700nm wave length**
 - D. It contain reaction center molecule and antenna complex

Briefly compare and contrast photosynthesis and Respiration

<i>Respiration</i>	<i>Photosynthesis</i>
Is catabolic process	Is anabolic process
Uses sugar as raw material and produces CO ₂ and H ₂ O	Uses CO ₂ and H ₂ O to produce sugar
Is an oxidative process	Is reductive process to produce sugar.
All organism respire to releases energy	Only photoautotrophic organism
Requires NAD and FAD as proton transfer	Requires NADP as proton transfer
Takes place in mitochondria	Takes place in chloroplast

How is carbohydrate synthesized in the light-independent reactions?

- Light-independent reactions uses product of light dependent reaction, ATP and NADP, to reduce carbon dioxide. .
- It doesn't require light but based on product of light dependent reaction.
 - It takes place in the stroma of the chloroplasts.
 - Is called Calvin cycle after the work of Melvin Calvin on unicellular algae, *Chlorella* (refer).
 - Is also referred to carbon fixation and reduction reaction.
 - Is a complex cycle catalyzed by different enzyme.

This reaction involves three consecutive steps.

Stages of Calvin cycle

a. Carbon fixation stage

- Is a beginning stage of Calvin cycle when carbon dioxide reacts with pre-existing five carbon molecule ribulose biphosphate (**RuBP**) in stroma of chloroplast.
- Form an immediate carbon six molecule Phosphoglyceric acid (PGA)
- Is catalyzed by **Rubisco** enzyme (RuBP carboxylase/oxygenase).

b. Reduction carbon dioxide

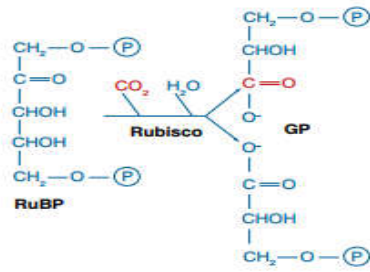
- Is when carbon dioxide is reduced to produce two carbon three molecule glyceraldehyde-3-phosphate (**G3P**).

- This stage uses energy from ATP and proton from reduced NADP of light reaction.
- Some G3P goes to synthesis glucose and other organic molecules, where else goes to regenerate RuBP.

c. Regeneration of RuBP from G3P

- Two G3P molecules needed to produce one glucose molecule.
- Regenerate original RuBP

The action of Rubisco



- Calvin cycle needs six turns to produce two G3P (**TP**, *Triose phosphate*) which is enough to make one glucose molecule.
- One turn of Calvin cycle needs 3ATP and 2 NADP to produce one glucose molecule.

Find how many NADP and ATP is needed to produce on glucose molecule?

4. How many ATP and reduced NADP molecules used up during reduction of carbon dioxide respectively?

- | | |
|----------------------|---------------------|
| A. 6 ATP and NDP | C. 18ATP and 12NADP |
| B. C24ATP and 12NADP | D. 3ATP and 2NADP |

The light independent reaction

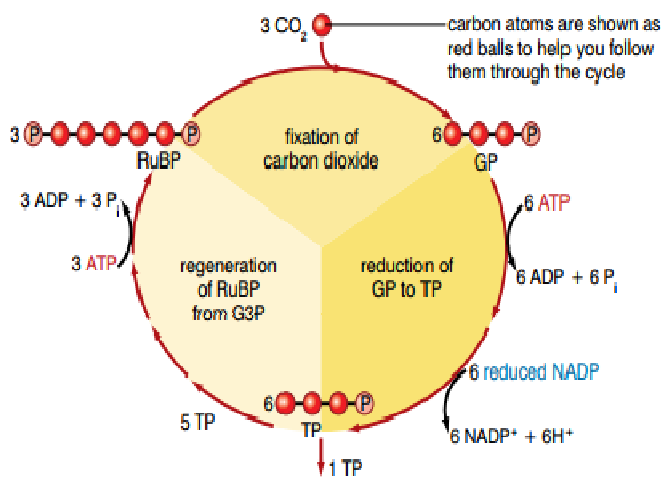
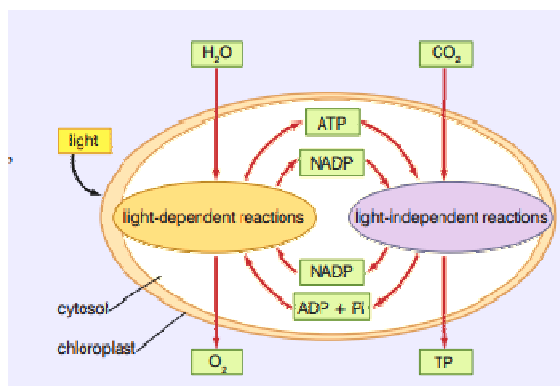


Illustration of how the light-dependent and light independent reactions are related.



What factors affect the rate of photosynthesis?

The rate of Photosynthesis is dependent on a many environmental factors. This includes:-

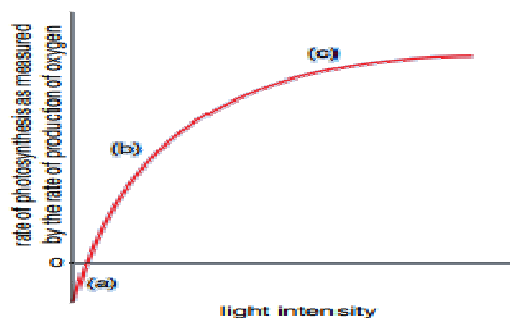
1. Light intensity

- It limits the rate of light dependent reaction directly by affecting number of electrons in reaction center molecule, **Photo-excitations**.

Explain how the effect of light on the following seasons where:

- On cold bright day (Arctic) – High light and low temperature.
- On warm cloudy day (summer) –Low light and high temperature
- On warm sunny day (summer)-Both light and temperature high

The rate of photosynthesis is limited by factor that is present in a limiting quantity (short supply), which is known as the **principle of limiting factors**.



The effect of light intensity on the rate of photosynthesis?

Based on the graph above:

a. Very low light intensities

Respiration is still occurring and is taking in oxygen faster than photosynthesis is producing it.

b. medium light intensities

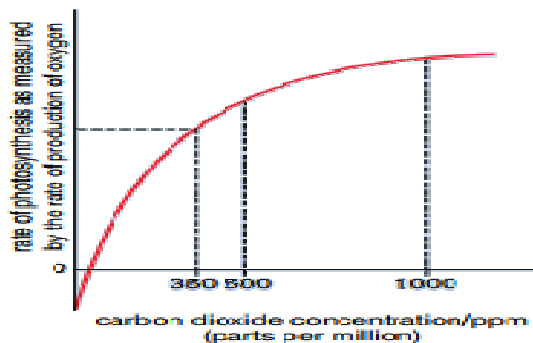
Photosynthesis is producing more oxygen than respiration uses, the rate of photosynthesis increases light intensity.

c. very high light intensities

The rate of photosynthesis is level out with light intensity; eventhouthgh other factor is probably limiting the rate.

2. Carbon dioxide concentration.

- It limits light independent reaction of photosynthesis by influencing initial reaction with RuBP.
- At low concentration of carbon dioxide little photosynthesis takes place, although respiration still uses oxygen. As $[CO_2]$ an increase so does rate of photosynthesis, until saturation of **Rubisco**.

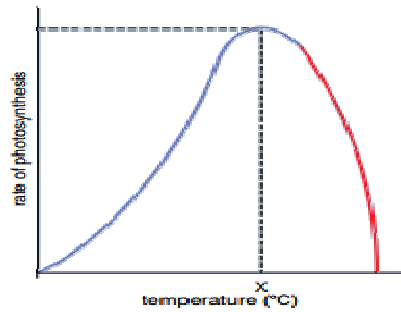


3. Temperature

- It limits the rate of both light dependent (ATP synthase) and independent (Rubisco) reaction of photosynthesis, as both reaction catalyzed by enzyme.
- But there is optimum temperature for photosynthesis enzyme which varies with geographical location.

Example:

- Enzymes of plants that live at arctic have lower optimum temperature than plants living in tropics.
- As temperature exceeds optimum, enzyme denatures and rate of photosynthesis decreases rapidly.



How all factors interact to influence the rate of photosynthesis?

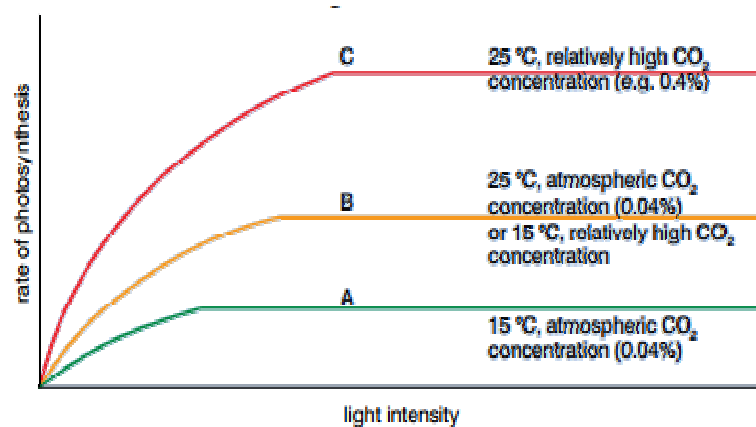
Increasing the light intensity increase:-

- The rate at which electron exited from reaction center, photo –excitation
- The rate at which ATP and reduced NADP are produced in the light-dependent reactions.
- Increase the rate at which the Calvin cycle can take place.

However, the rate at which the Calvin cycle can ‘turn’ could be limited by:

- Low temperature (limiting the rate of Rubisco enzyme)
- Low carbon dioxide concentration, this limits the rate at which ATP AND NADP reused in light dependent reaction and the whole process limited, even though light intensity increases.

Effect of several factors on rate of photosynthesis



The region indicated on the graphs above where light is non-limiting (horizontal lines), but other factors that are limits are:

- **A**-Both temperature and carbon dioxide; increasing either produces an increase in the rate of photosynthesis to level
- **B**-Temperature or carbon dioxide concentration (the factor that hasn't been increased from A); increasing the temperature increases the rate to level C.

4. Wavelength of the light

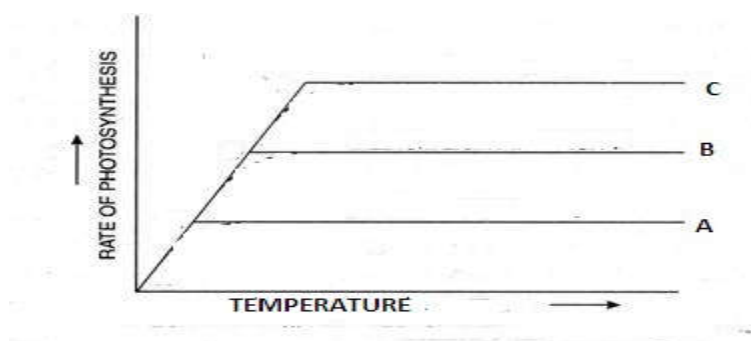
- Rate of photosynthesis is faster in 'red' and 'blue' wavelengths than else, because these wavelengths are absorbed more efficiently than others. Leaves appear green as they reflect green wavelengths.

5. Amount of chlorophyll

- Rate of photosynthesis increases with chlorophyll concentration.

Explain the role of principle of limiting factor in commercial plant grower?

5. The graph given below shows the effect of temperature on the rate of photosynthesis at different amount of light intensity and at different concentration of carbon dioxide. Question number and depend on this graph.



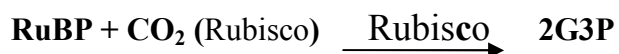
Line 'A' could indicate:

- A. High light intensity and low carbon dioxide concentration
- B. High light intensity and high carbon dioxide concentration
- C. Low light intensity and high carbon dioxide concentration
- D. Low light intensity and low carbon dioxide concentration**

Are there any other ways of photosynthesis?

C3 photosynthesis and photorespiration

- This type of photosynthesis takes place in plants growing in temperate environments, like Europe.
- They are vast majority of land plants (85%). E.g. Tomatoes, Potatoes, Spinach, Cassava, Rice, Barley .etc.
- Said **C3** because the first compound formed in LI reaction of Calvin cycle contains carbon three atoms (2G3P).



Leaves of C3 plants have the following major adaptation for photosynthesis:

- Have broad leaves to capture more light
- Palisade cell has chloroplast at upper surface of leaves to absorb more light.
- Their stomata are mainly at lower surface.
 - To minimize water loss (has waxy cuticle)
 - They open their stomata mainly at day time, to allow entry of CO₂ and close if water loss is great on hot day.
- Spongy mesophyll has air space.

That allows easy diffusion of CO₂ and O₂ between palisade and stomata.

Bundle sheath cell lacks chloroplast and CO₂ fixed ones

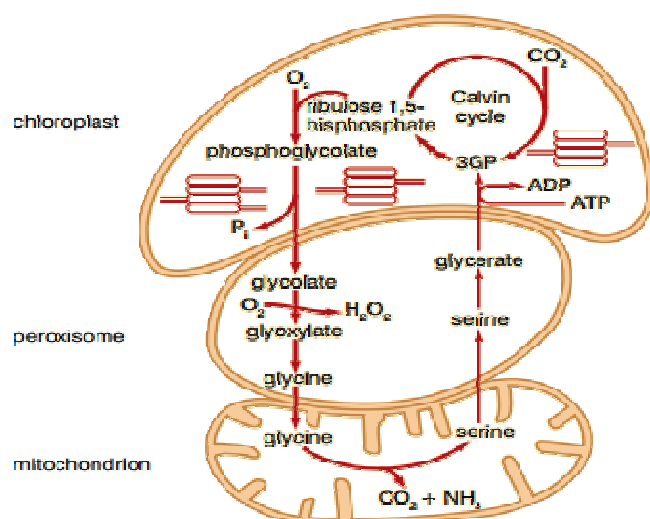
Photorespiration (C2 photosynthesis)

- It involves oxidation of carbon.
- Is catalyzed by Rubisco/oxygenase (oxygenation) instead of carboxylase.
 - In hot tropical conditions, C3 plants do not yield enough carbon dioxide for photosynthesis, this is because their stomata closed to prevent water loss that prevent entry of carbon dioxide. As a result, the concentration of carbon dioxide in the leaves falls.
- Rubisco behave in unusual way that RuBP binds with oxygen instead of carbon dioxide.
- Then RuBP is oxidized to one molecule of **GP** (not two) and one molecule of phosphoglycolate.



- Where 1GP formed in photorespiration re-enter the Calvin cycle and phosphoglycolate converted into GP for use in the Calvin cycle. These reactions (involves **chloroplast**, **peroxisomes** and **a mitochondrion**) catalyzed by complex net work of enzymes.
- In addition, carbon dioxide is produced in the process.

The reactions of photorespiration



Photorespiration reduces efficiency of photosynthesis by **25%** than C_3 plants .Because:

- Carbon is oxidized, which is the reverse of photosynthesis (reduction of carbon to carbohydrate).
 - Ribulose biphosphate must be resynthesised and the phosphoglycolate removed.
 - It also cost ATP and NADPH in the resynthesis of RuBP.
6. Which of the following statement is **not true** concerning C_3 plants?
- The first compound formed in the dark reaction contains three carbon atoms.
 - The stomata are mainly on the lower surface to minimize water loss.
 - The cell of bundle sheath contains many chloroplasts**
 - The palisade cells are nearest the upper surface of the leaf
7. Which of the following processes mainly reduce efficiency of photosynthesis?
- Photophosphorylation
 - Fixation of CO_2
 - Chemiosmosis
 - photorespiration**

C4 photosynthesis

- This pathway gets round the problem of photorespiration that reduces efficiency of photosynthesis.
- Takes place in plants that grow in tropical and sub tropical enviroment like Ethiopia. Examples of C_4 plants include; maize, crabgrass, sorghum and sugarcane.
- Named C_4 , because the first compound formed in the light independent reactions of photosynthesis contain carbon four (oxaloacetate), not GP that of C_3 plants.

- The light-dependent reactions are the same as in the C3 plants, but differ in how glucose is synthesized in the light-independent reactions.
- The structure of the leaf of C4 is essentially similar to that of a C3 plant, but differ in that:
 - ✚ Cells of the bundle sheath contain chloroplasts that C3 plants lacks.
 - ✚ No thylakoid means that the light-dependent reactions cannot occur here and oxygen is not produced in these chloroplasts.
 - ✚ This helps to prevent photorespiration and allows Calvin cycle to take place.
- The light-dependent reactions in C4 pathway take place in the mesophyll cells, which have chloroplasts with thylakoid.

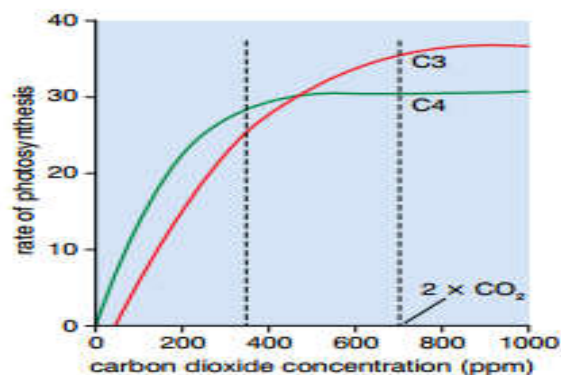
The following reactions take place:

1. CO₂ reacts with a C3 compound called PEP (Phosphoenol pyruvate) to form the C4 compound **oxaloacetate** in mesophyll cell.
 - Catalyzed by the enzyme PEP carboxylase (**Pepco**).
$$\text{PEP} + \text{CO}_2 \xrightarrow{\text{PEP carboxylase}} \text{Oxaloacetate}$$
 2. Oxaloacetate is converted into another C4 compound (**malate** in bundle sheath cell).

$$\text{Oxaloacetate} \longrightarrow \text{Malate}$$
 3. In the bundle sheath cell, malate is converted to pyruvate with the release of a molecule of CO₂ that starts Calvin cycle by binding with RuBP.

$$\text{Malate} \xrightarrow{\text{CO}_2} \text{Pyruvate}$$
 4. The pyruvate is back converted to PEP that enters Calvin cycle to synthesis sugar; this reaction requires ATP.
- C4 cycle uses two more molecules of ATP to deliver a molecule of CO₂ to Rubisco than does the C3 cycle.
 - This is not a problem in tropic, as the high light intensity that generates much ATP from the light dependent reactions.
 - C4 photosynthesis is most efficient under the following conditions:
 - Low carbon dioxide concentration
 - High light intensity
 - High temperature.
 - Plants grow in Ethiopia are C4plants and well adapted to hot and bright days. So, produce high yield when compared to C3 plants.
 - Why C4 plants experience low carbon dioxide concentration?
 - This is not due to composition air in tropics is different from other regions.
 - But, C4 plants (grass, maize ...etc) grow very close together and compete for CO₂ in the air reduces

This graph shows efficiency of C₃ and C₄ photosynthesis at different [CO₂]



8. Which of the following statement is **NOT true** about C₄ plants such as maize?
- A. Light dependent reactions occur in mesophyll cell.
 - B. The bundle sheath cell contains chloroplast.
 - C. Chloroplast of bundle sheath cell lack thylakoid.
 - D. They harvest CO₂ at night time.**

Comparison of C₃ and C₄ plants

Features	C ₃ Plants	C ₄ Plants
Bundle sheath cells	Lack chloroplasts	Have chloroplasts with no thylakoid
Enzyme used to fix CO ₂	Rubisco	Pepco (PEP carboxylase)
Optimum temperature	15–25 oC	30–40 oC
optimum CO ₂ concentration	700 ppm	400 ppm
Fixation of CO ₂	Mesophyll cells	Mesophyll cells
Calvin cycle	Mesophyll cells	Bundle sheath cells

Crassulacean acid metabolism (CAM photosynthesis)

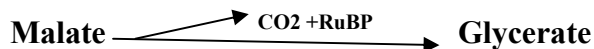
- This carbon fixation pathway experienced in s plants adapted to desert conditions.

Example: Succulent, xerophytes cacti. Agaphe...etc

- In this area their stomata closed during day time when temperature is high to reduce rate of evapotranspiration. So, if they don't open their stomata how they can obtain CO₂ needed for photosynthesis? They have temporal adaptation.
 - By opening stomata at night for carbon fixation the mesophyll cells.
 - They experiences the same set of reactions with C₄ plants, but differ in carrying reactions at times (seasonal isolation).
1. At night plants opens their stomata to allow carbon fixation that reacts with PEP in mesophyll cell forming oxaloacetate and malate as C₄plants.



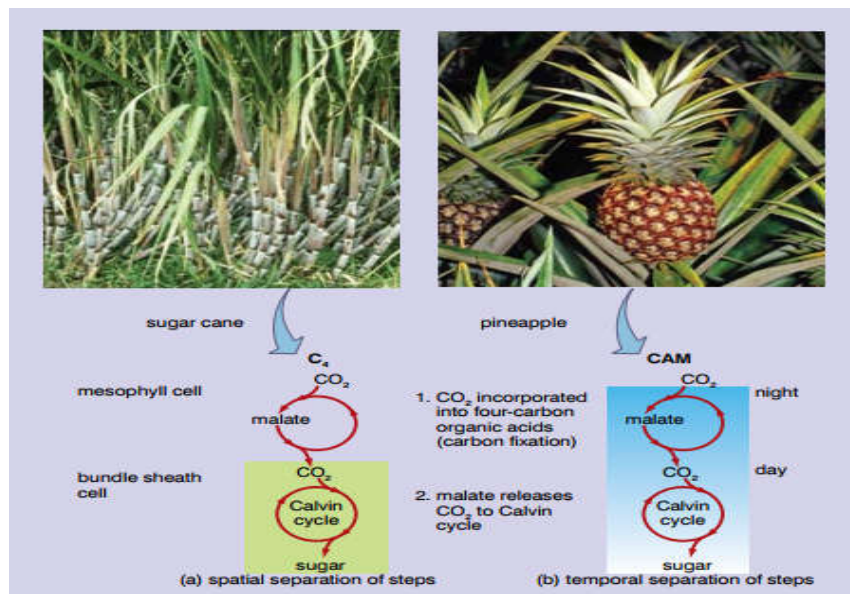
2. Malate stored in vacuole overnight.
3. During day, light dependent reaction generates ATP and NADP, so that Calvin cycle continues.
4. Malate released from vacuole and breakdown into glycerate, releasing CO₂ for Calvin cycle to produce sugar.



9. Which of the following is characteristic of Crassulacean Acid metabolism (CAM) plants?
 - A. Both stage of photosynthesis carried out them out at temporal isolation.
 - B. They are mostly adapted in temperate area than C₃ plants.
 - C. They have no secondary fixation of carbon dioxide.
 - D. They are more efficient than C-4 plants in the condition of low CO₂ concentration

Compares the C₄ pathway and the CAM pathway.

What is the evolutionary advantage of CAM plants in desert environment?



Answer the following multiple choice questions. Don't visit key before you try it on your own. Refer relative sources and contact your teacher where necessary.

- From the following identify the **wrong** statement about PsII and PsI?
 - Each photosystem linked with different ETC
 - PsI linked with 'fd' to transfer excited electron to NADP
 - PsII involved in both cyclical and non-cyclical phosphorylation
 - Each photosystem have reaction center and antenna complex
- Which one of the following pairs of plants is **NOT** photosynthesis by C₄ and CAM pathway respectively?
 - Sugar cane and pineapple
 - Sorghum and Cacti
 - Maize and Cacti
 - cacti and sorghum
- All of the following are generally true about photorespiration **EXCEPT** one?
 - It involves RuBP binding with oxygen
 - It lower photosynthetic efficiency
 - It is reductive process that consume ATP and NADP
 - It is a condition in low [oxygen] and common in C₃ plants
- Which one of the following pairs of photosynthetic pigment has maximum absorptive spectrum at which rate of photosynthesis is effective than else?
 - Violet and Blue
 - Blue and Red
 - Green and Red
 - Yellow and Red
- Which one of the following is **NOT** generally true about Non-cyclical phosphorylation?
 - Chlorophyll molecule is initial and final donor and acceptor of a chain
 - It involves one way electron flow from water to NADP
 - It involves both PSII and PSI
 - It ends up with generation of ATP, NADP and O₂

6. Which one of the following pairs of factors directly influences light dependent and light independent reaction respectively and indirectly influences each other viseversaly?
 - A. Light intensity and Carbon dioxide concentration
 - B. Temperature and chlorophyll molecule
 - C. Carbon dioxide concentration and light intensity
 - D. Temperature and light intensity
7. Which one of the following pigment in a photosystem is referred as a reaction center where light dependent reaction starts?
 - A. Xanthophylls
 - B. Chlorophyll b
 - C. Chlorophyll a
 - D. Carotenoids
8. Which one of the following is **NOT** true about CAM pathway?
 - A. Is adapted to desert (arid) condition with high temperature and low moisture
 - B. They open and close their stomata at different circadian rhythm.
 - C. They have needle leaved and long rooted plant with thick bark
 - D. They has no role in sugar production
9. Among the following reaction in C₃,C₄ and CAM Pathway , one similarly takes place in mesophyll cell:
 - A. Fixation of carbon dioxide
 - B. Calvin cycle
 - C. Generation of ATP and NADP
 - D. Sugar production
10. In light dependent stage of photosynthesis light is essential for:
 - A. Fixation of carbodioxide from the atmosphere
 - B. Synthesis of ATP and proton carrying coenzyme
 - C. Regeneration to ribulose biphosphate
 - D. Combination of carbodioxide and water
11. Which one of the following reactions commonly occurs in both Cyclical and Non-cyclical phosphorylation of photosynthesis in thylakoid membrane of chloroplast?
 - A. Production of reduced NADP
 - B. Generation of ATP
 - C. Generation of O₂ and water photolysis
 - D. Involvement of both PsII and PsI
12. In what way chloroplast in bundle sheath cell of C₄ plants adapted for the pathway?
 - A. Absence of Calvin cycle
 - B. Presence of thylakoid membrane
 - C. Absence of thylakoid membrane
 - D. Dependence on high oxygen concentration
13. Which one of the following organelles involved in photorespiration to convert phosphoglycolate into glycolate to be re-used in Calvin cycle respectively?
 - A. Chloroplast, Mitochondria and Perioxosome
 - B. Ribosome, Nucleus and Mitochondria
 - C. Chloroplast, Perioxosome and Mitochondria
 - D. Nucleus, Ribosome and Golgi body
14. What is the main advantage that a photosystem containing molecules of different types light sensitive pigments have?
 - A. To increase amount/number of photosystem.
 - B. To absorb light of the different wave length
 - C. To increase amount of ATP in cell
 - D. To Increase number of chlorophyll molecule in the cell

15. Which of the following is true regarding cyclic photophosphorylation?
- A. Only photosystem II involved
 - B. The flow of electron is from PSI to PSI
 - C. It produces oxygen & red. NADP
 - D. There is ATP production
16. Under what conditions do C4 plants have more photosynthetic efficiency than C3 plants?
- A. Low temperature
 - B. Low CO₂ concentration
 - C. low light intensity
 - D. high CO₂ concentration
17. Which of the following reaction represents photo respiration?
- A. $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$
 - B. $\text{CO}_2 + \text{RUBP} \rightarrow 2\text{G3P}$
 - C. $\text{O}_2 + \text{RUBP} \rightarrow 1\text{G3P} + \text{phosphoglycolate}$
 - D. $\text{CO}_2 + \text{phosphoenolpyruvate} \rightarrow \text{Oxaloacetic acid}$
18. Which one of the following molecules initially combines with CO₂ in stroma of chloroplast?
- A. Glycerate phosphate
 - B. Oxalo acetate
 - C. Phosphoenol pyruvate
 - D. Ribulose biphosphate
19. Which of the following plants carryout light dependent and primary CO₂fixation in separate time?
- A. C-3 plants
 - B. C-4 plants
 - C. CAM plants
 - D. Both CAM and C4 plants
20. Which of the following is the correct sequence of the reaction in Calvin cycle of photosynthesis?
- A. Regeneration of RuBP, reduction reaction and CO₂ fixation.
 - B. Regeneration of RuBP, CO₂fixation and reduction reaction.
 - C. CO₂fixation, regeneration of RuBP and reduction reaction.
 - D. CO₂fixation, reduction reaction and regeneration of RuBP

Answer Key

5.1.Respiration		5.2. Photosynthesis		
1. C	11. B	1. D	11.B	21. A
2. D	12.C	2. B	12.B	22. B
3. C	13. C	3. C	13.D	
4. B	14. B	4.D	14.C	
5. A	15. D	5.C	15.D	
6. A	16. B	6.C	16.B	
7. C	17.C	7.C	17.C	
8. D	18.D	8.D	18.D	
9. A	19.B	9.D	19.D	
10. B	20.D	10.D	20.A	