1. SPECTROPHOTOMETRY

The main principle of spectrophotometry is measuring the amount of the substance based on its absorption of light in a certain wavelength range. The Beer-Lambert law shows a linear relationship between absorbance and the concentration of the substance. According to the law, the absorbance of the solution is directly proportional to the concentration of the substance in the solution and the path length. Therefore, absorbance (or Optical Density, OD) can be used to determine the concentration of a solute. The Beer-Lambert law is expressed mathematically as follows:

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \epsilon \cdot c \cdot l$$

here:

A – absorbance
I_0 –incident light (intensity of light before sample).
I –transmitted light (intensity of light after sample).
ϵ – molar extinction coefficient, mostly in $L \cdot mol^{-1} \cdot cm^{-1}$.
c – the concentration of a substance in solution, mostly marked with mol/L.
l – path length, mostly in cm.

The research was conducted to study apoptosis of rat liver cells. An important step in the research is to determine the concentration of *cytochrome c* protein released from mitochondria during the early stages of apoptosis. Due to the heme group in *cytochrome c* protein, the optical density of this protein can be determined spectrophotometrically at a wavelength of 550 nm. During the research, mitochondria were isolated from hepatocytes, and *cytochrome c* protein was placed in a buffer solution. To determine the concentration of this protein, several calibration data were obtained by creating different concentrations of *cytochrome c* protein of known concentration and measuring them in a spectrophotometer. The information is given below:

Concentration, ***mM***	Optical Density (OD)
2	0.100
4	0.199
6	0.301
8	0.399
10	0.500

A cuvette with a light path length of 1 cm was used to measure the optical density of proteins in the spectrophotometer. The optical density of the extracted *cytochrome c* protein in the sample was **0.380**.

Q1. Find ***(ϵ)*** the molar extinction coefficient **($L \cdot mol^{-1} \cdot cm^{-1}$)** for *cytochrome c* protein.

Q2. Find the concentration ***(mM)*** of extracted *cytochrome c* protein during the research.

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a π-radical. It is frequently used to determine the radical scavenging activity of phenols, vitamins, flavonoids, as well as the antioxidant properties of food, beverages, or plant extracts. It is also soluble in ethanol or methanol. The antioxidant donates electrons to neutralize DPPH. Activation is determined by measuring the DPPH color change at 517 nm. Loss of color indicates antioxidant activity (change from purple to light yellow) (Figure 1). The **EC50** value is calculated which indicates a 50% reduction of the initial concentration.

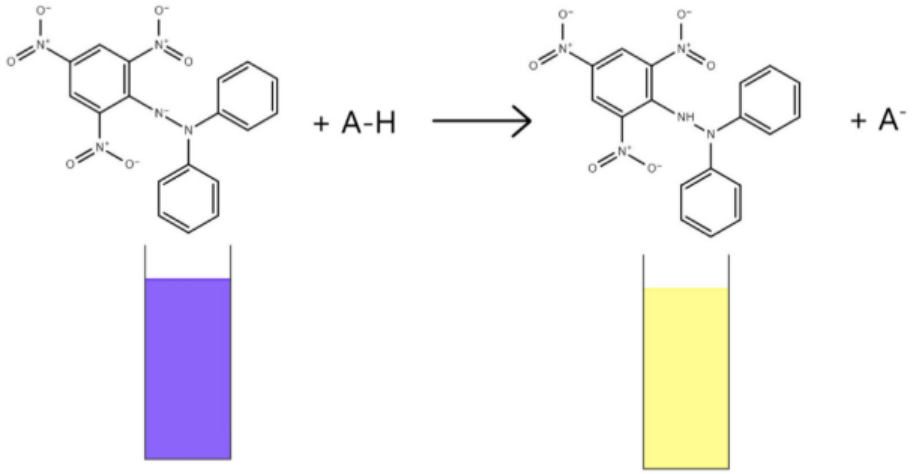


Figure 1. Color change as a result of the reducing activity of the DPPH solution.

The antioxidant activity is determined in proportion to the decrease in the absorbance of DPPH˙ at 517 nm. Using the decrease in absorbance, the percentage of the initial DPPH is scavenged can be calculated. As a negative control, the solvent of the sample is used, where no antioxidant activity is expected. A typical DPPH radical scavenging activity experiment design using a microplate is given in Figure 2. After the color change is determined as the absorbance value by the spectrophotometric method, the percent sweep is calculated for each concentration by comparing the absorbance value of the extract (A_{sample}) with the absorbance value of the blank (A_{blank}) with the formula below:

$$\text{DPPH scavenging effect}(\%) = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100$$

Negative control is used as a blank. A graph is created after calculating the percent inhibition value for each concentration used. Using the equation obtained, the concentration (EC50) at which the tested substance scavenges 50% of the DPPH radical will be calculated.

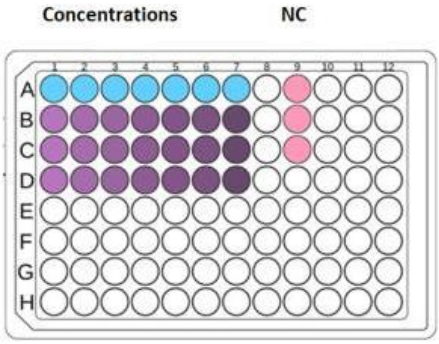


Figure 2. Free radical scavenging activity experimental plan. Only solvent and DPPH should be added to the negative control (NC).

In Uzbekistan culture, mistletoe (*Viscum album*) is the plant hung in all houses (Figure 3). *Viscum album* ssp. *album* (Loranthaceae) growing on various plants, has been used for the treatment of many diseases. To test whether mistletoe has antioxidant activity, plant leaves were collected and dried. It was kept in methanol for 2 hours. To determine the DPPH free radical scavenging activity, intermediate stocks were prepared at the concentrations specified below.



Figure 3. Mistletoe.

For plant extract, 1mg, 500 µg, 250 µg, 125 µg and 62.5 µg intermediate stocks were prepared. 100 µL of each intermediate stock solution was transferred to the microplate. 100 µL of DPPH solution was added to each well. It was incubated in the dark for 30 minutes and absorbance was measured at a wavelength of 517 nm on a Spectrophotometer. Three parallel results are given in Table 1. The average measured absorbance value for the negative control is 0.58.

Table 1. Absorbance of plant extract for DPPH activity

Amount	**Absorbance value**		
	Plant extract		
	1	2	3
1 mg	0.146	0.167	0.193
500 µg	0.352	0.366	0.358
250 µg	0.416	0.429	0.436
125 µg	0.481	0.496	0.487
62.5 µg	0.542	0.539	0.545

Q3. Fill in the table below. Round your answers to the 2nd place after the decimal point.

Amount	Average of Absorbance	% RS
1 mg		
500 µg		
250 µg		
125 µg		
62.5 µg		

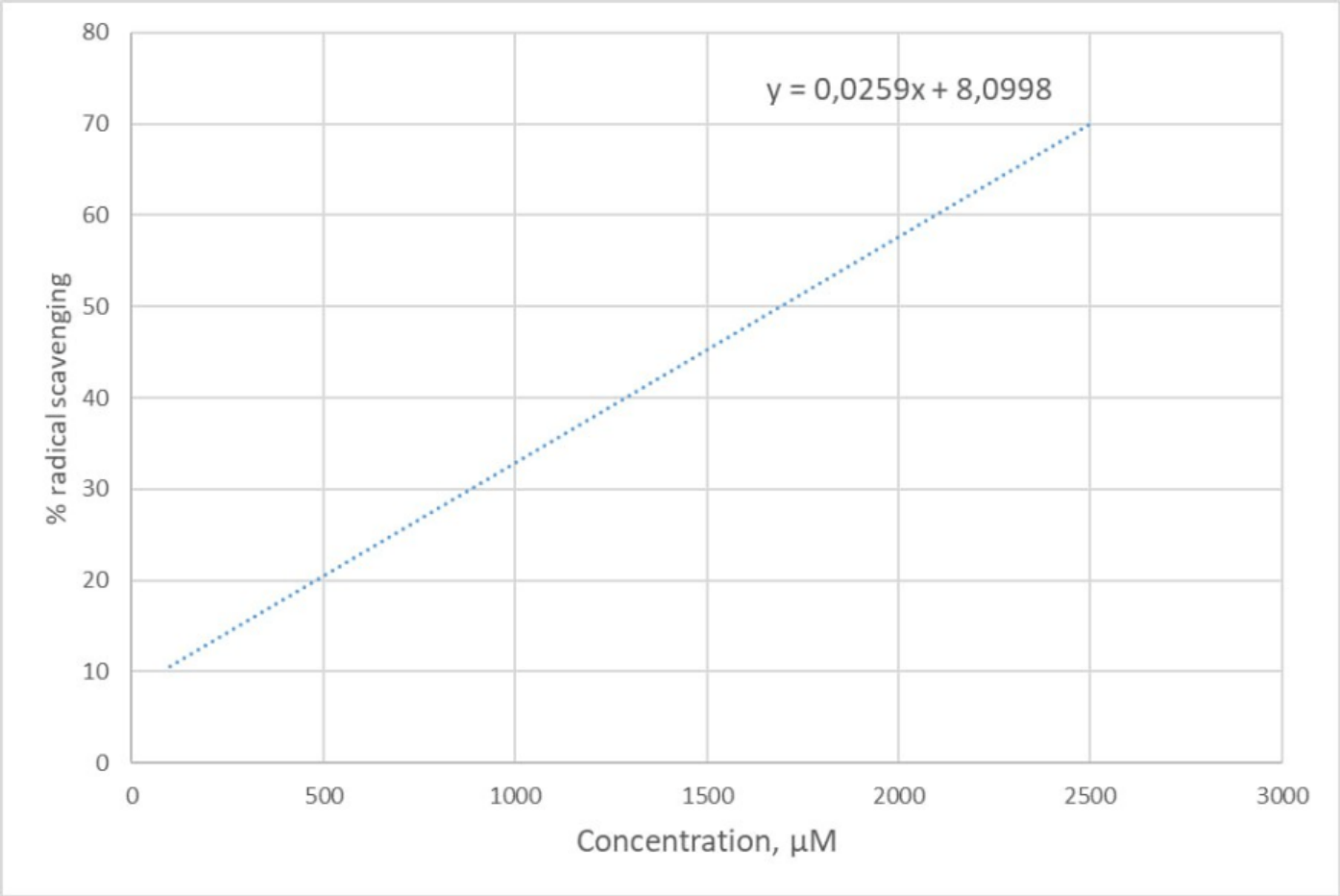


Figure 4.

The % radical scavenging graph against concentration for the plant extract was drawn (Figure 4). The slope was given.

Q4. Calculate the EC50 value for the extract. Show all your calculations clearly. Give your answers as a whole number in µM.

2. CHROMATOGRAPHY

Thin layer chromatography (TLC) is an easy-to-perform type of chromatography that allows separation of molecules in the mixture. In this chromatography method, two phases are used: a mobile phase (solvent) and a stationary phase (paper sheet) as depicted on Figure 1.

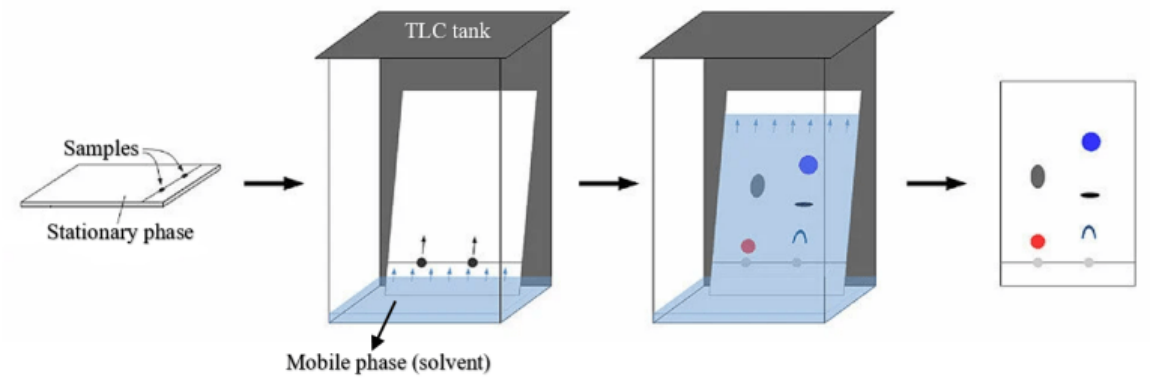


Figure 1: Thin layer chromatography experiment: samples are transferred to a thin layer of stationary phase. The stationary phase is placed in a tank with a mobile phase (solvent) at the bottom. As the solvent (mobile phase) moves up through the stationary phase (paper) by capillary effect, it carries the molecules in the samples along with it. Because the molecules move at different speeds, based on different affinity to the mobile phase, they are separated from each other. When the solvent reaches the top of the paper, the paper is removed from the container and the result is checked.

A chromatography experiment was conducted to check pigments in plant leaves, using hexane as a mobile phase and silica gel as a stationary phase. The place where the samples are loaded (origin), the starting line, is drawn on the bottom of the paper covered with silica gel. The liquid obtained after crushing the leaves was mixed with a little solvent and loaded to the designated line using a pipette. The paper was then placed in the tank and after some time, when the solvent reached the top of the paper, the paper was removed from the tank and the finish line was drawn (Figure 2).

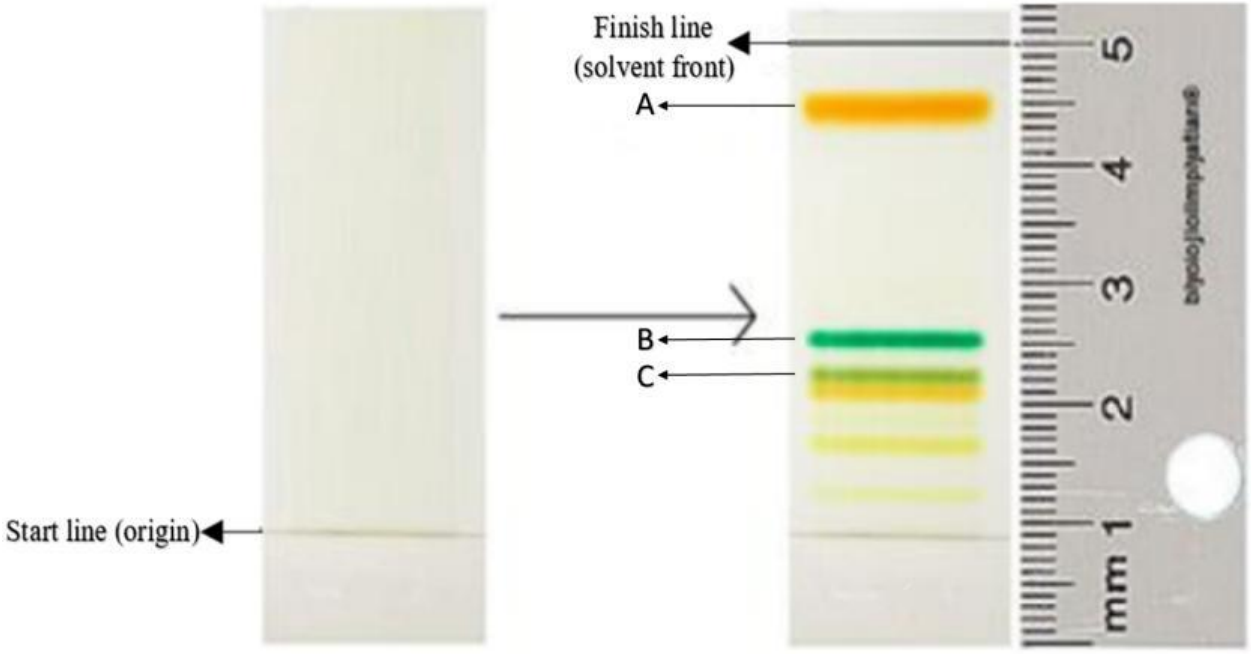


Figure 2: You can see TLC paper before chromatography (left) and after chromatography (right).

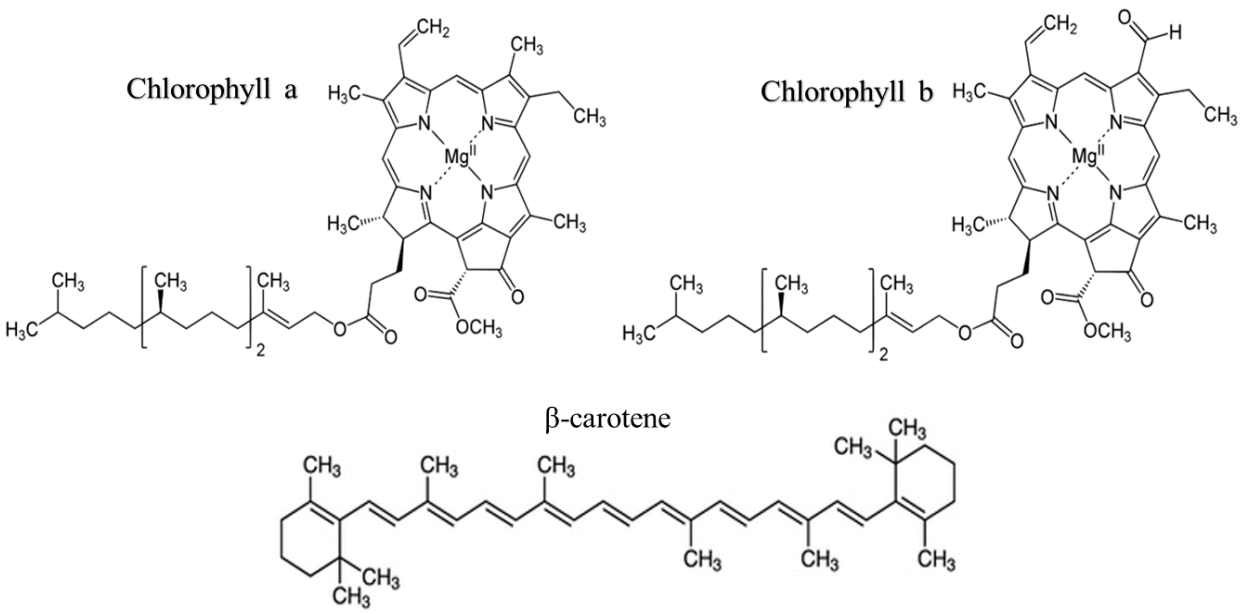


Figure 3: Chemical structure of some pigments in plant leaves.

The Rf value is used to numerically express the difference between the pigments traveling in the stationary phase. The Rf value is the ratio of the distance traveled by the pigment (the distance between the start line and the end point it reaches) to the distance traveled by the solvent (the distance between the start and finish lines).

Q1. Match the pigments A, B, and C in Figure 2 with chlorophyll a, chlorophyll b, and β-carotene.

	A	B	C
Chlorophyll a			
Chlorophyll b			
β-carotene			

Q2. Find the Rf value for chlorophyll a.

Q3. Find the Rf value for chlorophyll b.

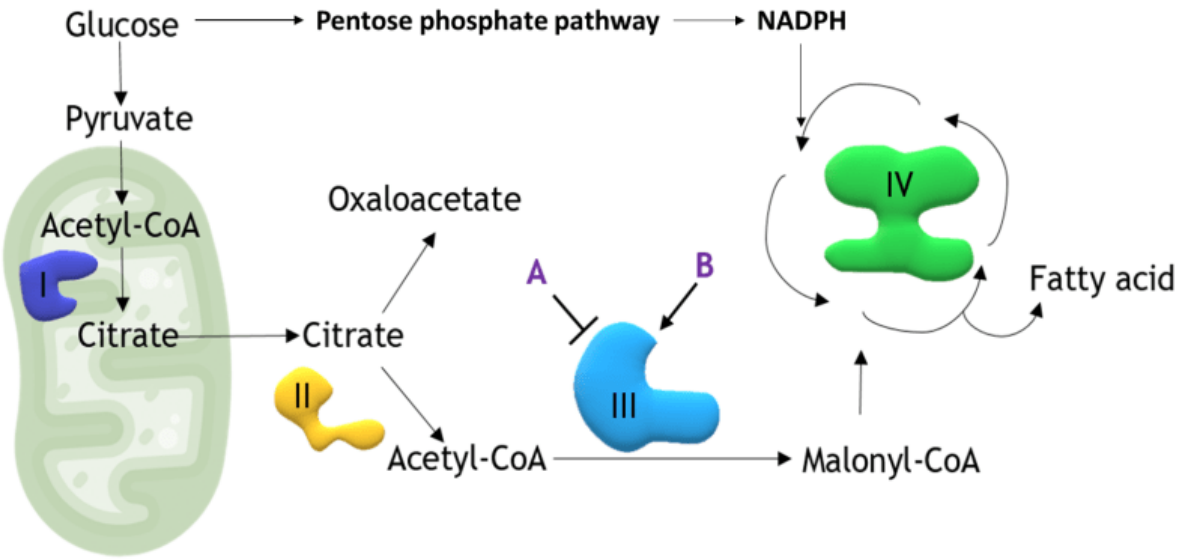
Q4. Find the Rf value for β-carotene.

Q5. Indicate if each of the following statements is true or false.

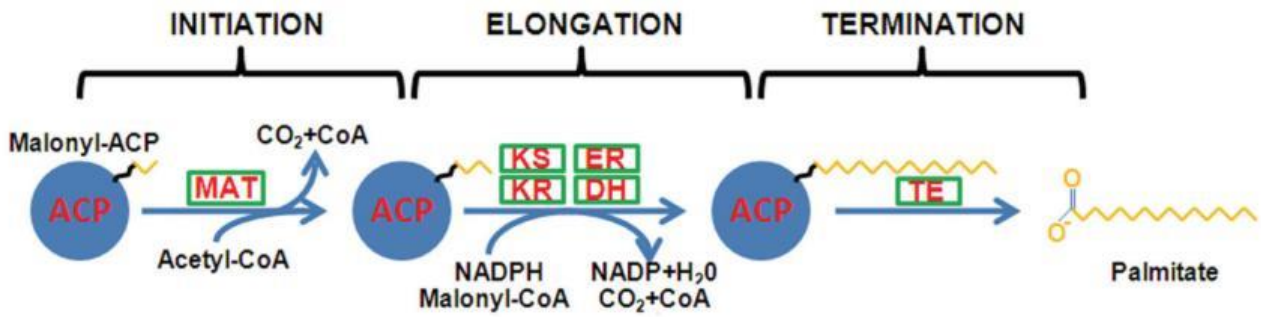
		True	False
A.	Chlorophyll a and chlorophyll b can be distinguished on a TLC plate not only by their Rf values but also by their color in UV light.		
B.	Chlorophyll b fluoresces at a different wavelength than chlorophyll a because it has an aldehyde group instead of a methyl group.		
C.	With TLC chlorophyll a, chlorophyll b and β -carotene pigments can be used for separation due to their polarity differences. β-carotene is a more non-polar pigment than chlorophyll pigments, so this pigment is attracted to the polar stationary phase and cannot go far on the TLC plate in a nonpolar solvent and has a low Rf value.		
D.	In this experiment, the silica gel used as the stationary phase is polar, and the hexane used as the mobile phase is nonpolar.		
E.	In this experiment, the β-carotene pigment has a smaller molecular mass than the chlorophyll a and chlorophyll b pigments, so the β-carotene pigment moves faster through the stationary phase than the chlorophyll pigments.		

3. METABOLISM OF FATTY ACIDS

Triacylglycerides are one of the main sources of energy in the body. In the human body, this substance is stored as a reserve in adipose (fat) tissue and is used as needed. These molecules can be obtained by the body through endogenous or exogenous ways. The mechanism of occurrence of one of these two ways is given below.



The following figure shows the formation of saturated fatty acid (palmitate) according to the same mechanism:



Enzyme III given above can be allosterically regulated using biologically active substances marked with the letter A or B.

Q1. Identify three of the given options that correspond to substance A.

Q2. Identify two of the given options that correspond to substance B.

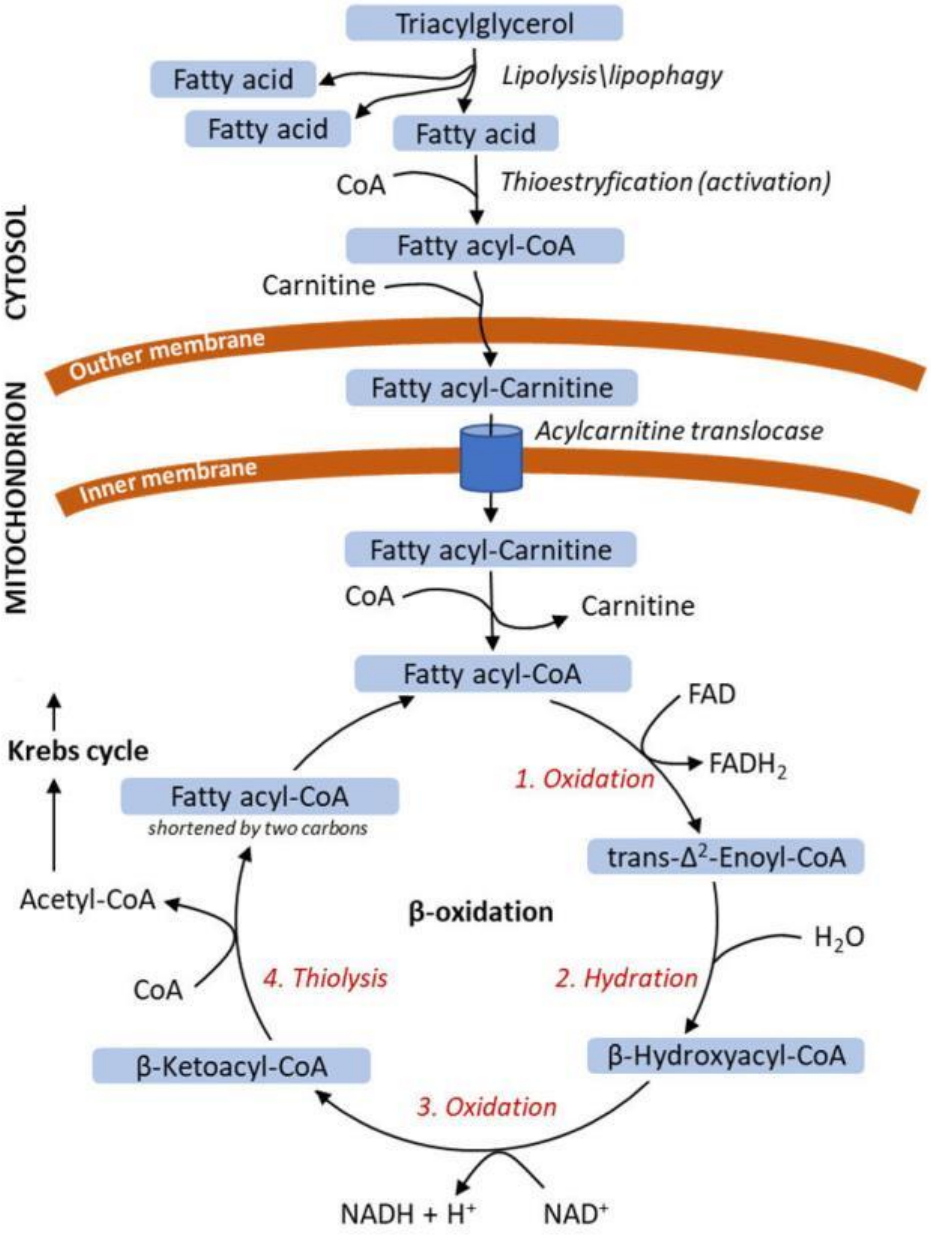
1. Epinephrine (Adrenaline)
2. Norepinephrine (Noradrenaline)
3. Insulin
4. Glucagon
5. Citrate
6. Adenosine diphosphate
7. Cortisol

Q3. Indicate if each of the following statements is true or false.

		True	False
A.	The release of citrate from the matrix to the cytosol is carried out with the help of proton pumps.		
B.	Reduction of the pentose phosphate pathway in hepatocytes also reduces the intensity of fat synthesis in the cytosol.		
C.	These processes occur rapidly in an organism suffering from diabetes type I (Diabetus milletus).		
D.	For the synthesis of fatty acids containing 16 C atoms, the cycle of fatty acid synthesis is repeated 8 times.		
E.	If the concentration of citrate increases in the matrix, it is transported to the cytosol, affects the enzyme acetyl-CoA carboxylase, and increases its velocity.		

Georg Franz Knoop discovered β-oxidation of fatty acids. In 1904, he conducted experiments using odd- and even-chain ω-phenyl fatty acids such as ω-phenylvaleric acid and ω-phenylbutyric acid. Knoop gave these compounds to dogs and analyzed their urine. He found hippuric acid (a combination of benzoic acid and glycine) in the urine of dogs fed odd-chain fatty acids, whereas phenaceturic acid (a combination of phenylacetic acid and glycine) was present in the urine of dogs fed even-chain fatty acids. From this he concluded that the metabolism of fatty acids proceeds by successive removal of two carbon fragments. The remaining fatty acid chain had to contain a carboxylic acid group. He hypothesized that the oxidation occurs at the β-carbon atom, which was unknown to organic chemistry yet. The scheme of β-oxidation for saturated fatty acids with an even number of C atoms, which occurs in a human cell, is described below.

	NADH	**$FADH_2$**
The amount of ATP on ETC (electron transport chain)	2.5-3	1.5-2



Q4. Complete the table below based on the beta-oxidation of myristic acid ($C_{14}H_{28}O_2$). Calculate considering the least efficiency of ETC given.

1.	Find the number of β-oxidation cycles.	
2.	Find the total amount of ATP produced in the mitochondria from NADH (assume that all acetyl-CoA produced by beta-oxidation enters the Krebs cycle).	
3.	Find the total amount of ATP produced from $FADH_2$ (assume that all acetyl-CoA from beta oxidation enters the Krebs cycle as well).	
4.	Find the amount of total acetyl-CoA formed.	

Q5. Indicate if each of the following statements is true or false.

		True	False
A.	In each cycle of β-oxidation, 1 molecule of ATP is used for fatty acid activation.		
B.	The β-oxidation process occurs rapidly in muscle tissue with well-developed fast glycolytic (type 2X) muscle fibers.		
C.	Because fat molecules are nonpolar molecules, they enter into mitochondria without any additional molecules.		
D.	Increasing the number of stored ATP molecules is a main factor of inhibition of enzymes in the β-oxidation process.		

4. ENZYME KINETICS

Enzymes are biological catalysts that speed up biochemical reactions by reducing the activation energy required for the reaction to occur.

The Michaelis-Menten equation is the basic equation of enzyme kinetics and indicates the rate of enzymatic reactions. The equation relates the reaction rate *(V)* to the substrate concentration *([S])* and the kinetic parameters of the enzyme:

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

here:

- ***V*** - the rate of the enzymatic reaction (initial velocity).
- V_{max} - the maximum rate of the reaction when the enzyme is saturated with the substrate (maximum velocity).
- K_m - the Michaelis-Menten constant and represents the substrate concentration at the reaction rate is half V_{max}.
- ***[S]*** – the concentration of substrate.

Lineweaver-Burk equation:

The Lineweaver-Burke diagram is a graphical representation of the Michaelis-Menten equation. This equation involves taking the reciprocal of both sides of the Michaelis-Menten equation, resulting in the following linear equation:

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}}$$

The Lineweaver-Burk plot is a double reciprocal diagram, with the x-axis representing **1/[S]** and the y-axis representing **1/*V***. The slope of the line is K_m/V_{max}, the intercept on the **Y** axis is equal to $1/V_{max}$, the intercept on the **X** axis is equal to $-1/K_m$. The advantage of the Lineweaver-Burk plot is that it linearizes the data, making it easy to determine V_{max} and K_m by visual inspection or linear regression analysis.

Q1. Inhibitors are substances that reduce enzyme activity. They can bind to enzymes and switch their activity to a passive state in various ways, which affects the rate of conversion of the substrate to the product. According to the mechanism of inhibition, inhibitors are divided into the following 4 groups.

A competitive inhibitor is similar to a substrate and competes with the substrate to bind to the active site of the enzyme. A competitive inhibitor does not affect V_{max}, but increases K_m.

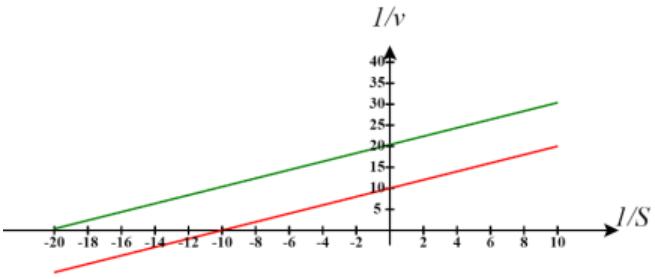
A noncompetitive inhibitor binds to an allosteric center in an enzyme or enzyme-substrate complex. A non-competitive inhibitor reduces V_{max}, but does not affect the enzyme's substrate affinity and leaves K_m unchanged.

An uncompetitive inhibitor binds only to the allosteric center of the enzyme to which the substrate is bound. A noncompetitive inhibitor reduces both V_{max} and K_m.

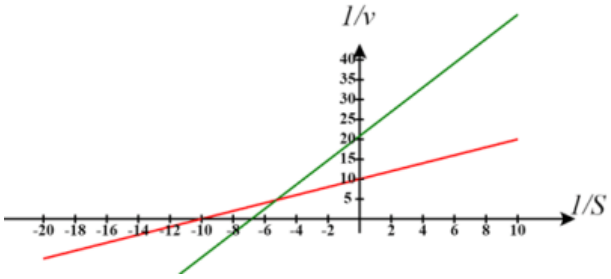
A mixed inhibitor can bind to a substrate complex with an enzyme or to a free enzyme, a mixed inhibitor does not bind to the active site. Their effect on enzyme kinetics is a combination of competitive and non-competitive inhibition, which reduces V_{max}, and the effect on K_m depends on the high affinity of the inhibitor to the enzyme or enzyme-substrate complex.

The activity of different enzymes without an inhibitor (red line) and with an inhibitor (green line) in the Lineweaver-Burk plot is shown below.

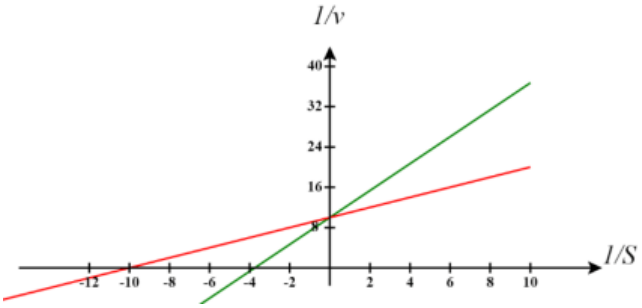
Graph 1:



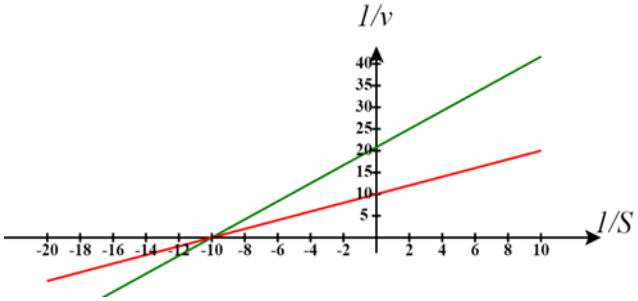
Graph 2:



Graph 3:



Graph 4:



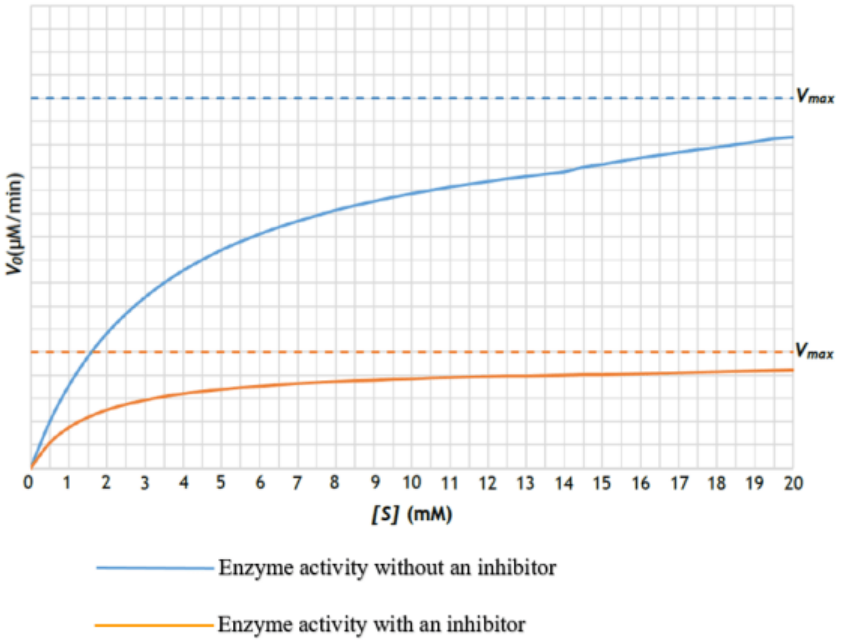
Based on the Lineweaver-Burk graphs, identify the type of inhibitor in each graph and complete the table below.

	Graph 1	Graph 2	Graph 3	Graph 4
Competitive inhibitor				
Noncompetitive inhibitor				
Uncompetitive inhibitor				
Mixed inhibitor				

Pyruvate kinase catalyzes the last step of glycolysis. This reaction is important in cell metabolism because the reaction ensures the formation of ATP. The empirical equation for the reaction catalyzed by pyruvate kinase is given below:

$$\text{Phosphoenolpyruvate (PEP)} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$$

The substrate for this enzyme is phosphoenolpyruvate; alanine inhibits this enzyme preventing excessive synthesis of ATP. The graph below shows the result of the experimental study of the enzyme activity in absence and presence of the inhibitor.



Q2. Find the value of K_m without an inhibitor.

Q3. Find the value of K_m (K_{app}) with an inhibitor.

Q4. If 0.75 mM inhibitor was used in the experiment above, calculate the K_i (inhibitor constant) value of the alanine inhibitor. Use the following formula:

$$K_{\text{app}} = \frac{K_m}{1+\frac{[I]}{K_i}}$$

here:

- K_{app} – K_m value with inhibitor
- K_m – K_m value without inhibitor
- $[I]$ – concentration of inhibitor
- K_i – inhibitor constant

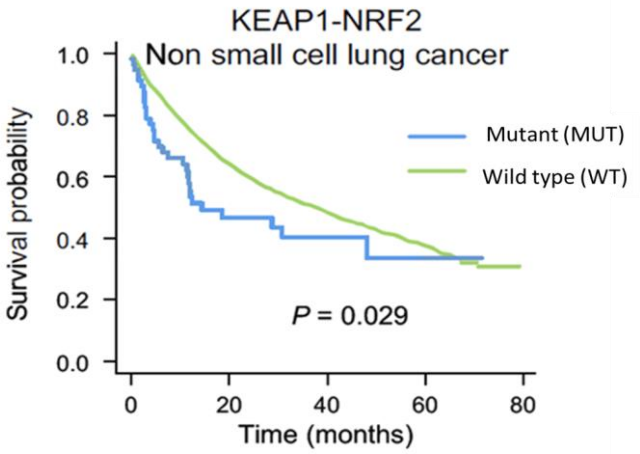
Q5. Identify the type of inhibiton.

A. Competitive inhibitor
B. Uncompetitive inhibitor
C. Noncompetitive inhibitor
D. Mixed inhibitor

5. KEAP1-NRF2 MUTATIONAL ANALYSIS

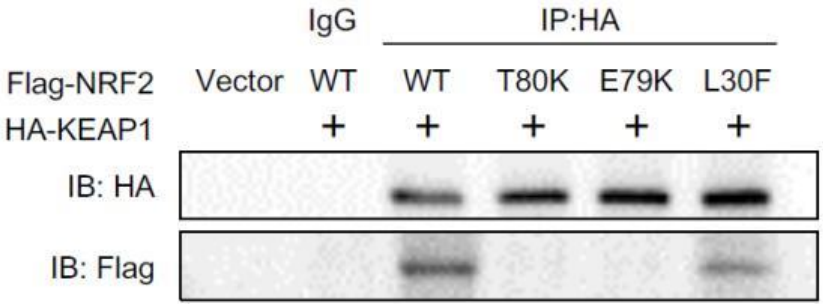
E3 ubiquitin ligases (E3s) are involved in cellular transformation and tumorigenesis by targeted protein degradation. Somatic mutations that alter interactions of E3s can be novel targets for the development of targeted protein degradation therapies.

KEAP1 is an adapter of E3 ligase that senses oxidative stress, and it mediates the degradation of NRF2, which is a key transcription factor in multiple cancer types. The figure below shows survival rates of patients with non-small cell lung cancer harboring mutations disrupting KEAP1 – NRF2 interaction.



KEAP1 recognizes NRF2 structurally through its conserved ETGE (aa 79-82) and DLG (aa 29-31) motifs. The molecular interaction of KEAP1 and NRF2 was determined through a co-immunoprecipitation assay in HEK293T cells. Thr80Lys and Glu79Lys mutations on the NRF2 ETGE motif and the Leu30Phe mutation on the NRF2 DLG motif were studied.
HEK293T cells were co-transfected with HA-tagged wild-type KEAP1 expressing vectors and either Flag-tagged wild-type NRF2, Thr80Lys, Glu79Lys, or Leu30Phe expressing vectors for 48 hours.

Cells were lysed with a lysis buffer on ice, and supernatants were incubated with anti-HA antibodies coupled with protein A/G beads overnight. Immunoprecipitated complexes were washed and were then eluted and subjected to Western blotting. The results are presented in the figure below.



Q1. Indicate if each of the following statements is true or false.

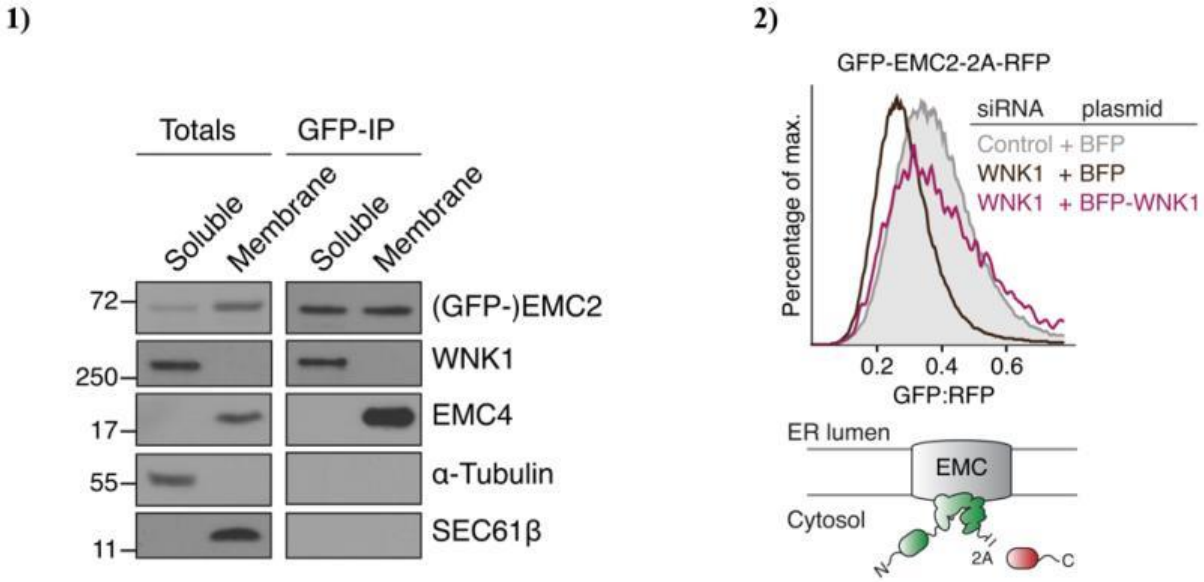
		True	False
A.	Mutations in the NRF2 ETGE motif partially sustain the binding of NRF2 to KEAP1.		
B.	Leu30Phe mutation in the NRF2 DLG motif disrupts the binding of NRF2 to KEAP1 completely.		
C.	Mutation of Thr80Lys protects NRF2 from ubiquitination and subsequent degradation.		
D.	The effect of Thr80Lys mutation would be antiproliferative in non-small cell lung cancer cells.		

6. ROLE OF WNK1 IN EMC COMPLEX STABILITY

The ER membrane protein complex (EMC) is a crucial ER-resident complex that is evolutionarily conserved and essential for the biogenesis of various membrane proteins. It plays a vital role in several cellular functions, such as inserting G protein-coupled receptors into membranes, integrating tail-anchored proteins for cholesterol regulation, and acting as a chaperone to stabilize multi-pass membrane proteins. Central to the EMC's functionality is the EMC2–8/9 heterodimer, which is predominantly influenced by EMC8. The proper function of the EMC requires the precise assembly of its nine subunits, which is tightly regulated to prevent undesirable interactions. Disruption of these core subunits leads to complex degradation via the ubiquitin-proteasome pathway, with EMC2 serving as a critical scaffold that stabilizes the entire structure.

Pleiner et al. used an elegant method to identify proteins that impact the stability of the EMC2-8 complex. They utilized native immunoprecipitation (IP) with anti-FLAG affinity resin, followed by mass spectrometry to pinpoint candidate proteins. Subsequent analysis involved western blotting for the candidate protein WNK-1 and additional proteins in total cell extracts and IP for GFP-tagged proteins (figure 1). They later created a stable cell line that expresses GFP-EMC2-2A-RFP, in which 2A is a cleavage site for endoproteases. They subsequently infected those cell lines with BFP or BFP-WNK1 plasmids, and WNK1 siRNA or control, and analyzed the cells with flow cytometry (figure 2).

Figure 1 and Figure 2



They later created three more transcripts: EMC4-GFP-2A-RFP, GFP-2A-RFP-SQS, and GFP-2A-RPF-VAMP2, where SQS is a tail-anchored (TA) EMC substrate, and VAMP2 is a TA WRB-CAML substrate (another TA complex independent from EMC).

Figure 3 summarizes the mechanisms of these three transcripts.

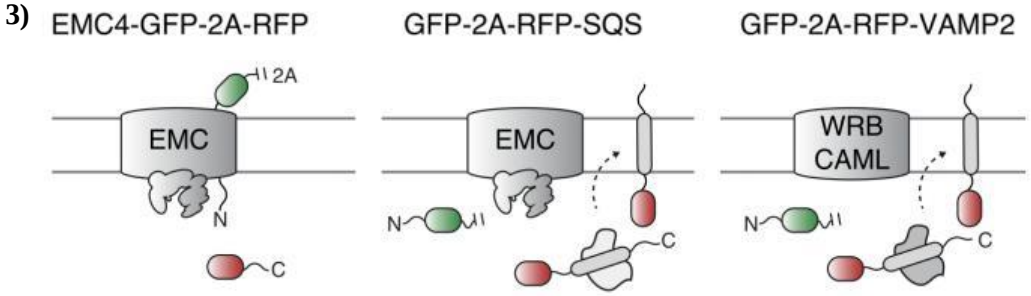
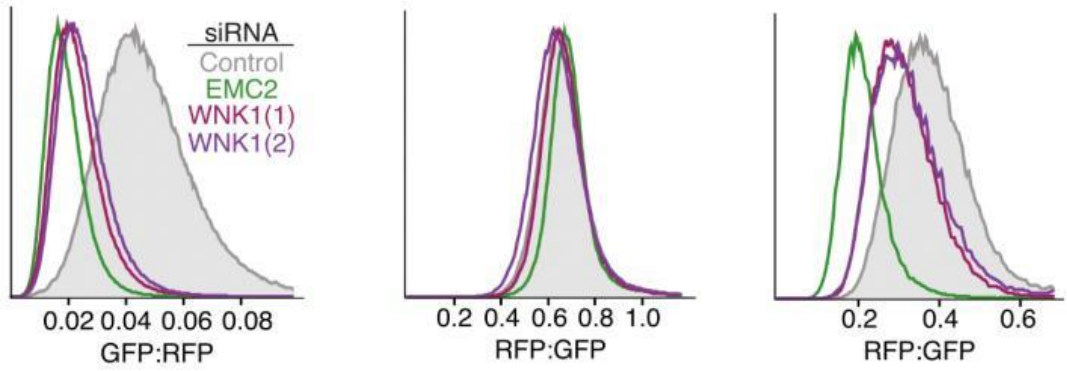


Figure 4 shows the flow cytometry results (EMC4-GFP-2A-RFP, A, and B) for those transcripts. WNK1 (1) and WNK1 (2) show 2 different siRNAs for WNK1.

4) EMC4-GFP-2A-RFP **A** **B**

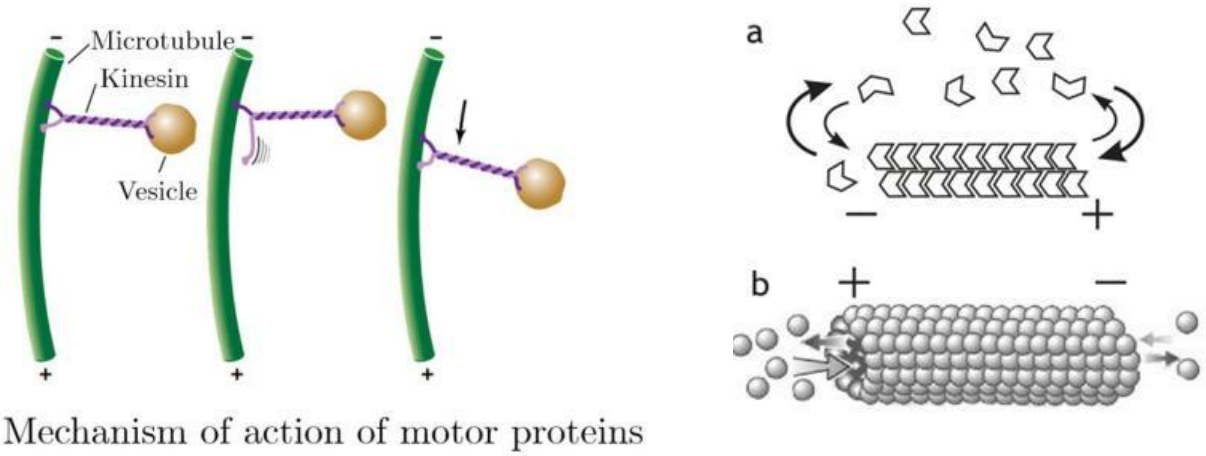


Q1. Indicate if each of the following statements is true or false.

		True	False
A.	SEC61β, together with WNK1, might have a role in the stability of EMC, as it interacts with the complex		
B.	It is expected that if a protein increases the stability of the EMC, the GFP: RFP ratio will be higher for these experimental settings		
C.	BFP-WNK1 transcript is resistant to WNK1 siRNA		
D.	A in Figure 4 shows the SQS transcript, while B shows the VAMP2 transcript		
E.	Inhibition of WNK1 with siRNAs completely blocks the function of the EMC complex		
F.	WNK1 mutant cell lines may have disrupted cholesterol regulation		
G.	WNK1 bounds to the membrane before interacting with EMC		

7. INTRACELLULAR TRANSPORT

Intracellular transport is the movement of vesicles and substances inside the cell. Intracellular transport is required to maintain intracellular homeostasis by responding to physiological signals. Proteins synthesized in the cytosol are distributed to their respective organelles based on a specific sequence of amino acid sorting. Intracellular transport occurs with the help of cytoskeletal components and motor proteins.
Microtubules are structures of protofilaments arranged along the circumference of a hollow cylinder, composed of 13 α- and β-tubulin heterodimers.



Mechanism of action of motor proteins

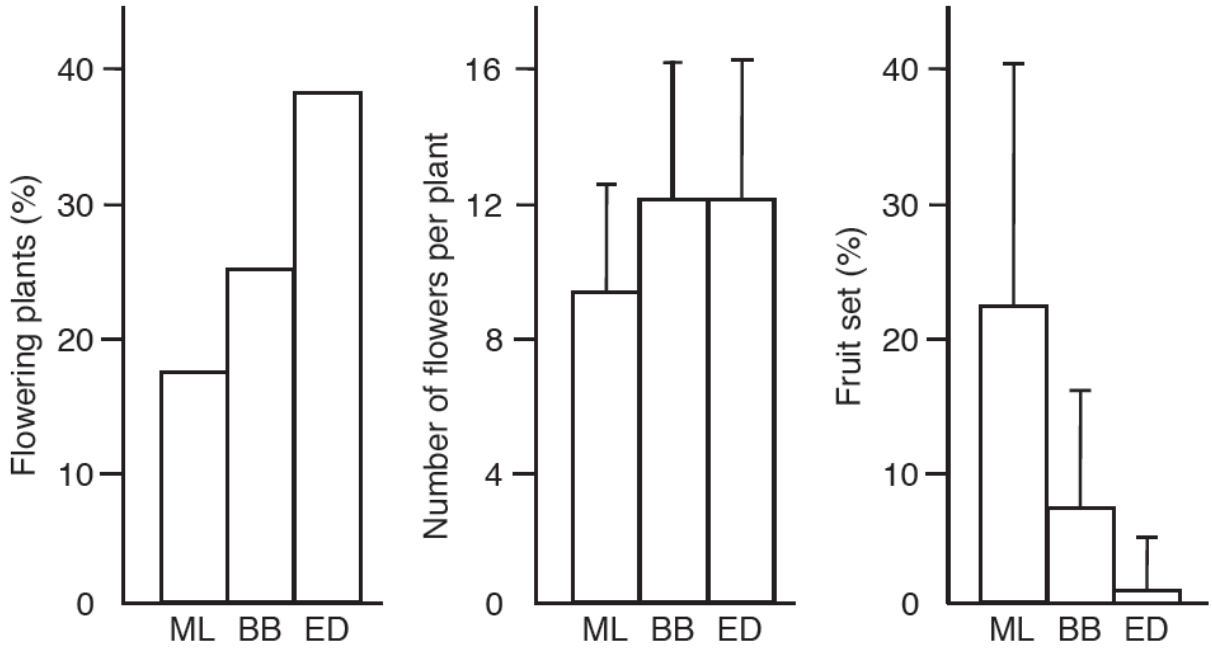
- Motor proteins include **dyneins, kinesins,** and **myosins.**
- They are carriers of macromolecules, organelles, vesicles, and other substances, thereby providing intracellular transport.
- Dyneins move along microtubules from the plus end to the minus end. Kinesins move along microtubules from the minus end to the plus end.
- Myosins move along actin microfilaments.

Q1. Indicate if each of the following statements is true or false.

		True	False
A.	The protein synthesized in the ribosomes of the rough endoplasmic reticulum is transferred to the Golgi apparatus with the help of dynein.		
B.	Amoeba feeds on bacteria, unicellular algae, and small protists through phagocytosis. Nutrients are captured because of pseudopodia. Actin and myosin are involved in the formation of pseudopodia.		
C.	In the telophase of mitosis, if β-tubulin formation is inhibited, cytokinesis stops.		
D.	When the glucose concentration in the blood is too high, the activity of kinesin proteins in α-cells of the pancreas increases.		
E.	When a cell is infected with a virus, the genetic material of the virus is delivered to the nucleus by dynein.		

8. POLLINATION OF FLOWERS

Some plants use an effective strategy to attract pollinators by emitting odors, such as rotting meat or manure. Such plants are pollinated by dung and other fly species. This tactic is common among orchids, such as perennial *Bulbophyllum variegatum*; three populations of this species (ML, BB, and ED) were studied in three different parts of Reunion Island. The results of the study are illustrated in the figure below.

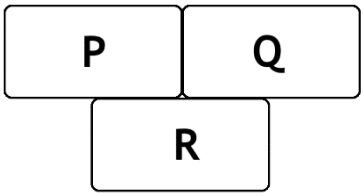


Q1. Indicate if each of the following statements is true or false.

		True	False
A.	The percentage of young individuals could be higher in the ML population than in ED and BB.		
B.	Compared with ML and BB populations, there could be limitations in the pollination potential of individuals in the ED population.		
C.	The number of fruits produced per plant was higher in the BB population than in the ML and ED populations.		
D.	Although the ED population had the highest percentage of blooming plants, this population had low reproductive success.		
E.	Although the ML population has a lower percentage of flowering plants, it has higher reproductive success than the BB and ED populations.		

9. WATER POTENTIAL

The water transport in any plant mostly depends on the water potential of the cells, which is defined as **Ψ***w*. The **Ψ***w* value of pure water is zero. Generally, when substances dissolve in water, **Ψ***w* is negative. **Ψ***w* is the sum of solute potential - **Ψ***s* and pressure potential - **Ψ***p***.** Due to the difference in **Ψ***s* potentials of neighboring cells, water moves from the higher **Ψ***w* indicator to the lower **Ψ***w* indicator until equilibrium is reached. Therefore, solute potential and pressure potential play a role in balancing **Ψ***w* in adjacent cells. In the hypothetical situation, plant cells P, Q, and R are arranged in the order shown on the right and all the cells have the same size.



Q1. Fill in the table using the information given for the starting time of cells P and R.

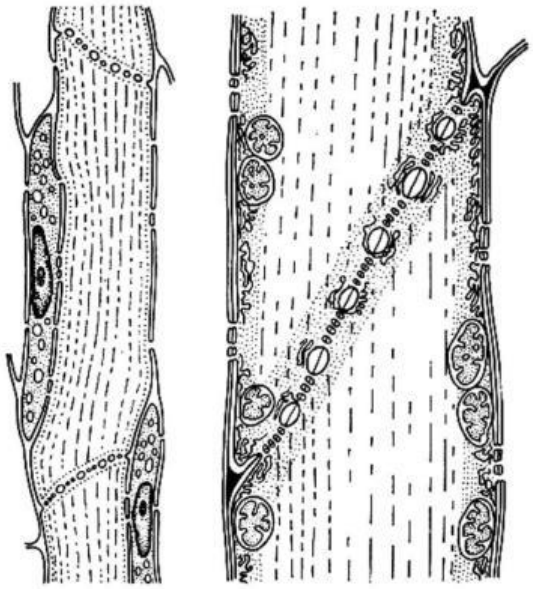
Cell	**Ψ***s (MPa)*	**Ψ***p (MPa)*	**Ψ***w (MPa)*
P	-8	2	……
R	……	2	-4

Q2. At equilibrium, when there is no additional pressure on these three cells and no additional solute is added, the water potential of the system is close to -6 MPa. In this case, find the water potential of the Q cell at the starting point.

Q3. With arrows, draw the movement of the water after the initial time in the cell diagram. Illustrate all possibilities on your diagram.

10. PLANT TISSUE IDENTIFICATION AND FUNCTION

The figure shows plant cells of the particular tissue.



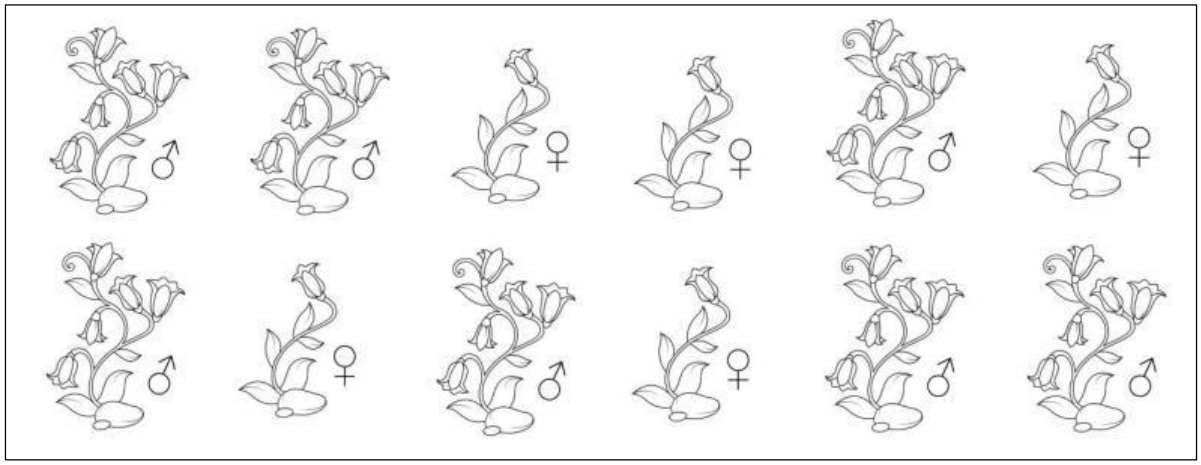
Q1. This tissue belongs to:
A. Ferns (Polypodiophyta)
B. Plauns (Lycopodiophyta)
C. Conifers (Pinopsida)
D. Flowering plants (Magnoliophyta)

Q2. Indicate if each of the following statements is true or false.

		True	False
A.	Plants usually use these cells to store nutrients till the next vegetation period.		
B.	The main function of these tissues is integumentary (protecting the plant from the outside).		
C.	The cells shown in the figure perform their main functions when they are alive (when the protoplast is functional).		

11. POLLEN/OVULE RATIO IN PLANTS

Pollen/Ovule ratio is one of the important indicators describing the characteristics of the reproductive biology of the plant. The figure below shows the entire population of flowering plants. It is known that one pollinator of this plant species produces an average of 3,000 pollen grains, and one carpel produces an average of 140 ovules. Formulas for this type of flower are also known:
♂ $K^5\ C^5\ A^{5+5}$ and ♀ $K^5\ C^5\ G^{(3)}$



Q1. Find the Pollen/Ovule ratio for this population.
P/O = _____

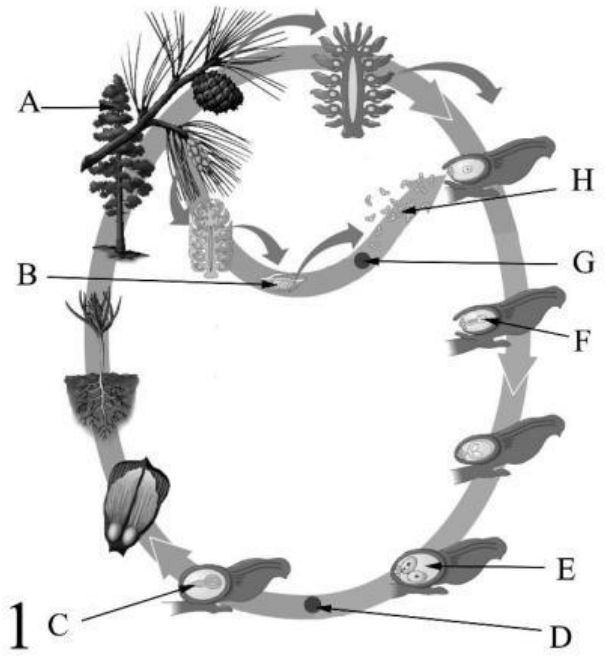
Q2. Based on the flower structure of this species, identify which of the following groups this species belongs to:
A. Monoecy
B. Dioecy
C. Gynomonoecy
D. Gynodioecy

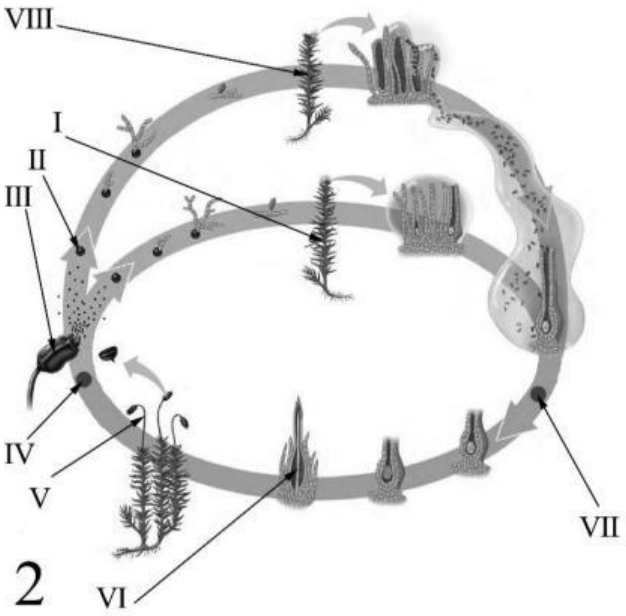
Q3. Indicate if each of the following statements is true or false.

		True	False
A.	The calculated P/O value could indicate that this plant species is pollinated by insects (entomophilia) rather than by wind (anemophily).		
B.	The percentage of bisexual plants in this population is about 60%.		
C.	Based on the structure of flowers, it is possible to say this species is a typical representative of monocotyledons.		

12. LIFE CYCLES OF PLANTS

Figures 1 and 2 illustrate the life cycles of plants belonging to two different systematic groups. Arrows (numbers and letters) indicate stages, structures, and processes in these cycles. Look at the figures and answer the questions.





Q1. Match stages/structures with a given figure.

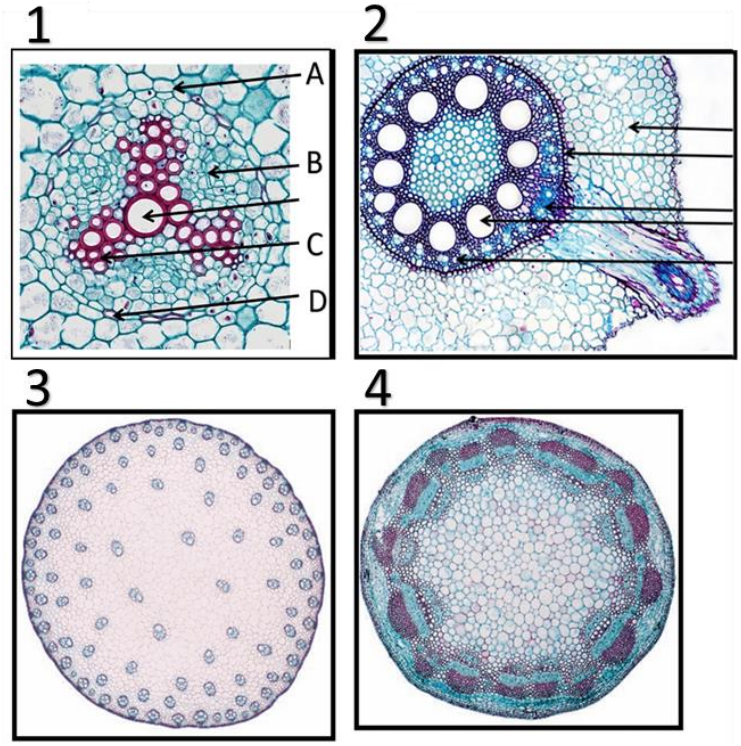
Stage	Plant 1	Plant 2
microgametophyte		
megagametophyte		
embryo of the sporophyte		
mature sporophyte		
spore		
meiosis		
fertilization		
sporangium		

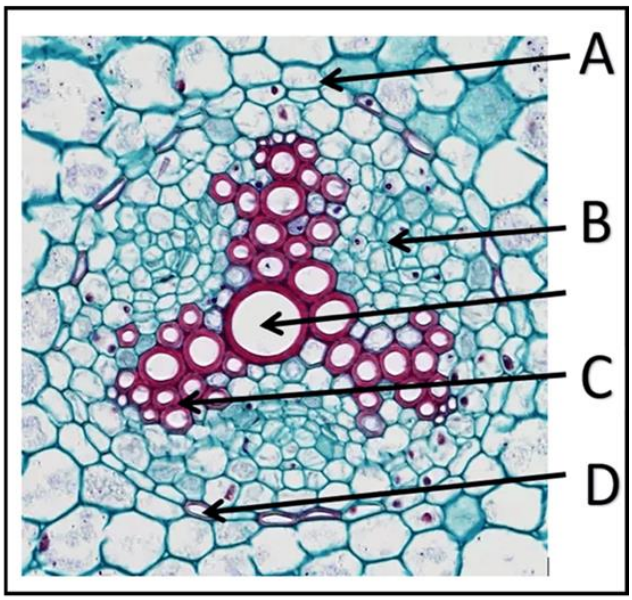
Q2. Indicate if each of the following statements is true or false.

		True	False
A.	Figure 1 shows the life cycle of a seed plant.		
B.	Figure 2 shows the life cycle of a heterosporous plant.		
C.	Both figures (1 and 2) show perennial free-living sporophyte plants.		

13. ANATOMY OF STEM AND ROOT

The cross-sections of several different vegetative organs of plants belonging to different groups are shown below.





ENLARGED FIGURE A

Q1. Using the photos above, fill in the table below.

	Dicotyledons	Monocotyledons
Root		
Stem		
Stele		

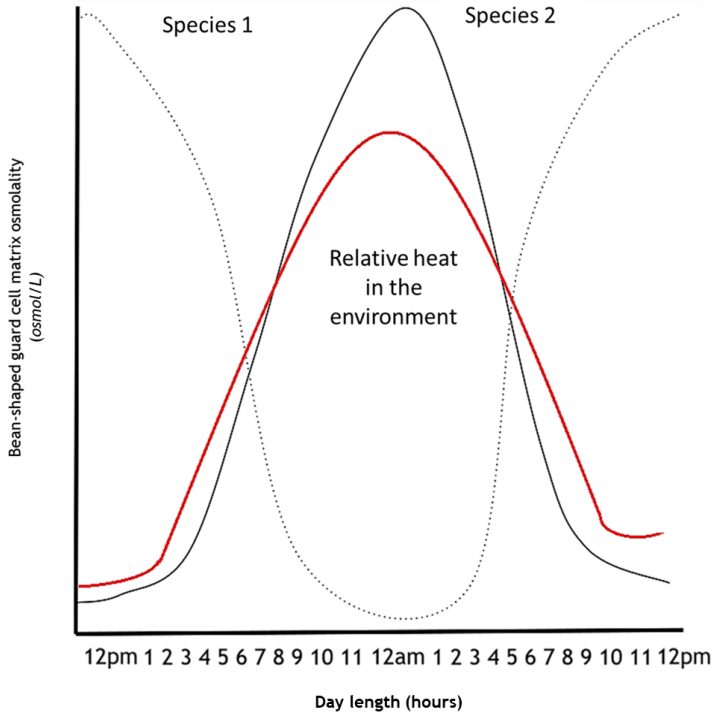
Types of stele:
I. Plectostele; **II.** Eustele; **III.** Atactostele; **IV.** Dictyostele

Q2. Match the tissues on the right figure with their specific functions according to the information given in the table below.

I.	This tissue consists of dead cells	
II.	This tissue consists of a collection of unspecialized cells.	
III.	This tissue is used to transport the products of photosynthesis.	
IV.	A layer of cells is involved in the formation of the Casparian line.	

14. MELVIN CALVIN'S DISCOVERY

In 1945, biochemist Melvin Calvin began work on plant photosynthesis. Until that time, it was not known how absorbed CO_2 turns into organic matter. Calvin discovered with several scientists that sunlight stimulates the chlorophyll molecule, and that the energy source for the so-called 'dark reactions' is the 'light reactions'. The process of the dark reaction was named the "Calvin cycle" after the scientist. This cycle can vary depending on the ecological environment in which plants grow.



Q1. Above is a graph of plants taken from two different ecological environments and the osmolarity of bean-shaped cells in them during the day. Using this information, identify the type of photosynthesis of plants. Write an '**X**' in the appropriate box.

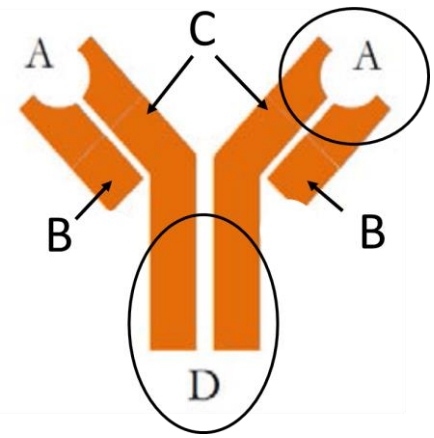
	Species A	Species B
C_3		
C_4		
CAM		

Q2. Indicate if each of the following statements is true or false.

		True	False
A.	In C3 plants, unlike C4 plants, the RuBisCO enzyme is located in the chloroplasts of mesophyll cells, where it can directly interact with atmospheric CO_2 entering the leaf through stomata.		
B.	When C3 plants remain in a warm environment, the synthesis of abscisic acid from their roots increases, making it possible for indirect photorespiration.		
C.	Unlike C3 plants, the Calvin cycle occurs only in the dark in CAM plants.		
D.	Unlike CAM and C3 plants, C4 plants do the Calvin cycle in bundle-sheath cells.		

15. IMMUNOGLOBULINS

The figure describes the structure of an antibody.



Q1. Match the definitions given below (I-IV) with the components of an antibody (A-D) respectively.

I. A shorter polypeptide that contributes to the specific binding of the molecule to the antigen.
II. A part that ensures precise recognition and binding of specific antigenic determinants (epitopes).
III. A part of an antibody that is recognized by a hunting macrophage.
IV. A polypeptide that interacts both with the pathogen and with macrophage.

	A	B	C	D
I				
II				
III				
IV				

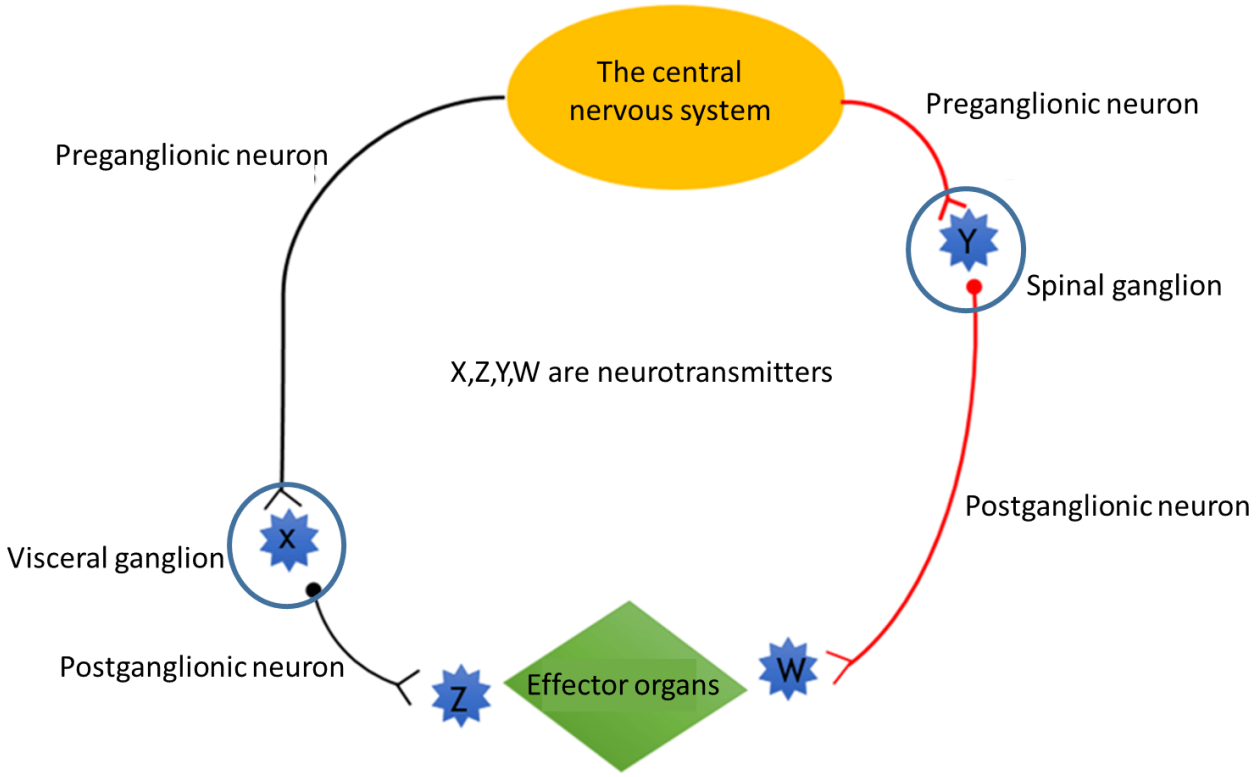
Q2. Indicate if each of the following statements is true or false.

		True	False
A.	Antibodies are produced only by T-lymphocytes in response to an antigenic challenge.		
B.	Macrophages are a part of the innate immune system that can present antigens from their surface to helper T after phagocytosing and processing pathogens.		
C.	Natural killer cells (a type of lymphocyte) can produce antibodies and have the same antigen-specific receptors as B-cell receptors.		
D.	The Fc region of the antibody is highly variable and it ensures the specific binding of the antibody to the antigen.		

16. AUTONOMIC NERVOUS SYSTEM

The central nervous system regulates the activity of organs at different levels, normalizes their work and ensures the maintenance of homeostasis. The figure shows nerve connections between the central nervous system and effector organs.

Figure 1



Q1. The activity of several types of neurotransmitters is necessary to ensure the above internal continuous communication. In the table below, match the neurotransmitters in Figure 1 accordingly.

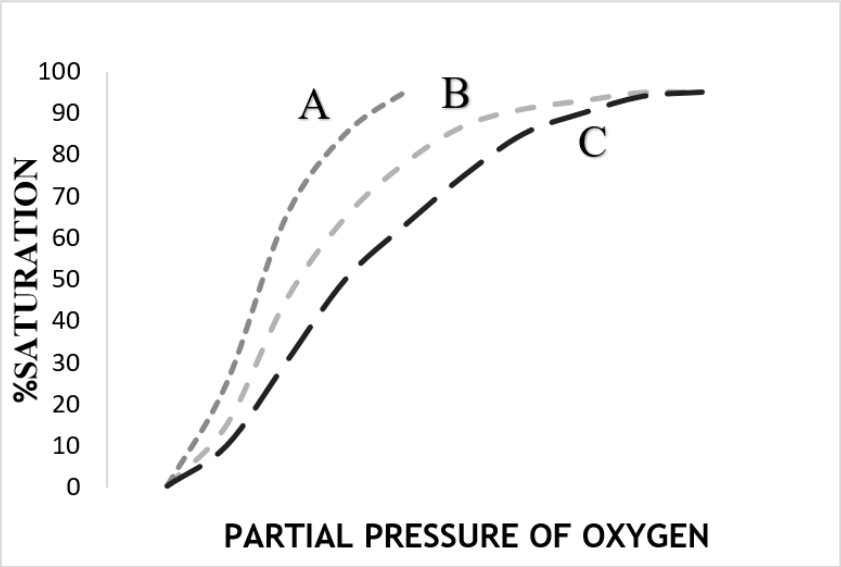
	Acetylcholine	Noradrenaline (Norepinephrine)	Glutamine	GABA (gamma-aminobutyric acid)
X				
Y				
W				
Z				

Q2. You can see five (I-V) different physiological processes that are provided by the pathways shown in Figure 1. Identify which neurotransmitters in the given nerve pathways are exocytosis in each process.

I.	Afferent nerve (entering the kidney) reduces the rate of glomerular filtration by stimulating the smooth muscles of the arterioles.	
II.	Contraction of the radial muscles of the pupil due to weak light.	
III.	It decreases the blood flow rate of the heart by influencing AV (atrioventricular) nodes.	
IV.	It reduces the fluid secretion in small and large intestines.	
V.	It stimulates JG (Juxtaglomerular) cells and increases the synthesis of renin in them.	

17. HEMOGLOBIN SATURATION

Hemoglobin (Hb) was accidentally discovered by Hunefeld in 1840 from earthworm blood samples. Hemoglobin is a protein that provides oxygen transport in blood. The binding of oxygen to a hemoglobin is depicted in the graph below. The affinity features of the hemoglobin molecule for O_2 can change based on certain principles.



Q1. Look at the graph and match the three given lines (A-C) with the following definitions.

1.	Adult Hb/O_2 line	
2.	Fetal Hb/O_2 line	
3.	The Hb/O_2 line in the case of low blood pH	

Q2. Indicate if each of the following statements is true or false.

		True	False
A.	The binding of oxygen molecules increases hemoglobin affinity to oxygen		
B.	The oxygen affinity of fetal hemoglobin is higher than adult hemoglobin to enable oxygen transfer from the mother's blood to the fetus's blood.		
C.	2,3-BPG facilitates oxygen dissociation from OxHb in the tissues at hypoxia		
D.	Increasing CO_2 gas-releasing process in the lung tissues causes a decreasing P_{CO_2} in the body and causes respiratory acidosis.		

18. KIDNEY METABOLISM

The kidney is an organ that maintains the pH and ion balance in the body and protects the body from unnecessary substances. These functions are provided by three different processes in the kidney: glomerular filtration, reabsorption, and secretion.

Young scientists investigated kidney filtration and reabsorption rates in a healthy person for one day. The results of the experiment are given in the following table:

Substances and ions	Filtered amount	Reabsorbed amount	Excreted amount
Glucose *(g/day)*	180	**X**	?
Bicarbonate ***(mEq/day)***	4320	4318	?
Sodium ***(mEq/day)***	25560	25410	**Y**
Chloride ***(mEq/day)***	?	19260	180
Potassium ***(mEq/day)***	756	?	92
Z	46.8	23.4	?
W	?	0	1.8

Q1. Identify the two unknown substances given below and mark them with an 'X' in the appropriate boxes.

	CREATININE	**URIC ACID**	**UREA**	**AMINO ACID**	**PHOSPHATE ANION**
W					
Z					

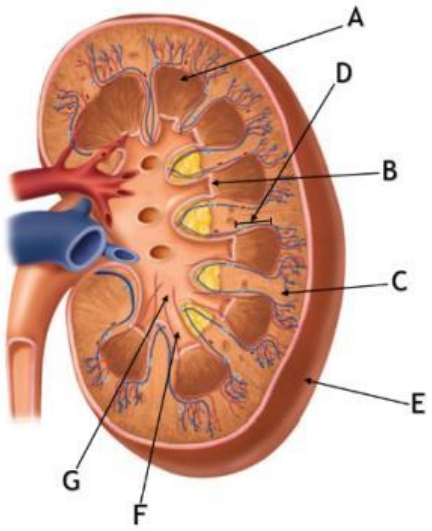
Q2. Find the two unknown numbers below and write your answer in the appropriate boxes.

X	
Y	

Q3.

		True	False
A.	The constriction of efferent arterioles causes a decrease in the filtration rate of the kidney.		
B.	A rise in antidiuretic hormone level leads to increased osmolality of liquid in the collective duct.		
C.	An increase in the concentration of noradrenaline enhances the excretion of urine by affecting the bladder.		
D.	Consuming a lot of salt leads to the decrease of atrial natriuretic peptide production by the "negative feedback" mechanism.		

Here you can see the anatomical structure of the kidney:

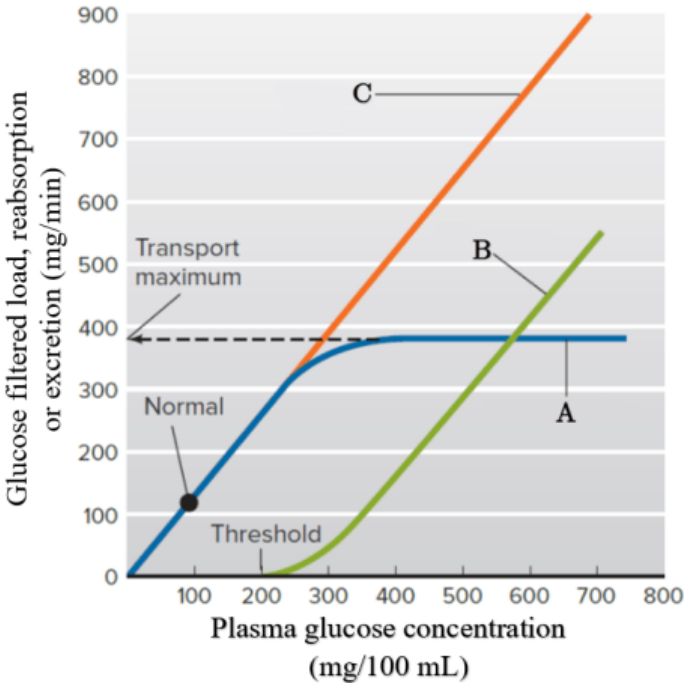


Q4. Match the parts of the kidney (A-G) with their names and complete the table below:

	A	B	C	D	E	F	G
Renal papilla							
Renal pyramid							
Minor calyx							
Renal capsule							
Major calyx							
Cortex							
Medulla							

(The answer is correct even if A and D are interchanged)

Q5. According to the graph, identify the processes (A, B, C) in the blood glucose control mechanism. One of the processes given in the table below is not depicted in the graph, leave this row blank to get full points.



	A	B	C
Filtration			
Reabsorption			
Secretion			
Excretion			

Various substances are cleaned from the blood through the kidneys at different levels, here is the formula used to find the level of purification of substances excreted through the kidneys.

$$C_s = \frac{U_s V}{P_s}$$

here:

- C_s = Clearance coefficient for substance S.
- U_s = Concentration of substance S in urine.
- V = Volume of urine per unit of time.
- P_S = Concentration of substance S in plasma.

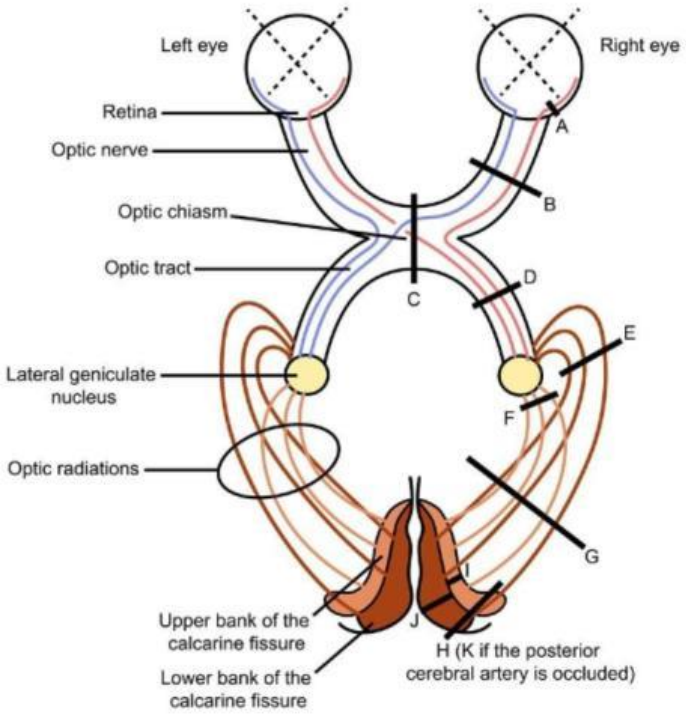
Q6. If the concentration of glucose in the blood of a healthy person is 5 mM/L, find the glucose clearance coefficient for this person using the above formula.

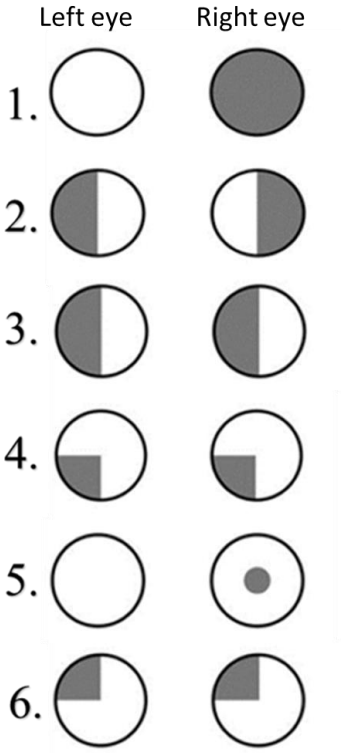
Q7. Indicate if each of the following statements about factors affecting filtration rate is true or false.

		True	False
A.	An increase in the amount of proteins in blood causes a decrease in the amount of filtered urine.		
B.	If high blood pressure increases the production of ADH, the produced antidiuretic hormone increases blood volume by increasing water reabsorption.		
C.	The osmolarity in the glomerulus is higher than the filtrate inside of capsule due to non-filtered proteins		
D.	The sympathetic nervous system increases the filtration by constricting the afferent blood vessels.		

19. VISION

Reflection of the image is transmitted as a nerve impulse to the optic nerve through the photoreceptors and bipolar cells located in the retina. Then, this nerve sends impulses to the brain cortex, where an image is formed. Several nerves are involved in this transmission. The figure below shows these signal pathways and the injuries that disrupt signal transduction (A-G). Damage to each nerve causes the loss of the image or some vision areas.





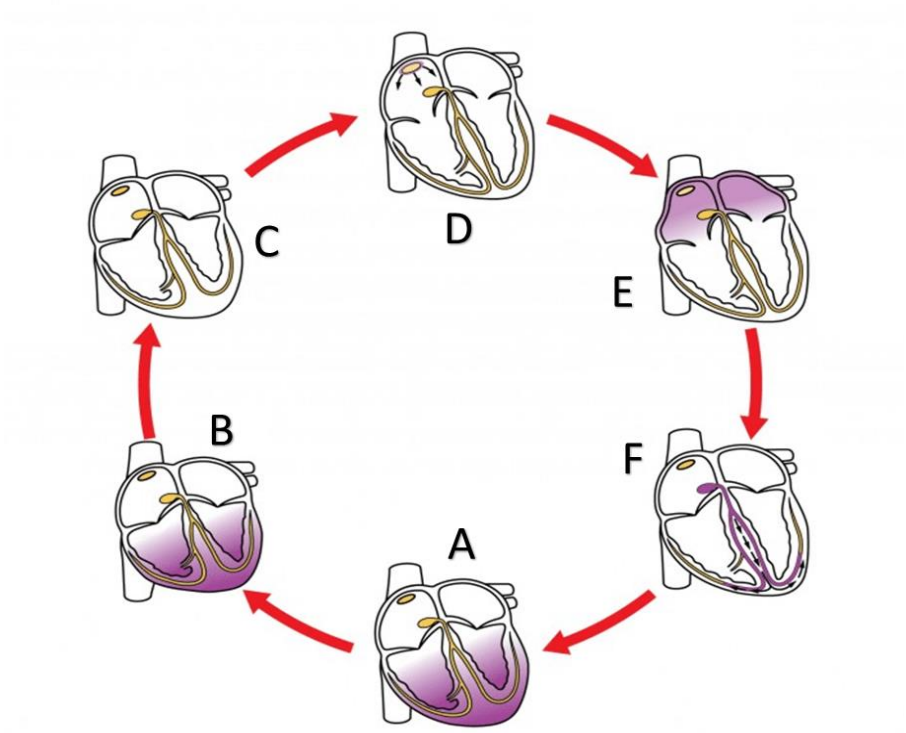
Q1. Match injuries (A-F) with conditionals (1-6).

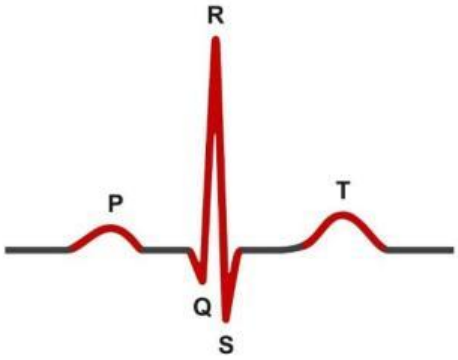
	A	B	C	D	E	F
1.						
2.						
3.						
4.						
5.						
6.						

20. PHYSIOLOGY OF CARDIOVASCULAR CIRCULATION

The heart is the main muscular organ that pumps blood with oxygen and nutrients to tissues. Any cardiac dysfunction will affect the entire organism. An electrocardiogram is one of the methods to check proper cardiac activity.

Q1. Match the following images of the cardiac cycle with normal ECG waves.

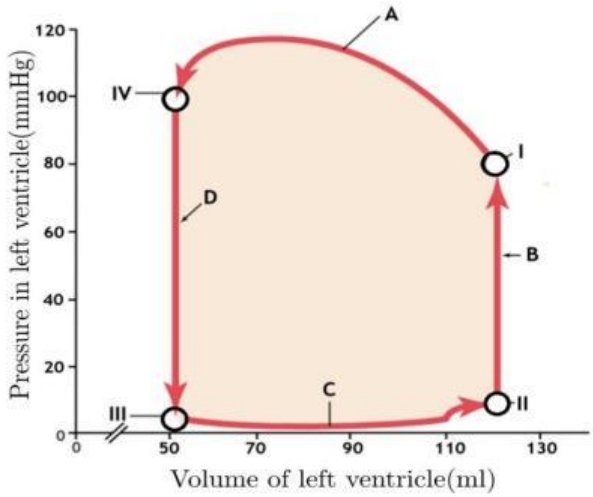




Waves	Images
P	
Q, R, S	
T	

Q2. During a heartbeat, the pressure in each chamber and its volume changes. The diagram of such a change of the left ventricle and ECG is shown below.

○ - heart valves





25 mm/sec

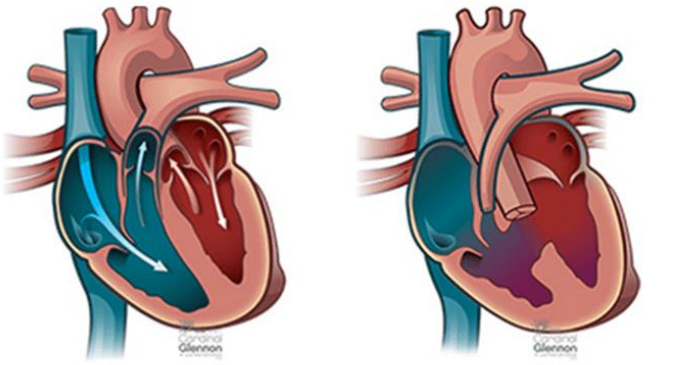
In the diagram above, the numbers I-IV represent the state of heart valves. Fill in the following table based on this information. If valve is located in the left side put on appropriate box under the **Left**.

X. Mitral valve
Y. Tricuspid valve
Z. Aortic valve
W. Pulmonary valve

	Valves	Open	Close	Left
I	X			
II	Y			
III	Z			
IV	W			

Q3. Look at the figure above and find the systolic volume for a minute of the heart (L/min).

The schematic representation of a normal heart and a heart with tetralogy of Fallot are given below. This is a polysymptomatic disease. Investigate the picture carefully by comparing size changes of specific parts.



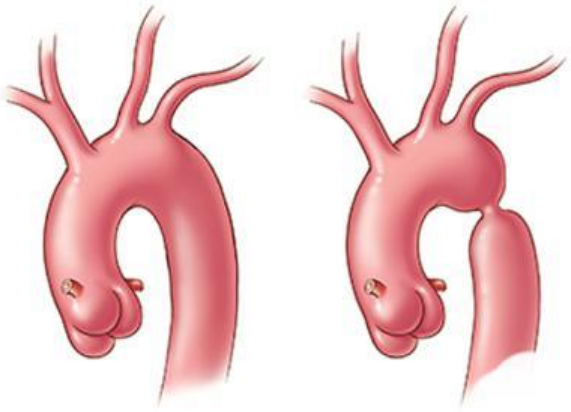
Healthy Heart

Tetralogy of Fallot

Q4. Look at the figure and identify how blood circulation changes in patients with tetralogy of Fallot and indicate if each of the following statements is true or false.

		True	False
A.	Due to the contraction of the pulmonary artery, more blood from the right ventricle flows into the aorta.		
B.	Because the blood pressure in the left ventricle is higher than the blood pressure in the right ventricle, part of the arterial blood in the left ventricle passes through the interventricular septal defect and flows to the pulmonary artery.		
C.	In a patient with an interventricular septal defect, the volume of blood in the pulmonary circulation is lower than in a healthy person.		
D.	In a patient with an interventricular septal defect, the amount of O2 in the arterial blood in the systemic circulation is lower than for a healthy person.		

The images of the aorta of a healthy individual and a patient with congenital coarctation of aorta (the narrowing in aortic arch).

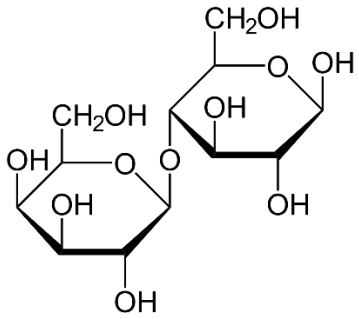
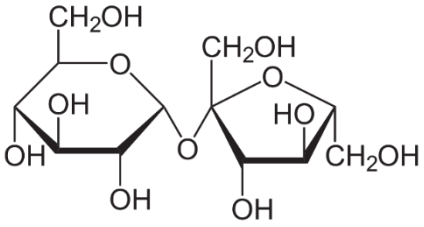
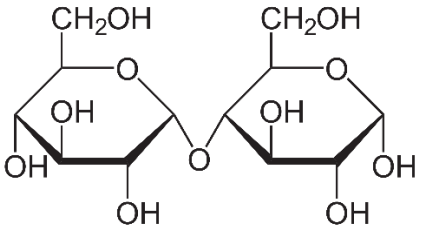
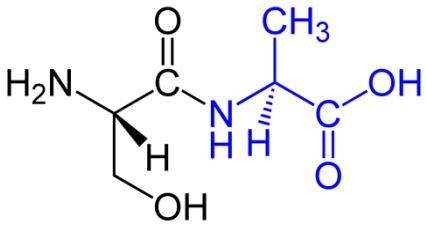
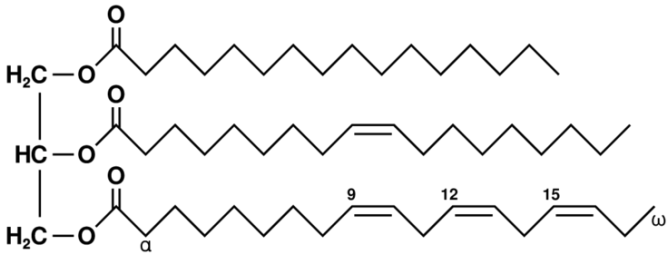


Q5. Using this image, you can infer circulatory hemodynamics disruption and indicate if each of the following statements is true or false.

		True	False
A.	Patients with this abnormality have less blood flow to their kidneys than healthy individuals.		
B.	The development of the arms and legs of patients with this abnormality tends to be disproportional.		
C.	Patients with this abnormality may develop right ventricular failure due to the right ventricle overload.		
D.	The pressure in the carotid arteries in patients with this abnormality is higher than in healthy people.		

21. DIGESTIVE SYSTEM PHYSIOLOGY

The digestive system is a tubular structure composed of several parts where different molecules are being hydrolyzed and absorbed. Here you may see some molecules that can be hydrolyzed in digestive system:

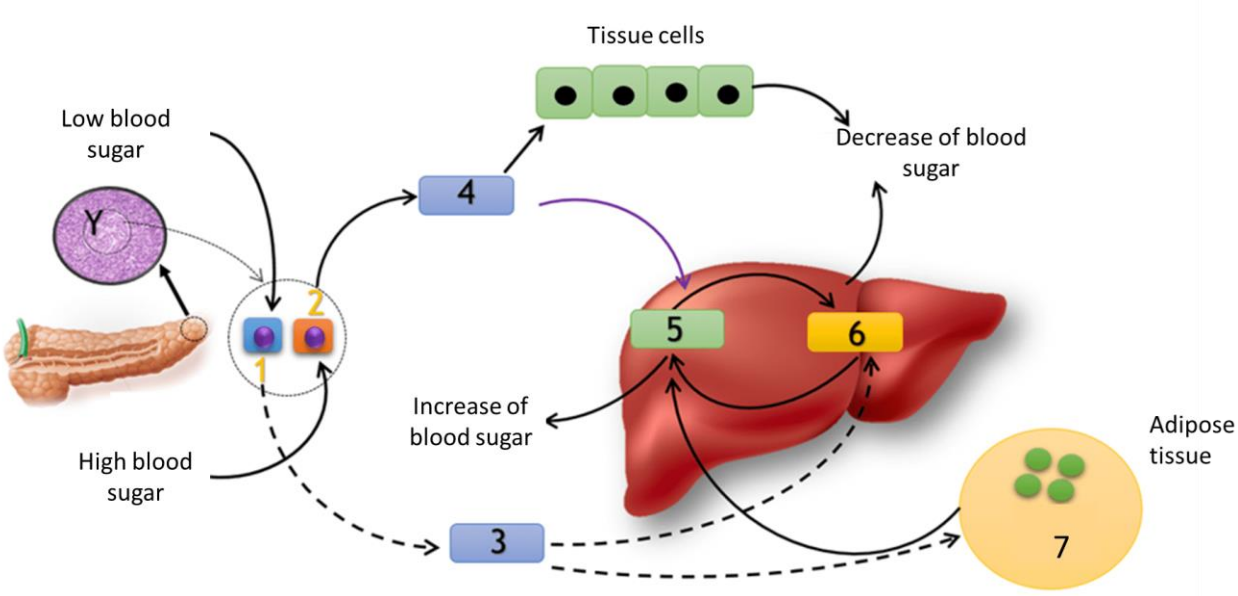
molecule I	molecule II	molecule III
		
molecule IV	molecule V	
		

Q1. Match the enzymes given above with their breaking down substrate.

	I	II	III	IV	V
Maltase					
Lipase					
Lactase					
Sucrase (Invertase)					
Dipeptidase					

22. CARBOHYDRATE METABOLISM

Carbohydrates serve as a primary source of energy in the human body. Here is a scheme of glucose level control in the blood.



A. Glycogenolysis	E. Glucagon	I. Alpha-cell	M. Glycerol
B. Gluconeogenesis	F. Insulin	J. Beta-cell	N. Glucose
C. Langerhans cell	G. Cortisol	K. Delta-cell	O. Fatty acid
D. Kupffer cells	H. Noradrenaline	L. Glycogenesis	P. Glycogen

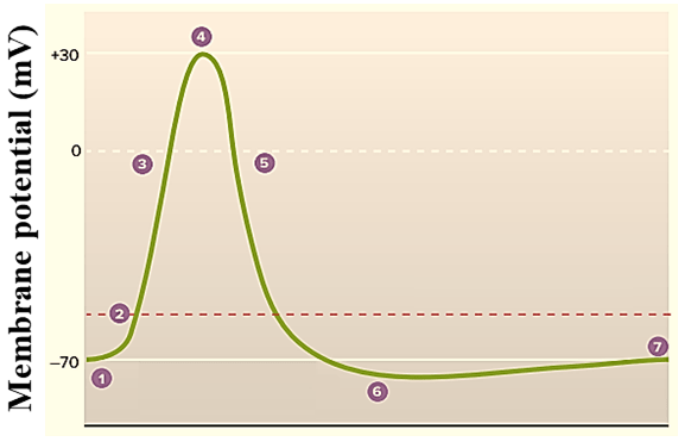
Q1. After looking at the scheme, find the appropriate answers to the given questions from the table below, and write an 'X' in the appropriate box.

1. Name the structure **Y**.
2. Name cell **1** (produces hormone **3**).
3. Name the process of **5** to **6** conversion.
4. Which hormones cause conversion of **7** to **5**.
5. Name cell **2** (produces hormone **4**).
6. Name the process of **6** to **5** conversion.
7. Name the process of **7** to **5** conversion.
8. Name the molecule **6**.

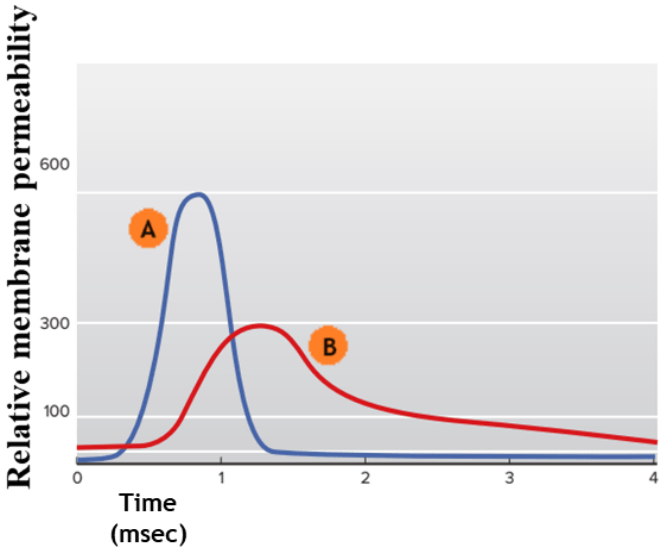
23. ACTION POTENTIAL

Sir Alan Hodgkin (1914-1998) and Sir Andrew Huxley (1917-2012) explained how action potentials are generated and spread.
1. At rest, the membrane is in a negatively charged state (resting potential).
2. If the potential reaches the threshold, the ion channels open and the voltage starts to become positive.
3. After a certain time, the channels will close again.
4. Axon cannot generate the next action potential until the membrane reaches the resting potential again.

a)



b)



Q1. Figure b shows the relative membrane permeability for ions (A, B) involved in the action potential. Respectively match the following according to the graph. (There are other ions that aren't appropriate answers, mark them with 'X').

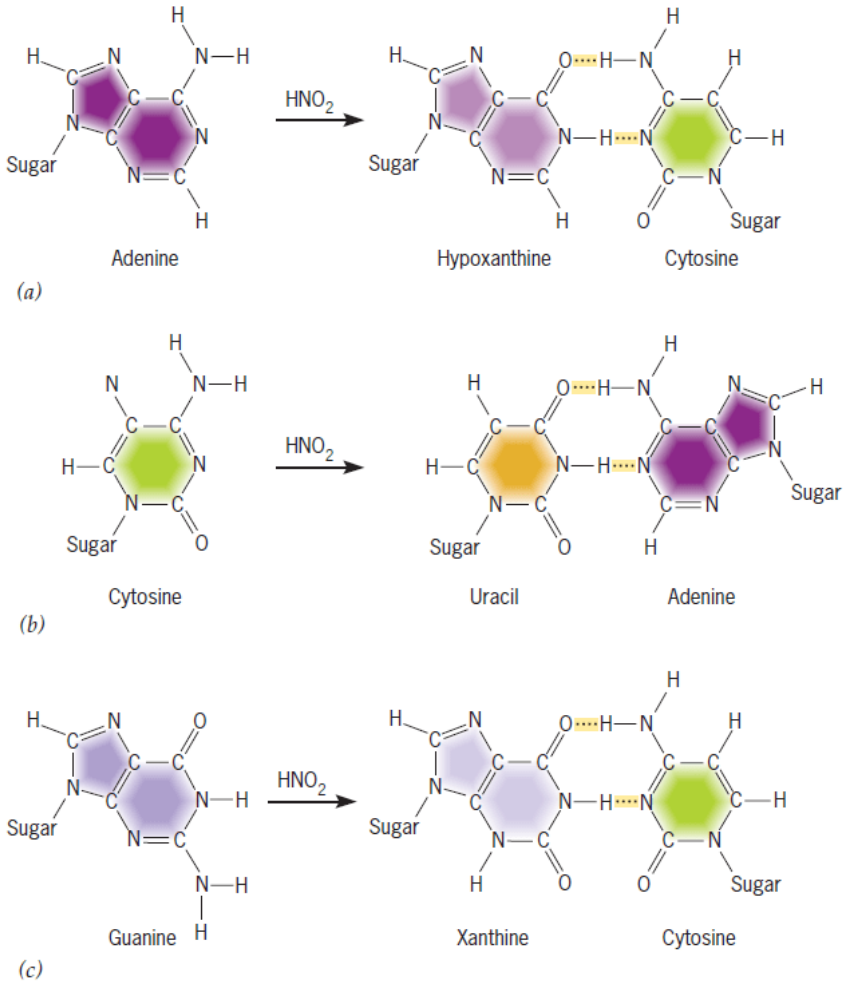
1.	Na^+	
2.	Ca^{2+}	
3.	K^+	
4.	Cl^-	

Q2. Figure **a** shows the mechanism of generation of action potential. Indicate if each of the following statements is true or false.

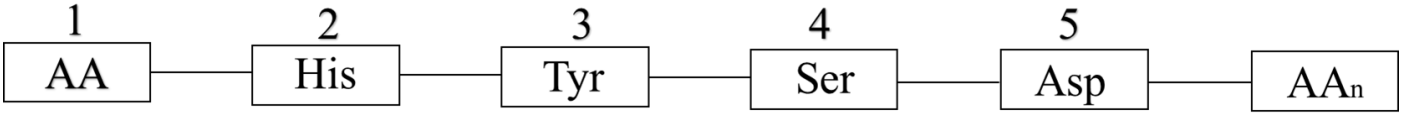
		True	False
A.	Increasing the permeability of Na^+ channels reduces the frequency of occurrence of action potentials.		
B.	Parts 3 and 5 in the figure represent the depolarization and repolarization phases, respectively.		
C.	The opening of Ca^{++} channels in the membrane provides a transition from 6 to 7 phases in the figure.		

24. TOBACCO MOSAIC VIRUS

Research has shown that some inorganic acids cause the deamination reaction of nucleotides which affects complementary interactions. Some example of deamination reactions is given below:



Scientists treated a population of non-replicating tobacco mosaic virus with nitric acid. If this tobacco mosaic virus has the following amino acid sequence, answer the given questions.



AA-amino acid

Q1. TMV was treated with HNO_2. According to the result of the experiment, if nitric acid only affects the 2nd amino acid codon what amino acid sequence(s) will be formed after the virus genome enters the host organism (write all possible peptides)?

25. SOMATIC CELL HYBRIDIZATION

Genetic hybridization is one of the most developed genetic methods. One of the promising areas of this field is somatic cell hybridization. Using this method, the location of several genes on the chromosome has been determined. Here you see the result of the hybridization of human and murine cells and enzyme Y expression in different hybrid lines.

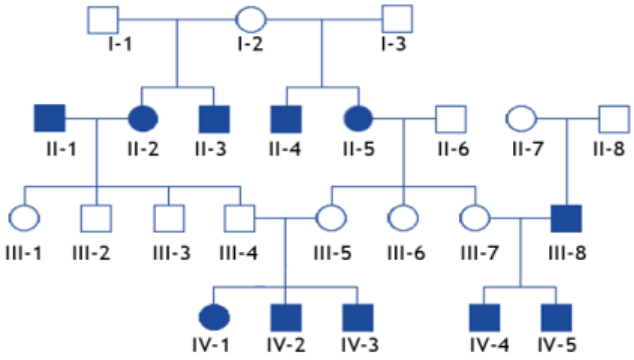
Cell hybrid line	**Enzyme Y**	**Human chromosomes present in cell hybrid line**							
		2	3	4	5	7	9	14	15
A	+	+	−	+	+	+	−	+	+
B	−	−	−	−	−	−	+	−	+
C	+	−	−	+	+	+	+	−	+
D	+	+	+	−	−	+	+	+	+
E	−	−	+	−	−	−	+	−	+
F	+	−	+	−	+	+	+	+	+

Q1. Indicate if each of the following statements is true or false.

		True	False
A.	Simultaneous presence of chromosomes 9 and 15 with a mouse cell leads to the promising synthesis of the Y enzyme.		
B.	The locus of the gene encoding the Y enzyme is located on chromosome 7.		
C.	The synthesis of the Y enzyme is controlled by the interaction of nonallelic genes located on different chromosomes.		
D.	The locus of the gene encoding the Y enzyme is located on chromosome 15.		

26. GENEALOGICAL METHOD. INBREEDING COEFFICIENT

Here you see a certain family tree. There was a marriage between relatives in this family. In addition, the inheritance of a unique trait was found in this family tree. Based on the results of the analysis, the genotypes of some organisms were recorded.



Q1. Determine the type of inheritance of the trait found in this family tree. Write 'X' in the appropriate box.

A.	Autosomal dominant	
B.	Autosomal recessive	
C.	X-linked dominant	
D.	X-linked recessive	
E.	Y-linked	
F.	Mitochondrial inheritance	
G.	Maternal effect	

Q2. Based on the family tree given above, find the inbreds among the organisms. Write 'X' in the appropriate box.

	1	2	3	4	5	6	7	8
I								
II								
III								
IV								

Q3. Calculate the inbreeding coefficient for each of the inbred organisms. Write your answer in the table below. Use the following formula: (the number of empty boxes may be greater than the number of inbred organisms.)

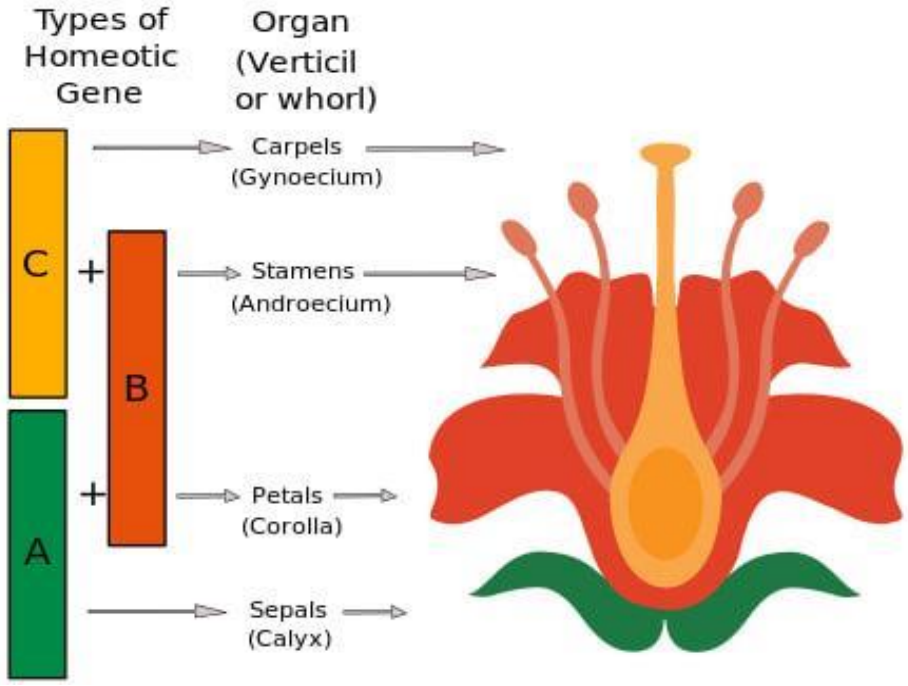
$$I = \left(\frac{1}{2}\right)^{n-1}$$

n – represents the number of individuals in the path that runs from an individual up to the common ancestor through one parent and back down to the other parent, without going through any individual twice.

Inbred organisms	Inbreeding coefficient

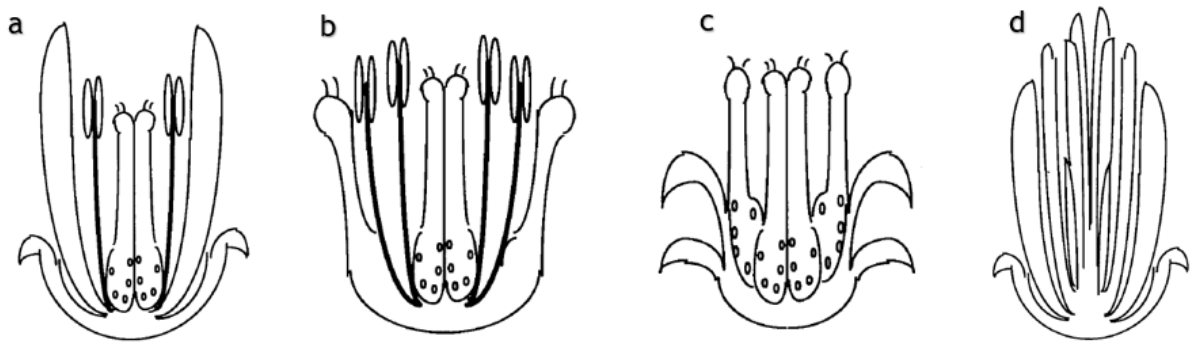
27. ABC MODEL

The flowers of the wild species *Arabidopsis thaliana* consist of four flower parts in the following order (from outside to inside): sepals, petals, stamens, and carpel. A vertical cross-section of the *Arabidopsis thaliana* flower and a horizontal cross-section is shown in figure below. Flower development is controlled by the interaction of A, B, and C components.



- Expression of the A gene ensures the development of sepals.
- Co-expression of genes A and B ensures the development of petals.
- Co-expression of B and C genes ensures the development of stamens.
- Expression of the C gene ensures the development of carpel(s).

The schematic illustrations (a-d) of four different types of flowers of *Arabidopsis thaliana* are given below.



The code tables you need to write your answers are given below (you can use the same code more than once). Organ identification code.

Existing organ(s)	Organ(s) code
sepals, petals, stamens, and carpel	1
Only sepals	2
Only stamens and carpels	3
Only sepals and stamens	4
Only sepals and petals	5
Only sepals and carpels	6

The active part(s) of a "homeotic" gene model	Gene(s) code
A, B, C	7
A, B	8
A, C	9
B, C	10
A	11
B	12
C	13

Genotype	Genotype code
Wild type (wt)	14
Mutant	15

Q1. Fill in the table below using the identification codes.

Samples	Organ identification code	The active part(s) of a "homeotic" gene model	Genotype code
a			
b			
c			
d			

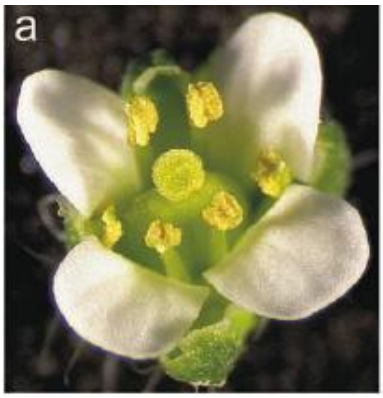
The phenotype codes for expressing flower morphology are shown below.

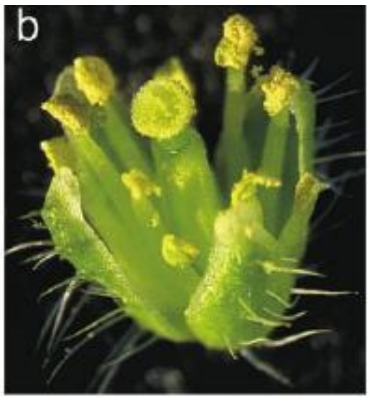
Phenotypes	Phenotype codes
Leaves in all four whorls	1
Sepals in all four whorls	2
Petals in all four whorls	3
Carpels in all four whorls	4
Stamens in all four whorls	5
Sepals in the 1st and 2nd whorls, Carpels in the 3rd and 4th whorls	6
Sepals in the 1st and 4th whorls, Petals in the 2nd and 3rd whorls	7

Q2. Fill in the table below using the phenotype codes.

	Phenotype code
Determine the flower morphology of a plant with mutant genes **B** and **C**.	
Determine the flower morphology of a plant with mutant gene **C**.	
Determine the flower morphology of a plant with mutant genes **A**, **and B**.	
Determine the flower morphology of a plant with mutant gene **B**.	
Determine the flower morphology of a plant with mutant genes **A, B, and C**.	

Q3. Natural photos (a-c) of *Capsella bursa* (a, b) and *Tulipa gesneriana* (c) flowers are given below.

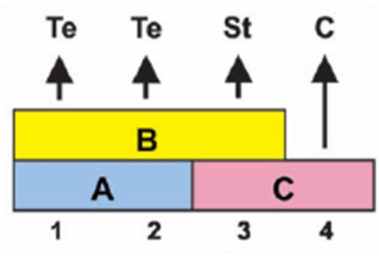




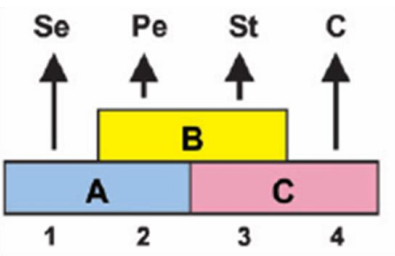


Here you can see 3 types of ABC gene models:

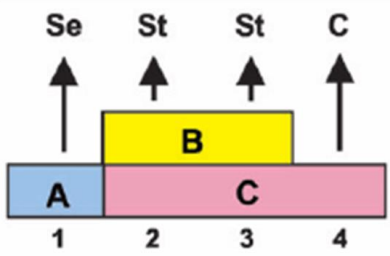
1



2



3

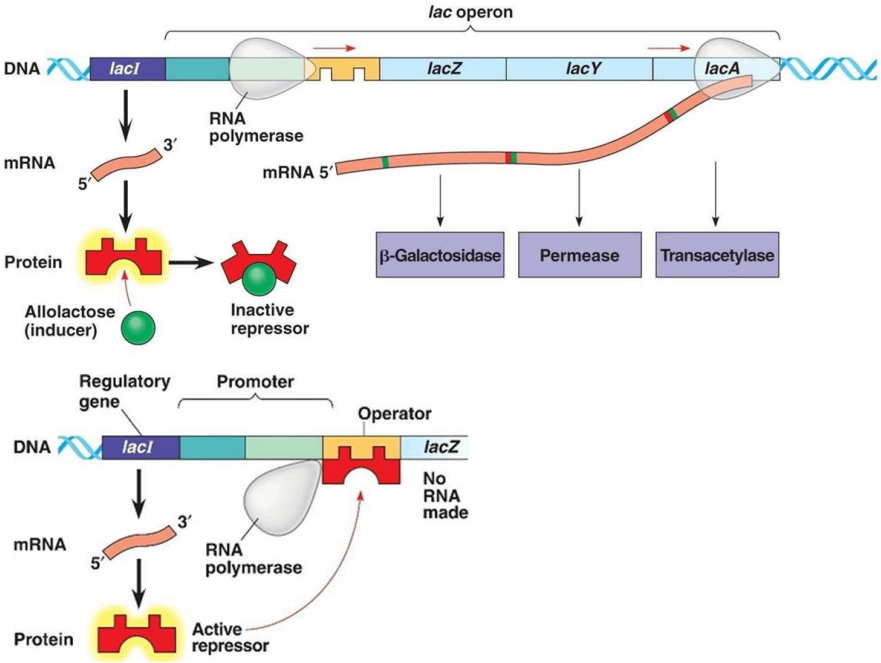


Match the figure of flowers with the appropriate model.

	a	b	c
1.			
2.			
3.			

28. LAC OPERON

Genes in prokaryotes are usually organized in complex transcriptional units called “operons”. One such operon is the Lac operon found in *E.coli*. The transcription of this operon is controlled by the presence or absence of lactose and downregulated by lac-repressor, coded in the *lacI* gene. Recently, in addition to the $lacI^+$ and $lacI^-$ alleles in this organism, the I^s gene was determined. This gene encodes a "super-repressor" that does not form a bind lactose and permanently suppresses the operator. There is another mutation O^C in the operator that prevents the binding of any repressor.



$lacY^+$ makes permease, an enzyme essential for the rapid transportation of galactosides from the medium to the interior of the cell. Its allele $lacY^-$ makes no permease. Lactose must enter the cell in order to induce the $lacZ^+$ gene to produce the enzyme β-galactosidase. The allele $lacZ^-$ makes a related but enzymatically inactive protein called lacCZ.

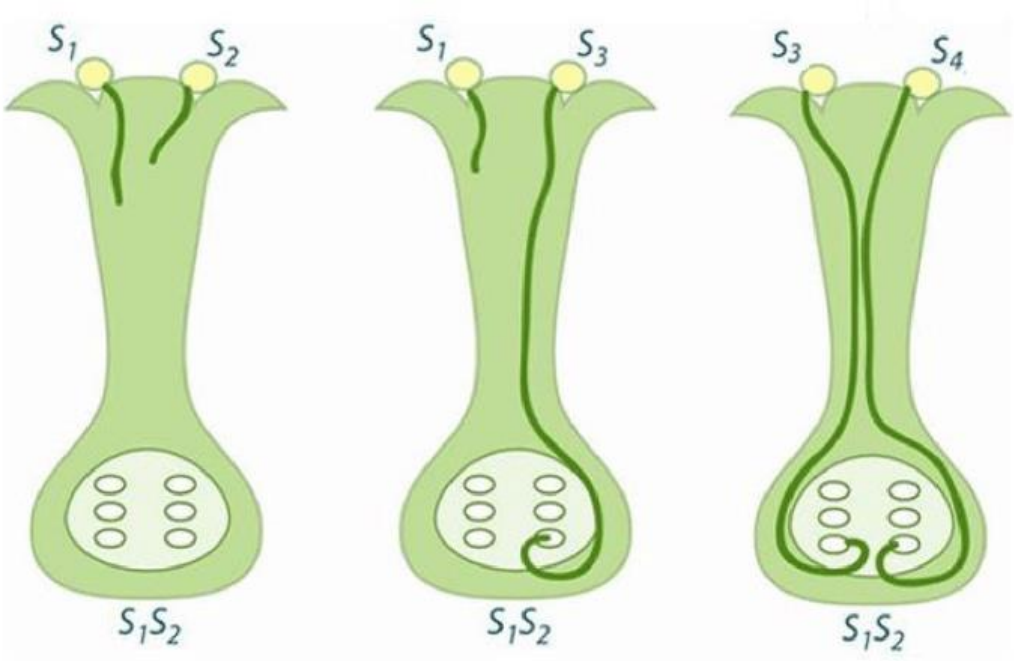
(P=permease, β-*gal*= β-galactosidase, lacCZ= protein lacCZ)

Q1. Depending on the presence or absence of lactose and given genotypes, fill in the table below by using the symbols: if the corresponding protein is not synthesized write “-”, if synthesized write “+”.

Genotypes		LACTOSE ABSENT			LACTOSE PRESENT		
		P	β-gal	lacCZ	P	β-gal	lacCZ
1.	$I^+ O^+ Y^+ Z^-$						
2.	$I^+ O^+ Y^- Z^-$						
3.	$I^s O^+ Y^- Z^+$						
4.	$I^+ O^c Y^+ Z^+$						
5.	$I^- O^c Y^- Z^+$						
6.	$I^s O^c Y^+ Z^-$						

29. DOUBLE FERTILIZATION

A number of incompatible alleles are known in Alfalfa (*Medicago sativa*). If the alleles in the style and the alleles in the pollen tube of a diploid plant are the same, then the sperm cell will not reach the ovule. This plant has 4 alleles that are incompatible with each other: *S1, S2, S3, S4.*



Determine the genotypes of the embryo (a) and endosperm (b) in the seeds resulting from the following crosses and determine the ratio of these genotypes. If there is no offspring out of pollination, put an "X" symbol.

	Carpel ♀	***Stamen*** ♂
Q1.	S_1S_2	S_1S_2
Q2.	S_1S_4	S_3S_4
Q3.	S_1S_3	S_2S_4
Q4.	S_2S_3	S_3S_4

	Embryo	Endosperm
Q1.		
Q2.		
Q3.		
Q4.		

30. POPULATION GENETICS

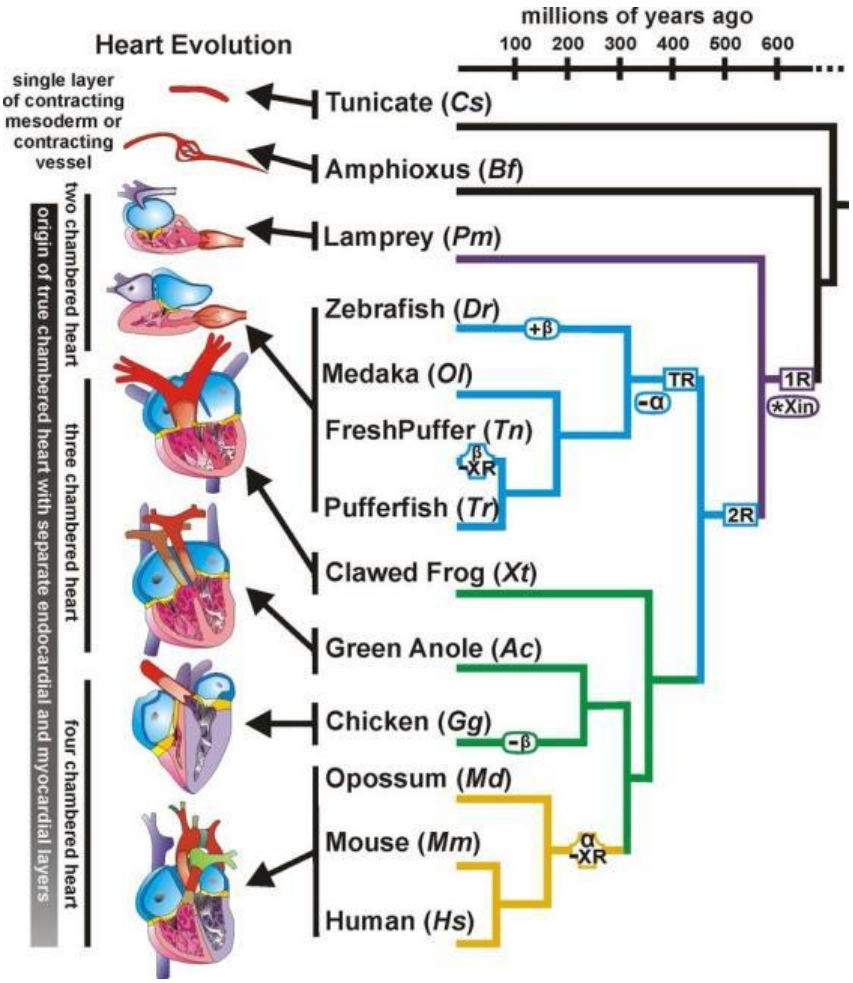
Several groups of scientists visited an island to conduct experiments on insects. After studying an insect species on the island, they found that the inheritance of pigments in the species is controlled by a single gene with two alleles. D represents dark color and d represents bright color. Dd is heterozygous and the color is intermediate. In a heterogeneous environment, the frequency of the D allele is 0.7, and the d allele is 0.3. The important reason for this polymorphism of colors is that some areas of the environment where they live are very dark, and some areas are very sunny. Due to a strong storm, a group of 1000 insects flew to an area with full sun. The probability of survival of alleles in this area (w) is equal to DD=0.3, Dd=0.7, dd=1.0.

Q1. Determine the frequency of each allele in the F1 generation after the storm.

Q2. Determine the frequency of genotypes in the F1 generation after the storm.

31. EVOLUTION OF THE HEART

Blood pumping function in primitive chordates is performed only by special blood vessels, while in birds and mammals, a four-chambered heart already exists. The phylogenetic tree below represents the evolution of the heart in chordates:



Q1. Based on an analysis of the evolution of the heart in chordates, indicate if each of the following statements is true or false.

		True	False
A.	Fresh Puffer *(Tetradon sp.)* and Zebrafish *(Danio rerio)*, share a common heart structure.		
B.	Zebrafish and Medaka forms a monophyletic group		
C.	The origin of homeothermic animals from poikilothermic animals implies changes in cardiac structure and function, increased metabolic demand associated with thermoregulation.		
D.	The -β mutation linked to the origin of the β-adrenergic receptor is common among all endotherms.		
E.	In the phylogenetic tree, the independent emergence of the four-chambered heart in mammals and birds is an example of convergent evolution.		

32. SHANNON INDEX

A group of ecologists conducted a study to measure and evaluate the level of biodiversity in two different ecosystems, A and B. They calculated the Shannon index for each ecosystem based on the biodiversity of species. The *Shannon index* ***(H)*** is a widely used indicator for measuring biodiversity that takes into account the richness of species and their even distribution (horizontal structure) in the environment. It is calculated by the following formula:

$$H = -\sum_{i=1}^{S}(p_i \cdot \ln(p_i))$$

here:

- ***H*** *Shannon index*
- ***S*** the total number of species in the ecosystem
- p_i the ratio of the number of individuals in each species forming the ecosystem to the total number of individuals in the ecosystem

The results of this study are shown below:

Ecosystem A:

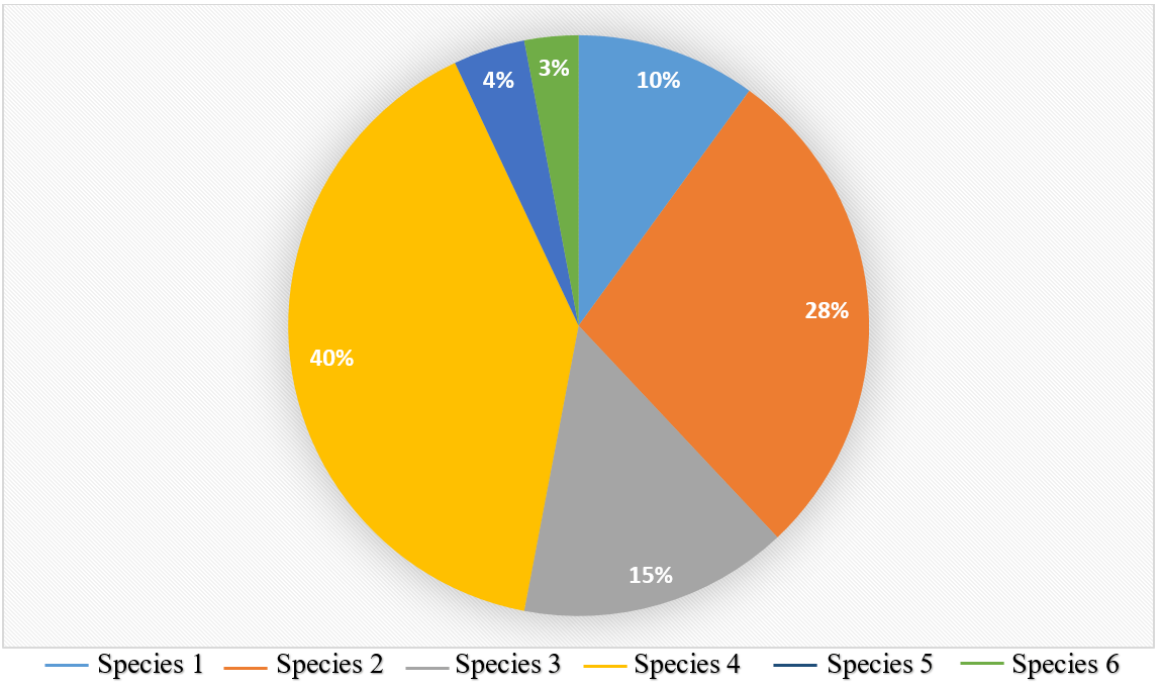
- Shannon index ***(H)***: 1.47

Ecosystem B:

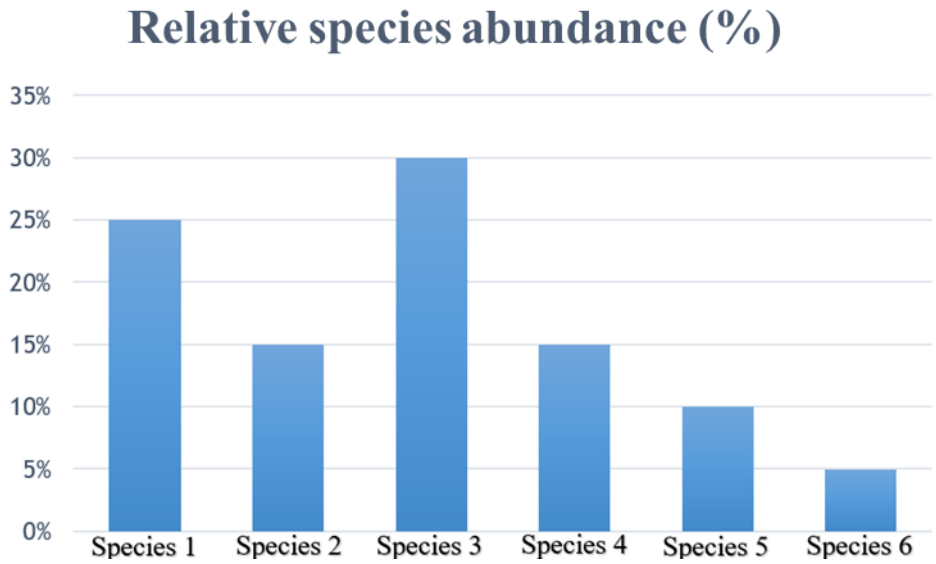
- Shannon index ***(H)***: 1.66

The following data are provided to represent the distribution of species in both ecosystems. Use graphs A and B for a visual representation.

Graph A: The pie chart representing the percentage distribution of species in an ecosystem A:



Graph B: The bar chart showing the relative abundance (richness) of each species in the ecosystem B:



Q1. Analyze the above information and indicate if each of the following statements is true or false.

		True	False
A.	If species 6 disappears in ecosystem B, the Shannon index will decrease.		
B.	The pie chart in Graph A and the bar chart in Graph B show that species are more evenly distributed in Ecosystem A compared to Ecosystem B.		
C.	Ecosystem A has a lower *Shannon index* than ecosystem B and it means that ecosystem A has a higher level of species biodiversity.		
D.	If in ecosystem A certain species are dominant, ecosystem A may have a high *Shannon index* despite having fewer species.		
E.	Ecosystems C and D have 4 species each, and in ecosystem C these species are distributed at proportion 1:1:1:1, and in Ecosystem D they are distributed in proportion 20:2:1:1 from this it can be concluded that Ecosystem C is more stable than ecosystem D, species are evenly distributed nd has a high Shannon index.		

Q2. The appearance of a new species in the area of **ecosystem A** was observed, and this caused a change in the number of individuals of the species in the ecosystem. Based on the following information, determine the *Shannon index* for the new **ecosystem A**.

Species	**Number of individuals**
1	20
2	25
3	15
4	40
5	10
6	5
7	25
8	24
TOTAL	164

33. MATRIPHAGY

Matriphagy is **the consumption of the mother by her offspring**, matriphagy is observed in the spider species *Stegodyphus lineatus*. A female spider is eaten by her offspring when she is a certain age. The young spiders then live in groups in the nest for a short time, and after the third molting, they disperse from the nest individually. Some mother spiders do not undergo matriphagy, which means that if the mother spider is not eaten by the first generation, she has a **40%** chance of reproducing a second time. The table below provides demographic information for this species.

	The total number of offspring with a 100% chance of breeding	Survival rate at 3rd molting	Body masses of spiders when they leave the nest	Survival rate from hatching to reproductive age
First generation when matriphagy is observed	120	95%	4.0 mg	30%
First generation when matriphagy is not observed	120	60%	2.5 mg	15%
Second generation when matriphagy is not observed	40	75%	4.0 mg	25%

Q1. If the mother spider does not undergo matriphagy and reproduces a second time, find the total number of her offspring.

Q.2 If the mother spider undergoes matriphagy, find the number of this mother's offspring that will reach reproductive maturity.

Q3. If the mother spider does not undergo matriphagy and breeds a second time, find the number of this mother's offspring that will reach reproductive maturity.

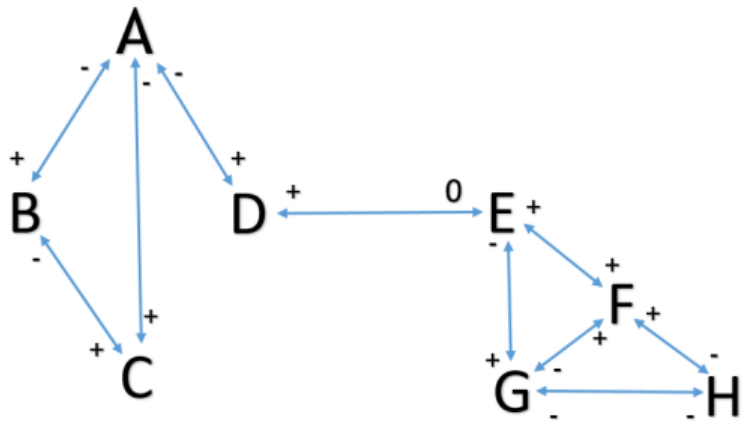
Q4. If the mother spider does not undergo matriphagy, and if she breeds a second time, find the sum of the total body masses of all the offspring that were able to leave the nest.

Q5. Identify the type of biological relationship observed in the spider species *Stegodyphus lineatus*.

A. Cannibalism
B. Amensalism
C. Mutualism
D. Competition

34. ECOLOGICAL RELATIONSHIPS

The diagram below shows the model of population interaction in an ecosystem. Capital letters represent populations. Double-headed arrows (↔) indicate a direct relationship between two populations. Interactions can be beneficial (+), detrimental (-), or neutral (0) for each population.



Determine how changes in one population affect (directly or indirectly) changes in another population in the ecosystem. Indicate your answer by placing an X in the appropriate space on the answer sheet.

How will a decrease in *population C* influence the other populations listed below?

Q1. To population F
Q2. To population B
Q3. To population A

How will an increase in *population E* influence the other populations listed below?

Q4. To population C
Q5. To population G
Q6. To population H

	Increases	Not change	Decreases	Unpredictable
Q1.				
Q2.				
Q3.				
Q4.				
Q5.				
Q6.				

35. HAMILTON'S RULE

Hamilton's rule predicts that individuals will be more likely to support closer kin altruistically, and thus more likely to get support from closer kin. Consider a study of a bee colony that aims to test Hamilton's rule. To perform this study several steps are needed: estimation of genetic relatedness, observation of altruistic behavior, and colony success measurement.

Firstly, young scientist Davron Tukhtaev took three bees from one colony and measured the relatedness between them using modern genetic technologies. He put the results of his measurements in Table1.

Table 1: Genetic relatedness matrix

Individuals	Bee 1	Bee 2	Bee 3
Bee 1	1.0	0.5	0.3
Bee 2		1.0	0.6
Bee 3			1.0

Q1. Davron asked you to help him calculate the average genetic relatedness (r) between the individuals in the colony based on the data in Table 1.

Then Davron counted acts of altruistic behavior and success scores. For the success score, Davron used the following formula:

$$\text{Score} = 0.4 \times (\text{Reproduction}) + 0.6 \times (\text{Foraging Efficiency})$$

here:

Reproduction: This represents the reproductive success of the colony. It can include indicators such as the number of new individuals hatched in a certain period.

Foraging efficiency: It measures how efficiently a colony gathers resources such as food or building materials. Foraging efficiency can be determined by factors such as the amount of resources collected per unit of time or the success rate of foraging trips.

The results of his experiment are listed in Table 2:

Table 2: Altruistic behavior in colonies and their success scores

Colony	Count of detected altruistic actions, A	Success score of the colony, B
1	15	85
2	20	92
3	10	78

Q2. Consider Table 2, which provides information on the frequency of altruistic behavior and the colony success score for different colonies. For Colony 1, determine the integral index ***(M)*** representing the overall performance of the colony. Use the following formula for this:

$$M = A + 0.6 \times B$$

here:

- **M** - integral index representing the overall performance of the colony.
- **A** - Frequency of altruistic behavior.
- **B** - Success score of the colony.

Q3. Analyzing the above information, indicate if each of the following statements is true or false.

		True	False
A.	Altruistic behavior is more observed among individuals with high genetic relatedness in the colony.		
B.	Genetic relatedness within a colony can only be clearly determined using morphological characteristics.		
C.	The frequency of altruistic behavior in a colony is positively correlated with colony success, including factors such as reproduction and foraging efficiency.		
D.	It can be concluded that in a colony with a high average degree of genetic relatedness, the degree of cooperation between individuals will in any case be higher than in colonies with a low average degree of genetic relatedness.		
E.	The integral index representing the overall performance of colony 2 is higher than 75 and the integral indicators of other colonies, on this basis, it can be said that the foraging efficiency and reproductive properties of colony 2 are higher than those of other colonies.		