BIOLOGICAL AND BIOORGANIC CHEMISTRY

Workbook for foreign students of specialization "Medicine"

Part 1: General Bioorganic Chemistry

КИЇВСЬКИЙ УНІВЕРСИТЕТ 2019

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GENERAL LAB SAFETY RULES AND GUIDELINES

These rules must be followed at all times!

1. Do not work alone in the laboratory.

2. UNAUTHORIZED EXPERIMENTS ARE NOT ALLOWED:

   ✓ Students who come to the laboratory session must have a complete understanding of the laboratory procedures to carry out and be familiar with both the physical and chemical properties of chemicals and reagents to be used.

   ✓ Before every class, be familiar with the properties of all chemicals used at the lesson. This includes their flammability, reactivity, toxicity, and proper disposal. This information may be obtained from your instructor and from the MSDS.

3. DRESS CODE SAFETY RULES:

   ✓ Always tie back hair that is chin-length or longer.

   ✓ Always wear lab clothes that cover most of your skin. Never wear shorts or skirts in the lab.

   ✓ Never wear sandals or other open-toed shoes in the lab. Footwear should always cover the foot completely.

   ✓ Wear disposable gloves when using potentially dangerous chemicals or infectious agents.

   ✓ Sometimes special care for eye protection is required. Safety glasses must be used when certain procedures are being carried out. The instructor will call the students' attention to those procedures. The use of contact lenses is not recommended, since they reduce the rate of self-cleansing of the eye.

4. HOUSEKEEPING SAFETY RULES:

   ✓ Always keep your work area(s) tidy and clean. Only materials you require for your work should be kept in your work area. Everything else should be stored safely out of the way.

   ✓ Eating, drinking and smoking in the laboratory are strictly prohibited.

   ✓ Become familiar with the location and the use of standard safety features in the laboratory. The laboratory is equipped with fire extinguishers, washes, fume hoods and first-aid kits. Any question regarding the use of these facilities should be addressed to your instructor.

   ✓ Never leave an ongoing experiment unattended. If you are the last person to leave the lab, make sure to lock all the doors and turn off all ignition sources. Make sure you always follow the proper procedures for disposing lab waste.

5. HANDLING GLASSWARE AND EQUIPMENT:

   ✓ Each time you use glassware, be sure to check it for chips and cracks. Notify your lab supervisor of any damaged glassware so it can be properly disposed of.

   ✓ Be especially careful with electrical equipment like stirrers, hot plates, and power supplies (electrophoresis, etc.). Before using any high voltage equipment (voltages above 50V rms ac and 50V dc), make sure you get permission from your lab supervisor. Always unplug before handling and avoid contact with water. Use only one hand if you need to adjust any high voltage equipment. It's safest to place your other hand either behind your back or in a pocket.

✓ Never use lab equipment that you are not approved or trained by your supervisor to operate. If an instrument or piece of equipment fails during use or isn't operating properly, report the issue to a technician right away. **Never try to repair an equipment problem on your own!**

✓ If open flames like those of burners are necessary, make sure there are no flammable solvents in the area.

5. PERSONAL PROTECTION SAFETY RULES:

✓ **When performing laboratory experiments, you should always wear a lab robe.**

✓ When handling any toxic or hazardous agent, always wear the appropriate gloves.

✓ After performing an experiment, you should always wash your hands with soap and water.

✓ When using lab equipment and chemicals, be sure to keep your hands away from your body, mouth, eyes, and face.

6. CHEMICAL SAFETY RULES:

✓ **All chemicals in the laboratory are to be considered dangerous.** Do not allow any solvent to come into contact with your skin. Do not touch, taste, or smell any chemicals unless specifically instructed to do so. The proper technique for wafting chemical vapors will be demonstrated to you.

✓ Check the label on chemical bottles twice before removing any of the contents. Take only as much chemical as you need. Never return unused chemicals to their original containers.

✓ Never use mouth suction to fill a pipet. Use a rubber bulb or pipet pump.

✓ When transferring reagents from one container to another, hold the containers away from your body.

✓ **Acids must be handled with extreme care.** You will be shown the proper method for diluting strong acids. **Always add acid to water,** swirl or stir the solution and be careful of the heat produced, particularly with sulfuric acid.

✓ Handle flammable hazardous liquids over a pan to contain spills. Never dispense flammable liquids anywhere near an open flame or source of heat.

✓ Never remove chemicals or other materials from the laboratory area.

✓ Take great care when transporting acids and other chemicals from one part of the laboratory to another. Hold them securely and walk carefully.

7. ACCIDENTS AND INJURIES

✓ **Report any accident (spill, breakage, etc.) or injury (cut, burn, etc.) to the instructor immediately, no matter how trivial it may appear.**

✓ If you or your lab partner are hurt, **yell out immediately and as loud as you can to ensure you get help.**

✓ If a chemical splashes in your eye(s) or on your skin, immediately flush with running water from the eyewash station for at least 20 minutes. Notify the instructor immediately.

✓ When an acid or alkaline solution gets on your skin, it should be rinsed with 1 % $NaHCO_3$ or 1 % boric acid, respectively.

✓ The burned spot on the skin should not be treated with water; rather, a special bandage should be used.

1. INTRODUCTION TO BIOCHEMISTRY LAB ACTIVITIES

PRACTICAL WORK 1

1.1. Using laboratory equipment

In most labs, you'll encounter the same basic apparatus. Here, you will find a picture of the most commonly used lab equipment:

SHEET FOR NOTES
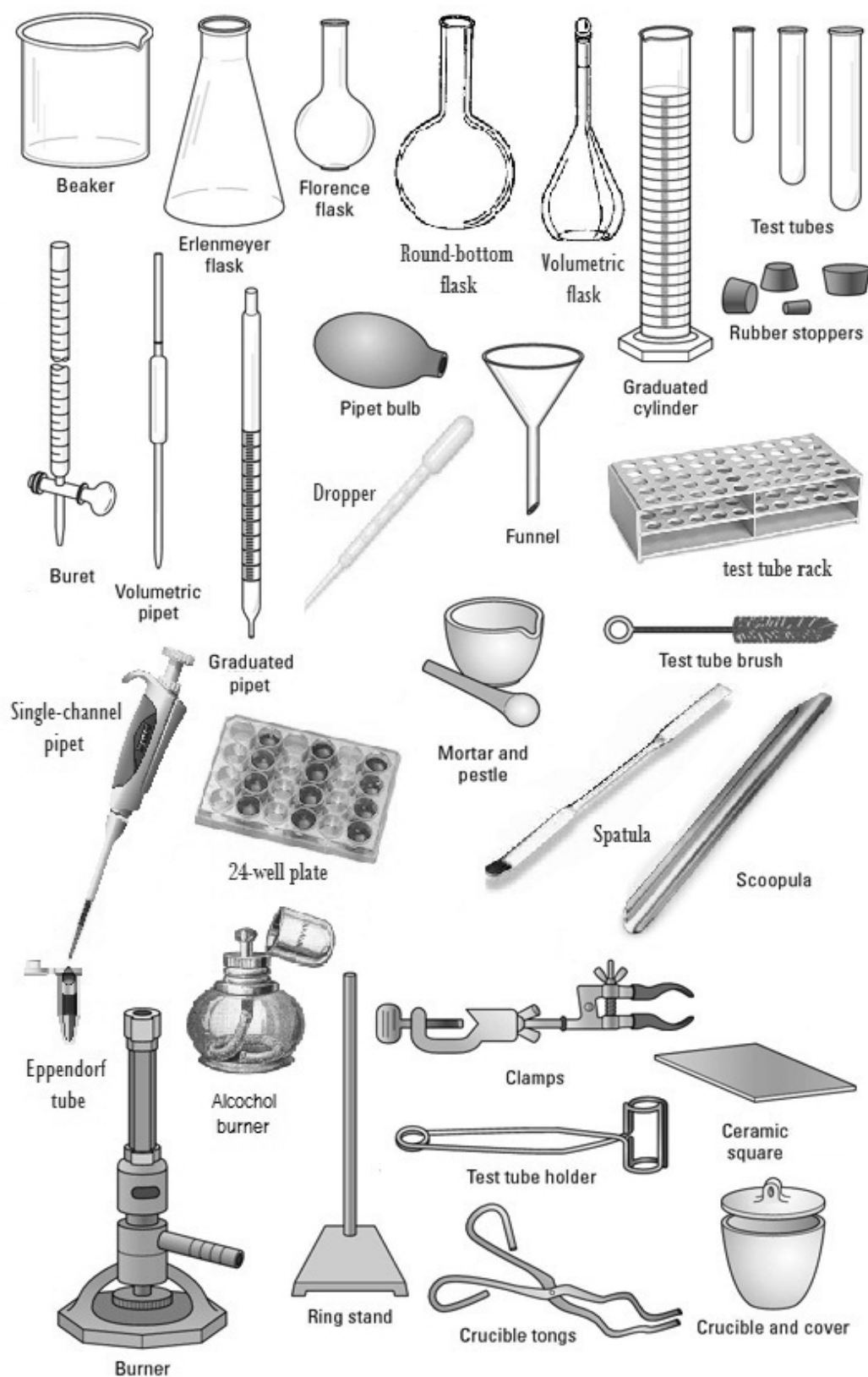
(listening to an explanation of your teacher write how different lab tools may be used)

Graduated cylinder activity

📖 A graduated cylinder (measuring cylinder) is a common piece of laboratory equipment used to measure the volume of a liquid. It has a narrow cylindrical shape. Each marked line on the graduated cylinder represents the amount of liquid that has been measured.

!!! A graduated cylinder can have numerous scales.

✏ *1. Determine the value for the minor grids on the cylinder:*

a)_____   b)_____   c)_____   d)_____
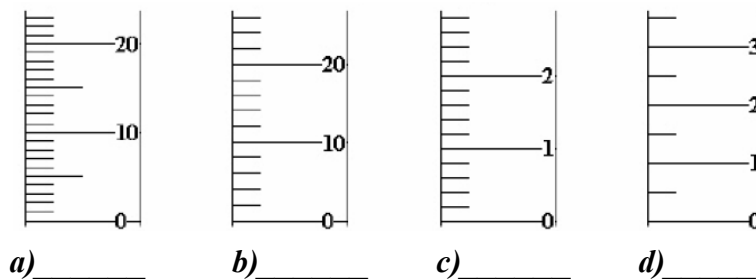
📖 When reading a graduated cylinder you need to keep the graduated cylinder on the desk and lower your eyes to the level of the **meniscus** and you read where the bottom of the meniscus is.
The main reason as to why the reading of the volume is done via meniscus is due to the nature of the liquid in a closed surrounded space. By nature, liquid in the cylinder would be attracted to the wall around it through molecular forces. This forces the liquid surface to develop either a **concave (A)** or **convex (B)** shape, depending on the type of the liquid in the cylinder. *Reading the liquid at the bottom part of a concave or the top part of the convex liquid is equivalent to reading the liquid at its meniscus.*

For example:

12.5 mL
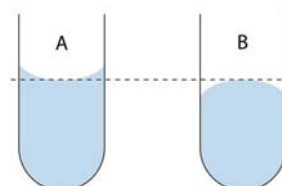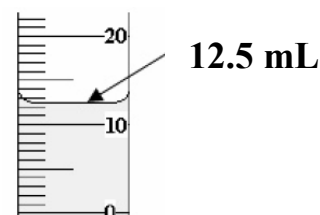
✏ *2. Determine the volume of line liquids in the following cylinders:*

a)_____   b)_____   c)_____   d)_____
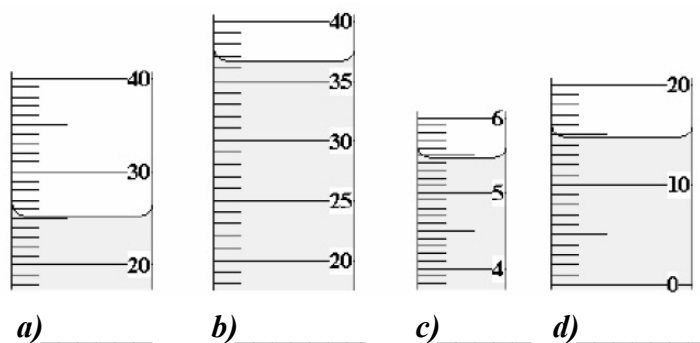
✏ *3. Draw in line meniscus for the following readings:*

a) 49.2 mL   *b) 18 mL*   *c)27.6 mL*   *d) 64 mL*
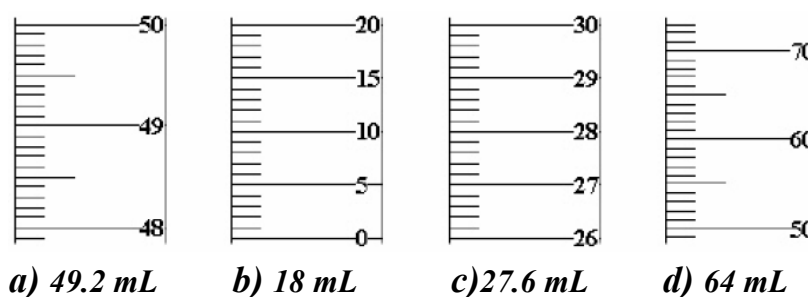
Micropipette activity

📖 Micropipettes are one of the primary tools of the laboratory. These micropipettes will allow you to accurately measure volumes as small as 1μl and as large as 1000μl.

NOTES:
➤ Do not attempt to set pipet for volumes larger than their maximum, or for volumes less than zero; doing so will damage the pipet.
➤ Never point a pipette up. This may cause liquid to run down into the pipette destroying it.
➤ When withdrawing liquids with the pipette, always release the plunger slowly. This prevents liquid from rushing into the end of the pipette and clogging it up. This is especially important with large volume pipettes.
➤ Be sure you use the proper size tip for each pipette.
➤ Always use a new tip for each different liquid.

Simple Check for Proper Calibration
Check the calibration of your micropipet by using the fact that 1 ml of distilled water has a mass of 1 g. Pipet a range of volumes spanning the pipette's useable range and weigh them on a top loading balance having at least 3 decimal place accuracy. Pipets having greater than 5 % error should be recalibrated.

https://di.uq.edu.au/community-and-alumni/sparq-ed/sparq-ed-services/using-micropipette
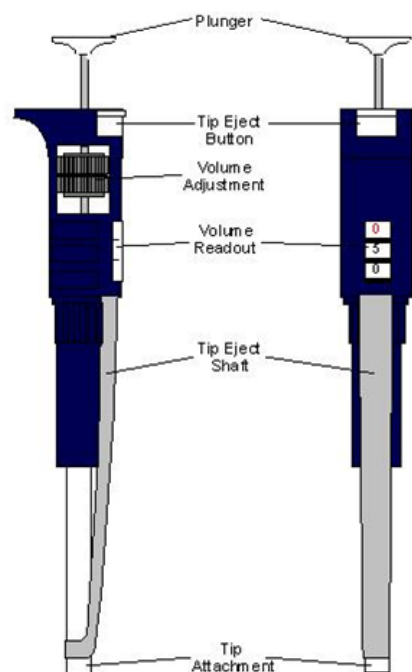
In this activity you will be using three micropipettes, P-10, P-100 and P-1000.
A *P-10* measures volumes of 1-10 μL. A *P-100* measures volumes of 10-100 μL. A *P-1000* measures volumes of 100-1000 μL.

!!! It is important to use the appropriate pipette for the intended volume.

✎ *Which pipette would you use if you wanted to measure the following volumes?*

a. 5 μL _____   d. 1.5 μL_____
b. 75 μL _____   e. 0.7 mL_____
c. 200 μL _____   f. 1.5 mL _____

✎ *Determine the following window settings and the appropriate pipet to be used given the following volumes.*

For example,

P-10
0
4.
7
4.7 μL

P-100
0
4
7
47 μL

P-1000
4
7
0
470 μL

A) P-1000
8
8
0
?_____ μL

C) P-?____
940 μL

E) P-10
0
4.
5
?_____ μL

B) P-100
0
9
8
?_____ μL

D) P-?____
50 μL

F) P-?____
29 μL

9

How to use a micropipette:

1. Make sure you are using the correct pipet for the intended volume. Turn the plunger until the volume you want to pipet is displayed in the window.
2. Next, you will need to select the appropriate disposable tip. Gently place the end of your pipet into a tip.
3. **Gently** press the plunger down and note that the pipet has **2 "stop" positions**.
4. *The first stop* is where you want to be when drawing up the appropriate volume of the sample. *The second stop* is to dispense the sample completely from the tip.
5. **Gently** press the plunger down to the first stop. Then insert the tip of the pipet into your sample solution. Begin releasing the plunger **very slowly** to prevent damaging the instrument.
6. Now you are ready to dispense the solution. Place the tip of the pipet into the container you are pipetting to and slowly dispense the solution. Do so by **slowly** pressing the plunger all the way down to the **second stop position.**
7. Keep your thumb at the second stop position until you have completely removed the pipet from the container to avoid drawing your sample back into your pipet.
8. When you are finished pipeting, simply eject your tip into a waste container by pressing down on the tip ejector button.

1 Liter (L) = 1000 milliliters (mL) = 1,000,000 µL

It is important to be able to carry out metric conversions.

1 µL = 0.001 milliliters = 0.000001 L

✎ *Using the following conversions, complete the problems below:*

1 µL = 1 x 10^{-3} mL = 1 x 10^{-6} L

1. 3 L = _____ mL
2. 3 µL = _____ mL
3. 10 mL = _____ µL
4. 100 µL = _____ mL

5. 100 mL = _____ L
6. 20 µL = _____ mL
7. 2000 mL = _____ L
8. 0.05 mL = _____ µL

FOR NOTES_____

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# 1.2. SOLUTIONS: concentrations and calculations

📖 In Chemistry, a **solution** is a homogeneous mixture of two things – a **solute** and the **solvent**. **Concentration** is a measure of how much solute is dissolved within the solvent.
For example, in water sugar solution, water is the solvent that dissolves sugar and sugar is the solute that is dissolved in water.

Concentration is the amount of solute in given solution.

If solutions can solve more solute at specific temperature, then we call them *unsaturated solutions.* If solution dissolves maximum amount of solute at specific temperature, then we call them as *saturated solutions.* If solutions contain more solute than its capacity, we call these solutions *supersaturated solutions.* We prepare them by heating solution and adding solute, after that we cool solution slowly. We can observe crystallization of solute in this solutions.

We can express concentration in different ways like concentration by percent or by moles.
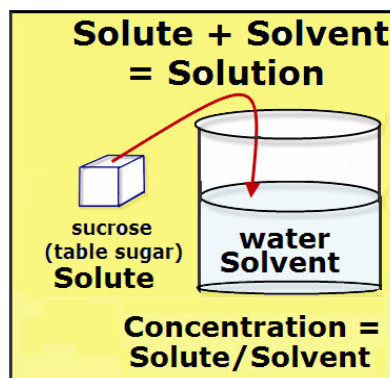
1) Concentration by Percent:

$$Percent\ by\ Mass = \frac{Mass\ of\ Solute}{Mass\ of\ Solution} \times 100$$

$$Percent\ by\ Volume = \frac{Volume\ of\ Solute}{Volume\ of\ Solution} \times 100$$

Example #1: 10 g salt and 70 g water are mixed and solution is prepared. Find concentration of solution by percent mass.
Solution:

Mass of Solute: 10 g

Mass of Solution: 10 + 70 = 80 g

80 g solution includes 10 g solute

100 g solution includes X g solute

Use simple proportion: X=10*100/80 = 12.5%

Example # 2: If concentration by mass of 600 g NaCl solution is 40 %, find the mass of solute in this solution.
Solution:

100 g solution includes 40 g solute

600 g solution includes X g solute

X=600*40/100 = 240 g NaCl salt dissolves in solution

2) Concentration by Mole:

$$Molarity\ (M) = \frac{Moles\ of\ Solute\ (moles)}{Volume\ of\ Solution\ (Liter)}$$

$$n\ (mol) = massa\ (g)\ /\ Mr \Rightarrow$$

$$m(g) = C_M\ (M) * Mr * V\ (L)$$

Example #3: What is the molarity of solution if you know that 0.40 moles of NaCl was dissolved in 250 mL to prepare it?
Solution:

Moles of Solute: 0.4 moles

Volume of Solution: 250 mL = 0.25 L

Molarity = 0.4/0.25 = 1.6 M

Example #4: How many grams of NaCl do you need to prepare 100 mL of 1 mM alanine solution?

Solution: m = 0.001 M * 58.5 * 0.1 = 0.00585 g =5.85 mg

Making Dilutions:

A solution can be made less concentrated by dilution with solvent. If a solution is diluted from V_1 to V_2, the molarity of that solution changes according to the equation:

$$M_1\ V_1 = M_2\ V_2$$

Example #5: How would you prepare 100 mL of 0.4 M $MgSO_4$ from a stock solution of 2.0M $MgSO_4$?
M1 = 2M   M2 = 0.4 M
V1 = ?   V2 = 100 mL

Solution: 2*X = 0.4*100 $\Rightarrow$ 0.4*100/2 = 20 mL

Making solution activity

1. How many grams of alanine are present in 100 mL of the 1 mM alanine solution?
   _____
   _____
   _____

2. How many milligrams of alanine are present in 100 mL?
   _____
   _____
   _____

3. What volume of a 5.0 M NaCl stock solution is necessary to prepare 500 mL of 0.16 M NaCl solution?
   _____
   _____
   _____
   _____

4. How would you prepare 250 mL of 70 % (v/v) of rubbing alcohol?
   _____
   _____
   _____
   _____
   _____

5. Calculate the amount of water, in grams, that must be added to prepare 16% by mass solution with 5.0 g of urea, (NH2)2CO ?
   _____
   _____
   _____
   _____

6. The density of ethyl acetate at 20 degrees is 0.902g/ml. What volume of ethyl acetate would be required to prepare a 2% solution of cellulose nitrate using 25g of cellulose nitrate?
   _____
   _____
   _____
   _____

7. You are required to make 750 mL of a solution that is 35.0% (w/v) NaCl. In the lab you have a solution that is 50.0% (w/v) NaCl and a solution that is 10.0% (w/v) NaCl. How would you make this solution?
   _____
   _____
   _____
   _____
   _____
   _____

8. You are required to make 100 mL 5% $Ca(NO_3)_2$. However, there is only $Ca(NO_3)_2 \cdot 4H_2O$ in the lab. How would you make this solution?
   _____
   _____
   _____
   _____
   _____

2. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF CARBOXYLIC ACIDS

📖 BACKGROUND

Carboxylic acids are structurally like aldehydes and ketones in that they contain the carbonyl group. However, an important difference is that carboxylic acids contain a hydroxyl group attached to the carbonyl carbon.

$$—COOH \quad \text{The carboxylic acid group}$$

This combination gives the group its most important characteristic; it behaves as an acid.

As a family, carboxylic acids are weak acids that ionize only slightly in water. As aqueous solutions, typical carboxylic acids ionize to the extent of only one percent or less.

$$R—COOH + H_2O \ \rightarrow \ R—COO^- + H_3O^+$$
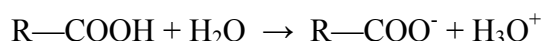
At equilibrium, most of the acid is present as un-ionized molecules. Water solubility depends to a large extent on the size of the R-group. Only a few low-molecular-weight acids (up to four carbons) are very soluble in water.
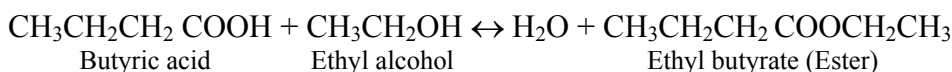
Although carboxylic acids are weak, they are capable of reacting with bases stronger than water. Thus while benzoic acid shows limited water solubility, it reacts with sodium hydroxide to form the soluble salt sodium benzoate. (Sodium benzoate is a preservative in soft drinks.)
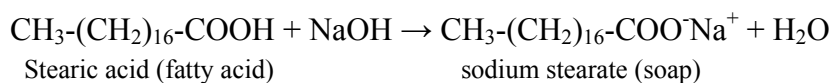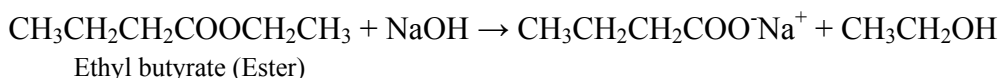
$$C_6H_5\text{-}COOH + NaOH \rightarrow C_6H_5\text{-}COO^-Na^+ + H_2O$$

Benzoic acid	Sodium benzoate
Insoluble	*Soluble*
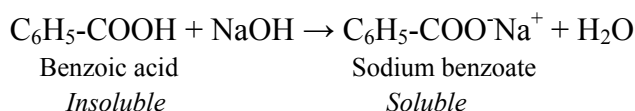
Sodium carbonate, Na_2CO_3, and sodium bicarbonate, $NaHCO_3$, solutions can neutralize carboxylic acids also.

$$CH_3COOH + Na_2CO_3 \rightarrow 2CH_3COONa + H_2O + CO_2$$

The combination of a carboxylic acid and an alcohol gives an ester; water is eliminated. Ester formation is an equilibrium process, catalyzed by an acid catalyst.

$$CH_3CH_2CH_2\,COOH + CH_3CH_2OH \leftrightarrow H_2O + CH_3CH_2CH_2\,COOCH_2CH_3$$

Butyric acid   Ethyl alcohol   Ethyl butyrate (Ester)

The reaction typically gives 60% to 70% of the maximum yield. The reaction is a reversible process. An ester reacting with water, giving the carboxylic acid and alcohol, is called *hydrolysis;* it is acid catalyzed. The base-promoted decomposition of esters yields an alcohol and a salt of the carboxylic acid; this process is called *saponification.* Saponification means, "soap making," and the sodium salt of a fatty acid (e.g., sodium stearate) is a soap.

$$CH_3CH_2CH_2COOCH_2CH_3 + NaOH \rightarrow CH_3CH_2CH_2COO^-Na^+ + CH_3CH_2OH$$

Ethyl butyrate (Ester)

$$CH_3\text{-}(CH_2)_{16}\text{-}COOH + NaOH \rightarrow CH_3\text{-}(CH_2)_{16}\text{-}COO^-Na^+ + H_2O$$

Stearic acid (fatty acid)   sodium stearate (soap)

A distinctive difference between carboxylic acids and esters is in their characteristic odors. Carboxylic acids are noted for their sour, disagreeable odors. On the other hand, esters have sweet and pleasant odors often associated with fruits, and fruits smell the way they do because they contain esters. These compounds are used in the food industry as fragrances and flavoring agents.

For example, the putrid odor of rancid butter is due to the presence of butyric acid, while the odor of pineapple is due to the presence of the ester, ethyl butyrate. Only those carboxylic acids of low molecular weight have odor at room temperature. Higher-molecular-weight carboxylic acids form strong hydrogen bonds, are solid, and have a low vapor pressure. Thus few molecules reach our noses. Esters, however, do not form hydrogen bonds among themselves; they are liquid at room temperature, even when the molecular weight is high. Thus they have high vapor pressure and many molecules can reach our noses, providing odor.

Carboxylic acids occur widely in nature. The fatty acids are components of glycerides, which in turn are components of fat. Peanut butter, cheese, chicken, and eggs - all of these contain fatty acids. Having the right amount and the healthy type of fat daily is great for our health because it can help our bodies function efficiently each day. Hydroxyl acids, such as lactic acid (found in sour-milk products) and citric acid (found in citrus fruits), and many keto acids are important metabolic products that exist in most living cells. Proteins are made up of amino acids, which also contain carboxyl groups.

Did you know?

Have you ever wondered what ingredient in our acne creams can make those pesky pimples go away? It is a carboxylic acid called salicylic acid.

Do you occasionally experience that inconvenient headache after a long day of work? You can say goodbye to that if you use aspirin, which contains a carboxylic acid called acetylsalicylic acid.

Carboxylic Acid	Chemical Structure	Uses
Salicylic Acid $C_7H_6O_3$		Facial cleanser Acne medication Anti-aging cream
Acetylsalicylic Acid $C_9H_8O_4$		Medication to relieve fever, inflammation, pain (Aspirin)

HOME PROJECT

(Write here your own example of carboxylic acid which has significant medical importance)

☠ **SAFETY CONCERNS:** sodium hydroxide, hydrochloric acid, sulfuric acid and glacial acetic acids are dangerous to skin, eyes, mucus membranes and clothes. Use these with caution. If contacted, wash with soap and copious amounts of tap water.

2.1. Odor, solubility and pH of carboxylic acids and their salts

Procedure:

Take 3 clean dry test tubes (any size) and label them 1, 2, and 3.

1. Into each tube place about 2 mL of deionized water.

2. Into the tubes add the following acids and mix each well:

Tube 1 - add 10 drops of glacial Acetic acid

Tube 2 - add about 0.1g of solid Benzoic acid.

Tube 3 - add about 0.1g of solid Succinic acid.

3. Note its odor by wafting (moving your hand quickly over the open end of the test tube) the vapors toward your nose. *Note the odor of each acid solution into the table below.*

4. In the non-shaded boxes of the report sheet, *record the solubility of each acid* in water at room temperature: S = soluble, I = insoluble, PS = partially soluble.

5. Take a glass rod and dip it into the solution. Using wide-range indicator paper (pH 1-12), test the pH of the solution by touching the pH paper with the wet glass rod. Determine the value of the pH by comparing the color of the pH paper with the chart on the dispenser. *Record the pH* of each soluble or partially soluble acid solution.

6. To each carboxylic acid solution add 3 mL of 3M NaOH. Stopper each tube and shake vigorously to mix. Determine the pH of each solution as before. If a solution is not basic, add more NaOH dropwise and mix until the solution is basic. *Record the final pH.*

7. *Note the solubility and the odor* of the now basic salt solutions and compare each to the solubility and odor of the solutions before the addition of base.

8. To each basic salt solution add 3 mL of 3M HCl and stopper & shake to mix. Determine the pH of each. If a solution is not acidic, add more HCl dropwise until the solution becomes acidic. *Record the final pH.*

9. *Record your observations.* (Does the original odor return? Original solubility?)

10. Using condensed structural formulas *write the equations for the reactions* of each acid with NaOH and then equations for the reactions of each salt formed with acid. *Include names of the sodium salts formed.*

✎ *Recorde the observations in the table*

1. Acetic Acid			
Another name for the Acid = _____ Name of the Salt = _____			
Property	**A. Water Solution**	**B. NaOH solution**	**C. HCl Solution**
Draw the structural formulas for the missing organic compounds	Acid + NaOH ⟶	Solt + HCl ⟶	?
Odor (strong S, mild M, none N)			
Solubility/ pH (soluble S, insoluble I)	Solubility / pH	Solubility / pH	Solubility / pH

2. Benzoic Acid

Another name for the Acid = _____ Name of the Salt = _____

Property	A. Water Solution		B. NaOH solution		C. HCl Solution	
Draw the structural formulas for the missing organic compounds	Acid + NaOH →		Solt + HCl →		?	
Odor (strong S, mild M, none N)						
Solubility/ pH (soluble S, insoluble I)	Solubility	pH	Solubility	pH	Solubility	pH

3. Succinic acid

Another name for the Acid = _____ Name of the Salt = _____

Property	A. Water Solution		B. NaOH solution		C. HCl Solution	
Draw the structural formulas for the missing organic compounds	Acid + NaOH →		Solt + HCl →		?	
Odor (strong S, mild M, none N)						
Solubility/ pH (soluble S, insoluble I)	Solubility	pH	Solubility	pH	Solubility	pH

Conclusion _____
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2.2. Formation of salts

Procedure

A. In the test tube mix 1 mL of 50 % acetic acid with equal amounts of 5 % sodium carbonate.

✎ *What do you observe?*_____

✎ *Complete the equation:*

$$CH_3COOH + Na_2CO_3 \rightarrow$$ _____

B. Add to the test tube 3 ml of 50 % acetic acid. Place into the tube one zinc bit and heat the reaction mixture.

✎ *What happens?*_____

✎ *Complete the equation:*

$$CH_3COOH + Zn \rightarrow$$ _____

Conclusion_____
_____
_____
_____
_____

2.3. Preparation of esters

Procedure:

1. Take 4 clean dry test tubes and label them 1, 2, 3 and 4.
2. Into the appropriate test tube, pour the correct amount of carboxylic acid and add the corresponding alcohol as indicated below:

   tube #1: 1 mL of glacial acetic acid (CH_3COOH) and 1 mL of ethanol (CH_3CH_2OH);

   tube #2: about 0.2g of benzoic acid and 2 mL of ethanol (CH_3CH_2OH);

   tube #3: 1 mL of glacial acetic acid (CH_3COOH) and 1 mL of butanol (C_4H_9OH)

   tube #4: about 0.2g of benzoic acid and 2 mL of butanol (C_4H_9OH)

3. Add 4 drops of concentrated sulfuric acid to each test tube.
4. Pour about 150 mL of tap water in a 250 mL beaker. Place the test tubes loosely closed with the stoppers in the water and heat the water on a hot plate to a temperature of 60-80°C. Leave the test tubes in the hot water bath for 15 minutes.
5. Cool the test tubes by immersing them in cold water in another beaker.
6. Add 5 mL of distilled water to each of the test tubes.
7. Carefully note the odor of the contents of each of the test tubes in the Table below. Hold the test tube about 30 cm away from your nose and gently waft the vapors towards your nose without inhaling deeply. Each of the odors should be somewhat familiar to you. Alternatively, the contents of the test tube may be poured into a beaker half full of water and the odor above it detected carefully.

NOTE: The reason for adding water to the contents of the test tube is to separate the esters from the reactants used. Esters are soluble in alcohol, but insoluble in water, and they generally have a density less than that of water, enabling them to separate and float to the top of the liquid mixture. This makes the detection of the odor more reliable.

✒ Write the equations for the esterification reactions you performed including the structures and names of the products formed	Odor

Tube #1
    Acetic acid   ethanol   ester
 	
                +   ⇒
Name of Ester:	

Tube #2
    Benzoic acid   ethanol   ester
 	
                +   ⇒
Name of Ester:	

Tube #3
    Acetic acid   butanol   ester
 	
                +   ⇒
Name of Ester:	

Tube #4
    Benzoic acid   butanol   ester
 	
                +   ⇒
Name of Ester:	

Conclusion _____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Teacher's signature _____ (_____)
full name

SOME MORE QUESTIONS:

1. Which of the following statements is INCORRECT about carboxylic acids?

a) They are nonpolar substances.
b) They are organic compounds.
c) They tend to have higher boiling points than water.
d) They are weak acids.

✎ *Explain you answer (try to write 1-2 sentences on each statement):*_____
_____
_____
_____
_____
_____
_____
_____

2. Which of the following is a carboxylic acid?
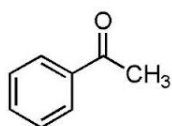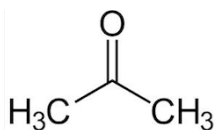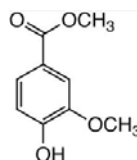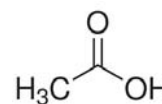
a	b	c	d

✎ *You answer is*_____

✎ *Explain your decision:*_____
_____
_____
_____
_____
_____

3. Which acid and alcohol are needed to prepare Methyl butanoate? Show the reaction and name the starting materials.

Carboxylic Acid		Alcohol		Ester	Water
	+		H⁺ →		+ H—O—H

Name_____   Name_____   **methyl butanoate**   water

19
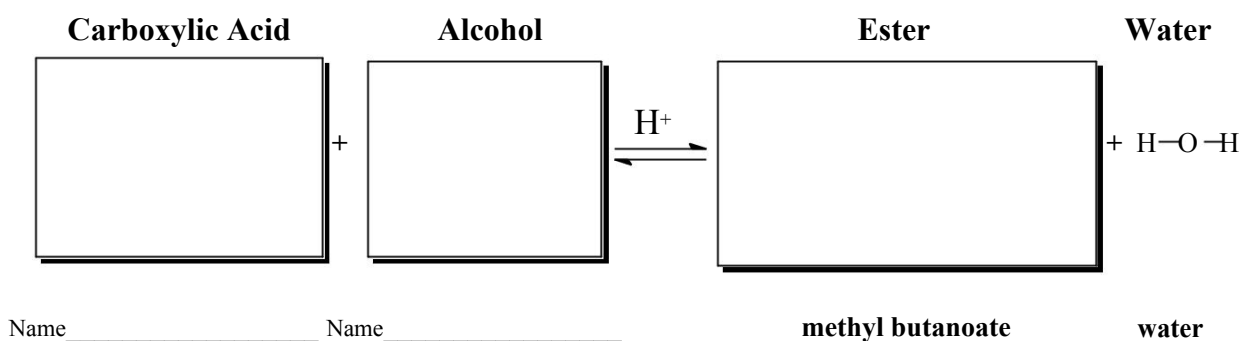
3. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF LIPIDS
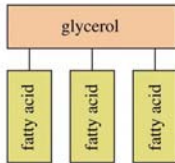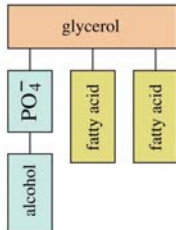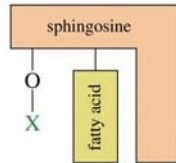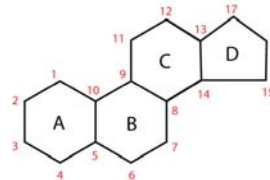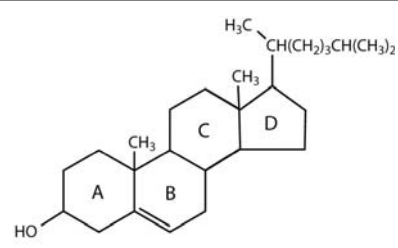
📖 BACKGROUND

LIPIDS – very important biomolecules which is insoluble in water but soluble in organic solvents and other lipids

FUNCTIONS OF LIPIDS

- ✓ Storage molecules for ENERGY (fats and oils): 1 gram of fat = 38.9 kJ
- ✓ Structural components of cellular membranes
- ✓ Protective molecules (waxes)
- ✓ Hormones and vitamins
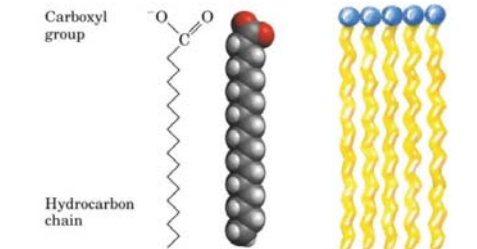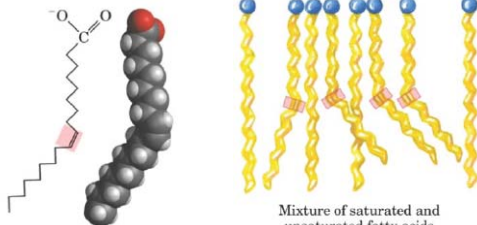- ✓ Intracellular messengers
- ✓ Pigments
- ✓ Insulation

FOUR MAIN CLASSES OF LIPIDS

1. **Triacylgylcerols** – storage lipids (non-polar). Also known as **triglycerides;** 2. **Phosphoacylglycerols** – membrane structural lipids (polar). Also known as **glycerophospholipids;** 3. **Sphingolipids** – membrane structural lipids (polar)	*These three have the basic structure of a <u>fatty acid</u>* **Figure 10-6** **Lehninger Principies of Biochemistry, Fifth Edition** © 2008 W.H.Freeman and Company
4. **Non-saponifiable lipids** – steroids, hormones, cholesterol *Based on a fused ring structure rather than fatty acids*	

FATTY ACIDS (FAs) – long chain **carboxylic acids:**

- ✓ 12-20 hydrocarbon LINEAR chains (the hydrocarbon chain is almost always unbranched);
- ✓ Most carbon-carbon bonds are single: $-H_2C-CH_2-$ (*saturated FAs*); however, they oftern contain one, two, or more carbon-carbon double bonds: $-HC=CH-$ (*unsaturated FAs*);
- ✓ <u>Fatty acid chain length and degree of unsaturation affect on **melting point** and **fluidity** of lipids;</u>
- ✓ No hydrogen bonds form between the carboxylic acid functional group. Fatty Acids interact through HYDROPHOBIC INTERACTIONS;
- ✓ By nature, fatty acids are *AMPHIPATHIC* – have both hydrophilic and hydrophobic parts;
- ✓ pKa of carboxylic acid is ~ 4-5; therefore deprotonated at physiological pH.

THE MAIN CHARACTERISTICS OF TWO TYPES OF FAs

SATURATED FATTY ACIDS – hydrocarbon chain has NO double bonds ✓ Pack close together ✓ Less fluid (FAs can't move as freely) ✓ Higher melting temperature because it takes more energy to break interactions ✓ Likely to be solids at room temperature	
UNSATURATED FATTY ACIDS – hydrocarbon chain has ONE or MORE DOUBLE BONDS Double bonds are most often "cis" configuration. Cause a kink or bend in the chain ✓ Do NOT pack as closely ✓ More fluid than saturated ✓ Lower melting temperature than saturated ✓ Likely to be liquid at room temperature	 **Figure 10-1** *Lehninger Principies of Biochemistry, Fifth Edition* © 2008 W.H.Freeman and Company ✓ For monounsaturated FAs, the double bond is usually bitween carbons 9 and 10. ✓ If more then one carbon-carbon double bond is present they are not conjugated but are separated by a methylene unit.

NOMENCLATURE OF FAs

1) ***Numbering from the carboxyl end*** - carboxyl C is No 1

Referred to as a system of numbers: **# of carbons: # of double bonds $\Delta^{x, y, z}$ (position of double bonds)**

For example: oleic acid:

(a) 18:1(Δ^9) *cis-9-Octadecenoic acid*
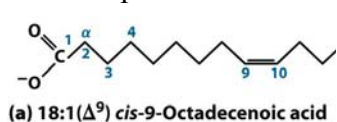
# of carbons is counted from the carbonyl end and includes the carboxyl carbon. Double bond starts at number written, therefore between 9 and 10 in the example.

2) ***Numbering from the methyl end*** - methyl C is marked ω. The position of the first double bond is counted from the methyl end. For example: linoleate (C18:2$\Delta^{9,12}$) bilongs to ω6 family of FAs:
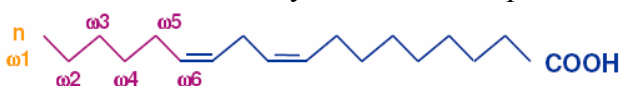
✎ *Complete the table with the structure of common, naturally occurring FAs*

| Number of carbons	Common Name	Abbreviated Symbol	Structure	ω class
16	Palmitic acid	16:0		
16	Palmitoleic acid	16:1Δ^9		
18	Stearic acid	18:0		
18	Linoleic acid	18:2$\Delta^{9,12}$		
18	Linolenic acid	18:3$\Delta^{9,12,15}$		
20	Arachidonic acid	20:4$\Delta^{5,8,11,14}$		

1. TRIACYLGLYCEROLS (TAGs)

TAGs are made up from 3 fatty acids ester linked to glycerol
- Each –OH on glycerol can react with a fatty acid
- Start with C1 $\Rightarrow$ C2 $\Rightarrow$ C3
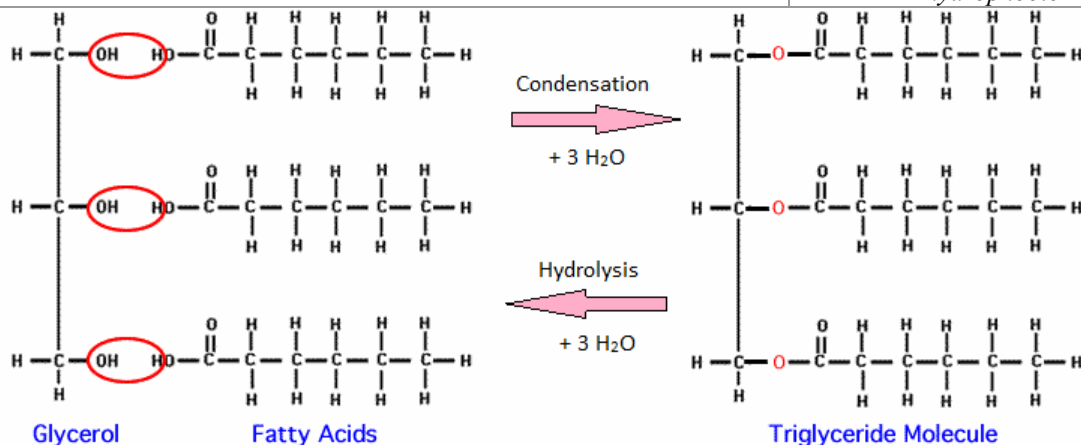- Release H_2O upon formation of ester linkage

How are they broken down?

Hydrolyzed into 3 fatty acids and 1 glycerol
<u>Physiologically in body:</u> enzyme called a **LIPASE** present in adipocytes and intestines
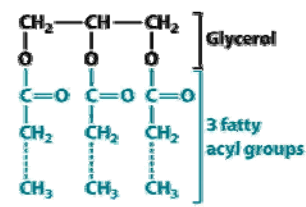
From the structures it is observed that (TAGs) are neutral (no ionic groups), non-polar and hydrophobic

from *http://science.halleyhosting.com/sci/ibbio/chem/notes/chpt3/triglyceride.htm*

Triacylglycerols as STORAGE LIPIDS comprise fats and oils !!! Rich source of energy

OILS (usually from plants) contain more **unsaturated** fatty acids – liquid at room temperature. *Except coconut oil*

FATS (usually from animals) contain more **saturated** fatty acids. Found in oily droplets in the cytoplasm of **adipocytes.** *Recommended:* consume **more** unsaturated than saturated fats. Saturated fat leads to atherosclerosis, heart disease and cancer.

HOME PROJECT

Do you know what SAPONIFICATION is?

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

2. PHOSPHOACYLGLYCEROLS (Phospholipids; Phosphoglycerides)

Very similar in structure to triacylglycerols except one of the alcohols of glycerol is esterified by **phosphoric acid** instead of a fatty acid = **phosphatidic acid (PA)**

Position 1 favors SATURATED FAs
Position 2 favors UNSATURATED FAs

✓ The phosphoric acid group is then **esterified** by a **second alcohol** to form the **phosphoacylglycerol.**
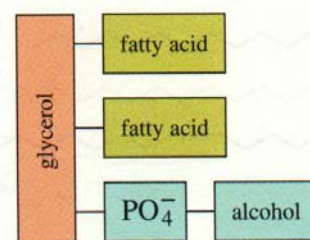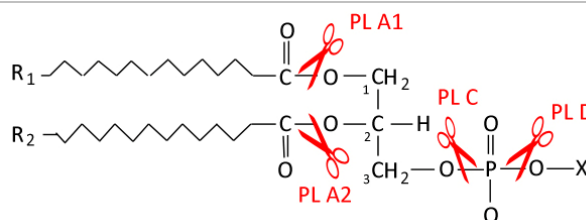
Glycerophospholipids

Figure 10-1
Lehninger Principies of Biochemistry, Fifth Edition
© 2008 W.H.Freeman and Company

These alcohols give very different properties to the phospholipids due to different structures

Name of **X**	Structure of **X**	Name of Glycerophospholipids
(a) Hydrogen	—H	Phosphatidic acid
(b) Ethanolamine	—CH_2—CH_2—$\overset{+}{N}H_3$	Phosphatidylethanolamine
(c) Choline	—CH_2—CH_2—$\overset{+}{N}(CH_3)_3$	Phosphatidylcholine
(d) Serine	—CH_2—CH—$\overset{+}{N}H_3$; COO^-	Phosphatidylserine
(e) Inositol	(inositol ring structure)	Phosphatidylinositol

Figure 8-7 part 2 Concepts in Biochemistry, 3/e
© 2006 John Wiley & Sons

Glycerophospholipid (general strucure)

Saturated fatty acid (e.g., palmitic)
Unsaturated fatty acid (e.g., oleic)

Phospholipids can be degraded to their component parts by a family of enzymes called **PHOSPHOLIPASES (PL).**
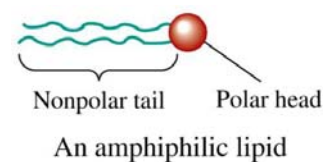
from *https://ru.m.wikipedia.org/wiki/Файл:Phospholipase.jpg*

Phospholipids are MUCH MORE **amphiphilic** (has both hydrophilic and hydrophobic regions) than triacylglycerols due to CHARGED groups at neutral pH

Therefore we can say that phospholipids have:
   ✓ One **POLAR HEAD**
   ✓ Two **NON-POLAR TAILS**

Nonpolar tail   Polar head
An amphiphilic lipid

FOR NOTES _____

_____

_____

_____

_____

3. SPHINGOLIPIDS

- ✓ Much more amphiphilic than triacylglycerols
- ✓ Membrane lipids based on the core structure of SPHINGOSINE, a long chain amino alcohol
- ✓ Fatty acid linked to sphingosine at an **AMINO** group at position 2
- ✓ 2nd esterification takes place at the **HYDROXYL** (-OH) on sphingosine
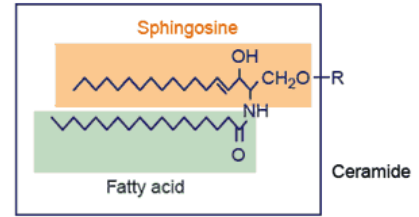
If R = H → ceramide
If R = sugar → cerebroside
If R = phosphocholine → sphingomyelin
If R = complex oligosaccharide → ganglioside

Sphingomyelin
- ✓ Insulates nerve axons
- ✓ Major lipid of myelin sheaths

Cerebrosides and Gangliosides (glycolipids)
- ✓ Abundant in brain and nervous system membranes
- ✓ Improper degradation results in many metabolic diseases

Tay-Sachs Disease
Gangliosides accumulate in nerve cells, brain and spleen ⇒ Death!

Gaucher Disease
Accumulation of glucocerebrosides
- Enlarged liver and spleen
- Bone pain
- Anemia

Name of **X**	Structure of **X**	Name of Sphingolipid
Hydrogen	—H	Ceramide
Phosphocholine	$-P(=O)(O^-)-O-CH_2CH_2\overset{+}{N}(CH_3)_3$	Sphingomyelin
Glucose	(glucose ring structure)	Glucosylcerebroside
Complex oligosaccharide	(galactose–glucose / Sia)	Ganglioside

Figure 8-8 part 2 Concepts in Biochemistry, 3/e
© 2006 John Wiley & Sons

4. NON-SAPONIFIABLE LIPIDS/STEROIDS

- ✓ Based on a fused ring system – **RIGID** structure
- ✓ No ester linkages
- ✓ Includes **HORMONES** (testosterone, progesterone, estrogen)

Cholesterol
- ✓ Common membrane lipid
- ✓ Almost exclusive to animal cells
- ✓ Very hydrophobic but amphiphilic
  - Hydrophilic group is the -OH on ring A
- ✓ Serves as the starting point for synthesis of steroid hormones

✎ *Give your own example of non-saponifiable lipid. Describe structure and function:*

PRACTICAL WORK 3

☣ **SAFETY CONCERNS:** Chloroform, methanol, n-hexane, ether and acetic acid are the volatile toxic liquids. *THEY SHOULD BE HANDLED UNDER A FUME HOOD.*

3.1. Separation of the lipids by thin-layer chromatography on silufol plate

📖 Thin-layer chromatography consists of a *stationary phase* immobilized on a glass or plastic plate and a *solvent*. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. The constituents of a sample can be identified by simultaneously running the standards with the unknown samples.
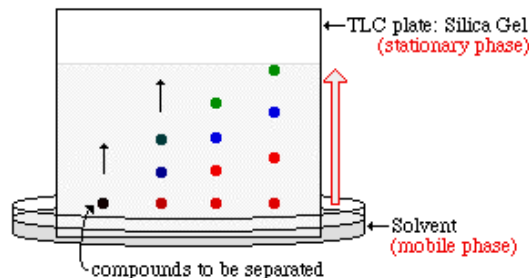
One edge of the plate is then placed in a solvent reservoir and the solvent moves up the plate by capillary action. When the solvent front reaches the other edge of the stationary phase, the plate is removed from the solvent reservoir. The separated spots are visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their partioning behavior between the mobile liquid phase and the stationary phase.

Reagents. Lipid extract in mixture of chloroform and methanol (2:1), mixture of n-hexane, ether and acetic acid (80:20:1), crystalline iodine.

*Equipment.* Chromatographic chamber, silufol plates (20x10 cm).

Procedures:
1. Apply a drop (≈ 0,005 mL) of the lipid extract on the silufol plate at the distance of 2 cm from the lower edge (start line) and carry out the upward separation in the solvent to a distance of 10 cm.
2. After that, take the chromatographic plate out of the chamber and dry under a draft hood. Detect the results of the sepatation in the vapor of iodine.
3. Put the silufol plate into the beaker with several crystals of iodine and covere it with a glass. Leave it at room temperature for 3-5 minutes.
5. Few minutes later lipids appear as the yellow or yellow-brown spots on the white or weak-yellow background of the plate.
6. Using the ruler, determine the distances that the solvent (solvent front, h_f) and the components of the lipid extract (h_1, h_2, h_3, h_4, h_5) passed. For each lipid component, calculate the distribution coefficient Rf by the formula:

$$Rf = h/h_f$$

h – the distance moved by the component of the lipid extract *(h_1, h_2, h_3, h_4, h_5);*
h_f – distance moved by the solvent front.
Serum lipids on the chromatogram are located by decreasing the Rf value: cholesterol esters > triacylglycerols > fatty acids > cholesterol > phospholipids.
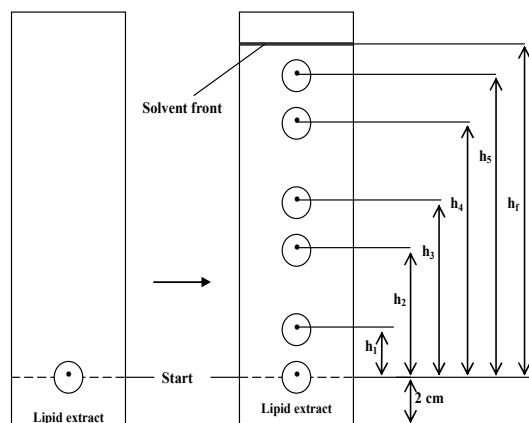
✎ *Record the obtained data to the table below.*

$h_f =$ _____

The order number of lipid extract component (count down from the start line)	The sample of lipid estract #1		The sample of lipid estract #2	
	h	R_f	h	R_f
1	$h_1 =$	$Rf_1 =$	$h_1 =$	$Rf_1 =$
2	$h_2 =$	$Rf_2 =$	$h_2 =$	$Rf_2 =$
3	$h_3 =$	$Rf_3 =$	$h_3 =$	$Rf_3 =$
4	$h_4 =$	$Rf_4 =$	$h_4 =$	$Rf_4 =$
5	$h_5 =$	$Rf_5 =$	$h_5 =$	$Rf_5 =$

Conclution _____

_____
_____
_____
_____
_____
_____
_____
_____

Teacher's signature _____ (_____)

full name

SOME MORE QUESTIONS:

1. A developed TLC plate has a solvent front of 6 cm and a solute front of 3 cm. What is the Rf value of the compound?

- 3
- 2
- 0.5
- 0.2

2. What is the maximum Rf value for any molecule in paper chromatography?

- 0.1
- 1.0
- 10
- Infinity

3. What does Rf value stands for:

- Retention factor
- Retardation factor
- both Retention factor & Retardation factor
- Relative factor

4. What does it mean when the Rf value of a molecule is 1?

- The molecule has more affinity to mobile phase.
- The molecule is more adsorbed onto stationary phase.
- The interaction between molecule and mobile phase is weaker
- None of the above.

5. When the thickness of adsorbent layer is increased, the Rf value will?

- Remains the same
- Decrease
- Increase
- None of the above.

4. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF AMINO ACIDS

📖 BACKGROUND

AMINO ACIDS – are the building blocks of proteins. There are 20 different amino acids in the proteins that make up living cells. Amino acids are organic compounds containing *amine* ($-NH_2$) and *carboxyl* (-COOH) functional groups, along with a *side chain* (R group) specific to each amino acid.
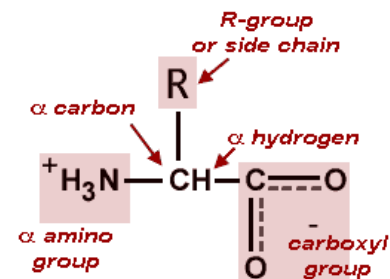
PROPERTIES OF AMINO ACIDS
- ✓ Only L-α-amino acids occur in proteins;
- ✓ Amino acids may have positive, negative, or zero net charge. At its isoelectric pH (pI) an amino acid doesn't bear net charge;
- ✓ Amino acids are weak acids;
- ✓ All amino acids are soluble in water and alcohol (polar solvents); but insoluble in nonpolar solvents (benzene).

GENERAL STRUCTURE OF AMINO ACID

All amino acids include five basic parts:	
1. α-carbon atom 2. α-hydrogen atom 3. an amino group – consisting of a nitrogen atom and two hydrogen atoms 4. a carboxyl group – consisting of a carbon atom, two oxygen atoms and one hydrogen atom 5. an R-group or side chain - consisting of varying atoms	 from *http://www.biology.arizona.edu/biochemistry/problem_sets/aa/basicstruct.html*

The R-group (side chain) is what makes each amino acid unique. Each of the 20 amino acids has a different side chain structure. Side chains contain mainly hydrogen, carbon, and oxygen atoms. Some amino acids have sulfur or nitrogen atoms in their R-groups.

The "R" group side chains on amino acids are VERY important:
- ➤ Determine the properties of the amino acid itself
- ➤ Determine the properties of the proteins that contain those amino acids;
- ➤ Dictate what a protein can and cannot do and how it folds.

AMINO ACIDS CAN ASSEMBLE INTO CHAINS (peptides, polypeptides, proteins):

- ✓ Amino acids are linked by **COVALENT BONDS = PEPTIDE BONDS;**
- ✓ Peptide bond is an **amide linkage** formed by a **condensation reaction** (loss of water);
- ✓ Brings together the **alpha-carboxyl** of one amino acid with the **alpha-amino** of another;
- ✓ R groups remain **UNCHANGED** – remain active;
- ✓ N-terminal amino and C-terminal carboxyl are also available for further reaction;
- ✓ Reaction is NOT thermodynamically favorable (not spontaneous). Need energy and other components and instructions.

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!!! You have to know the structures of 20 amino acids as well as their three letter and one letter codes.

NON-POLAR

Glycine (Gly / G)

Alanine (Ala / A)

Valine (Val / V)

Cysteine (Cys / C)

Proline (Pro / P)

Leucine (Leu / L)

Isoleucine (Ile / I)

Methionine (Met / M)

Tryptophan (Trp / W)

Phenylalanine (Phe / F)

+ CHARGE

Lysine (Lys / K)

Arginine (Arg / R)

Histidine (His / H)

POLAR

Serine (Ser / S)

Threonine (Thr / T)

Tyrosine (Tyr / Y)

Asparagine (Asn / N)

Glutamine (Gln / Q)

− CHARGE

Aspartic Acid (Asp / D)

Glutamic Acid (Glu / E)

from *http://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/24-proteins/amino-acids.html*

HOME PROJECT
What do you know about *ESSENTIAL AMINO ACIDS?*

AMPHOLYTE AND ISO-ELECTRIC POINT

1. Amino acids can exist as ***ampholytes*** or ***zwitterions*** (German word "zwitter" = hybrid) in solution, depending on the pH of the medium.

2. The pH at which the molecule doesn't carry the net charge is known as ***iso-electric point*** or isoelectric pH (pI).

3. In acidic solution they are cationic in form and in alkaline solution they behave as anions.
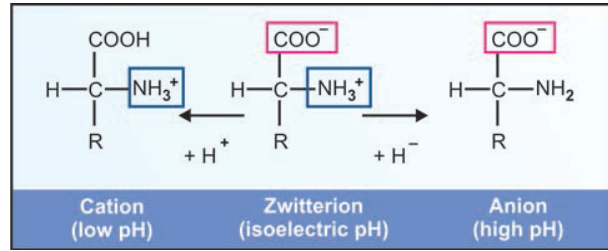
Figure 3-13
Textbook of Biochemistry for Medical Students
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4. At iso-electric point the amino acid will carry no net charge; all the groups are ionized but the charges will cancel each other. Therefore at isoelectric point, there is no mobility in an electrical field. ***Solubility and buffering capacity will be minimum at iso-electric pH.***

5. To such a solution if we add hydrochloric acid drop by drop, at a particular pH, 50% of the molecules are in cation form and 50% in zwitterion form. This pH is pK1 (with regard to COOH). If more HCl is added, more molecules become cationic in nature and solubility increases.

6. On the other hand, if we titrate the solution from iso-electric point with NaOH, molecules acquire the anionic form. When 50% of molecules are anions, that pH is called pK2 (with respect to NH_2).

7. From the graph it is evident that the ***buffering action is maximum in and around pK1 or at pK2 and minimum at pI.***

9. In the case of amino acids having more than two ionizable groups, correspondingly there will be more pK values (pK1 (COOH), pK2 (NH_2), pK3 (R-group)), e.g. Aspartic acid:

from   http://schoolbag.info/chemistry/mcat_biochemistry/2.html
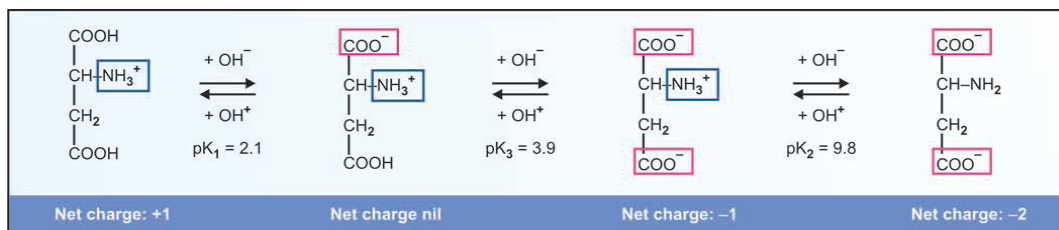
Figure 3-15
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The pK values of amino acids:

Name	pK_1	pK_2	pK_R
Glycine	2.4	9.8	
Alanine	2.3	9.7	
Valine	2.3	9.6	
Leucine	2.4	9.6	
Isoleucine	2.4	9.7	
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.0	10.6	
Serine	2.1	9.2	
Threonine	2.6	10.4	
Cysteine	1.8	10.8	8.3
Asparagine	2.0	8.8	
Glutamine	2.2	9.1	
Tyrosine	2.2	9.1	10.9
Tryptophan	2.4	9.4	
Aspartate	2.0	10.0	3.9
Glutamate	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

Table 3-2 Concepts in Biochemistry, 3/e
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How can you calculate pI?
1. Draw out the complete ionization of amino acid
2. Determine net charge on each ionized form
3. Find the structure that has no net charge
4. Take the average of the pKa's that are around the structure with **NO NET CHARGE**
pI = (pKa1 + pKa2)/2
5. Note do **NOT** just take the average of all pKa's.

For example: pI (Ala) = (2.3+9.7) = 6
What about Asp?? pKas: 2, 3.9, 10 (from Table)
     pI = (2+3.9)/2 = 2.95

!!! The pK value of imidazolium group of histidine is 6.0, and therefore effective as a buffer at the physiological pH of 7.4. The buffering capacity of plasma proteins and hemoglobin is mainly due to histidine residue.

PRACTICAL WORK 4

4.1. Thin layer chromatography for separation of amino acids

📖 Amino acids differ from each other in their side chains or R groups, which vary in structure, size, and electric charge. The interaction of the amino acids with the stationary phase like silica depends on their 'R' groups. The amino acid that interacts strongly with silica will be carried by the solvent to a small distance, whereas the one with less interaction will be moved further. By running controls (known compounds) alongside, it is possible to identify the components of the mixture. Since amino acids are colourless compounds, ninhydrin is used for detecting them. Ninhydrin reacts with α- amino acids that results in purple coloured spots. *Rf* values can be calculated and compared with the reference values to identify the amino acids.

☗ **SAFETY CONCERNS:** You have to take Ninhydrin reagent carefully. This reagent is a strong oxidizing agent so be attentive not to spill it on your hands or other body parts. Accidental spill of this reagent will cause severe itching sensation. Wash the spilled area with cold water and inform the lab assistant immediately.

Reagents. Mixture of amino acids and their standart solutions (0.1% solution of glutamine, glycine and leucine). Solvent mixture of normal butanol, acetic acid and water in the ratio 4:1:1 by volume (Ninhydrin reagent is added to this mixture).

Procedure:
1. Pour the solvent mixture into the TLC chamber and close the chamber. The chamber should not be disturbed for about 30 minutes so that the atmosphere in the jar becomes saturated with the solvent.
2. Cut the plate to the correct size and using a pencil (never use a pen) gently draw a straight line across the plate approximately 2 cm from the bottom.
3. Apply 5 µl of the amino acid on the line. Allow the spot to dry.
4. Spot the second amino acid on the plate. Enough space should be provided between the spots.
5. Repeat the above step for spotting other amino acids and their mixture.
6. Hold the TLC plates by their side. Ensure that you do not touch the developing part of the TLC plate, because your finger prints will also get developed causing the result to be unclear!!!
7. Place the plate in the TLC chamber as evenly as possible and lean it against the side (immerse the plate such that the line is above the solvent).
8. Allow capillary action to draw the solvent up the plate until it is approximately 1 cm from the end. Make certain that the spots applied to the plate are above the surface of the eluting solvent!!!
9. Remove the plate and immediately draw a pencil line across the solvent top.
10. Under a hood dry the plate with the aid of a blow dryer.
11. Place the plate above the flame. Ninhydrin will react with the faded spots of amino acids and make them visible as purple colored spots.

12. After some time, mark the center of the spots, then measure the distance between the center of the amino acid spots (h₁, h₂, h₃) or solvent front (h_f) and start line.
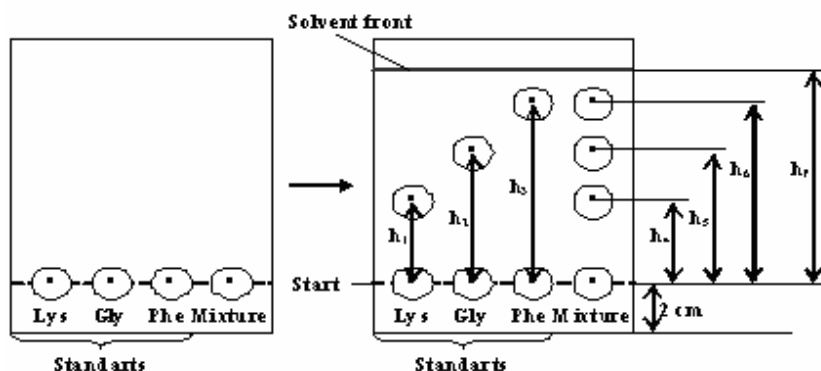
13. Calculate the *Rf* values using the formula:

$$Rf = \frac{distance\ moved\ by\ the\ substance\ from\ origin}{distance\ moved\ by\ solvent\ from\ origin}$$

✏ *Recorde the observations in the table:*

$h_f=$ _____

	Amino acids	h	Rf
Amino acid standards	1 - **Glu**	h1 =	Rf1 =
	2 - **Gly**	h2 =	Rf2 =
	3 - **Leu**	h3 =	Rf3 =
Amino acid of the mixture	4	h4 =	Rf4 =
	5	h5 =	Rf5 =
	6	h6 =	Rf6 =

Comparing Rf for amino acids of the mixture (Rf4, Rf5, Rf6) with Rf of the amino acid standards (Rf1, Rf2, Rf3), find the spots with the same Rf and identify the amino acids present in the mixture and make conclusion.

✏ **Conclusion** _____
_____
_____
_____
_____
_____
_____
_____

4.2. Xanthoproteic test

📖 Xanthoproteic test is a specific test used for the detection of aromatic amino acids
✏ *Write the names of aromatic amino acids which you know*

_____

Actually, it is a test for benzoid radicals. For example, the reaction of tyrosine nitration:

Quinoid form of dinitrotyrosine reacts with sodium hydroxide producing the *yellow coloresodium salt of dinitrotyrosine:*
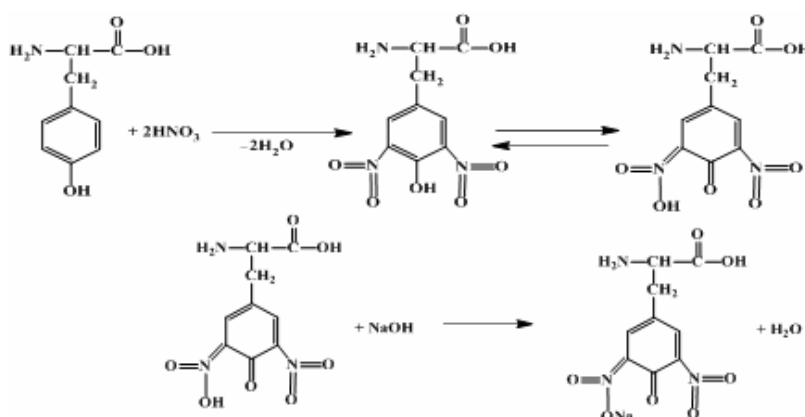
Reagents:
- Concentrated nitric acid;
- 0,01% tyrosine, glacine and protein water solution;
- 10% sodium hydroxide water solution.

☠ **SAFETY CONCERNS:** Now here are some of the things that you need to take care of while performing a Xanthoproteic test: heat the solution carefully; pay attention not to direct the test tube to any human while heating; take the test tube away from the fire when the solution starts boiling, that it wouldn't turn out; place it one more time when it goes down.

Procedure: Add to the test tubes appropriate reagents according to the scheme below:

Test tube 1	Test tube 1	Test tube 3
3 mL of tyrosine solution	3 mL of glycine solution	3 mL of protein solution
1 ml of strong nitric acid		
✎ *Write the results of the observations in each test tube:*		
Heat to boil for a half minute		
✎ *Write the results of the observations:*		
Cool under the tap water		
Slowly add sodium hydroxide solution (aproximetly 1 mL)		
✎ *Write the results of the observations:*		

Conclusion_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

4.3. Biuret test

📖 The Biuret test is often used to determine the presence of peptide bonds in protein. Peptides which have not less than two peptide bonds (—CO—NH—) in the alkaline medium involving copper (II) sulfate generate purple colored complex. Its name is derived from the fact that the test is also positive for the compound biuret, $H_2NCONHCONH_2$ obtained from urea by heating. Amides of amino acids, such as asparagine, can form similar complex with copper, as far as they have amide bonds –CO–NH_2.
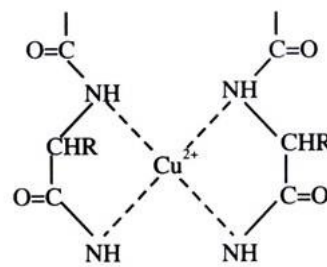
Copper (II) hydroxide, which is necessary for biuret test, is obtained in the reaction between copper (II) sulfate and sodium (or potassium) hydroxide:

✎ *Complete the equation:*

$CuSO_4 + 2NaOH \rightarrow$ _____

Reagents:
- 0.01 % asparagines, glycine and protein water solution;
- 10 % sodium hydroxide water solution;
- 10 % copper sulfate water solution.

Procedure: Add to the test tubes appropriate reagents according to the scheme below:

Test tube 1	Test tube 2	Test tube 3
3 mL of asparagine solution	3 mL of glycine solution	3 mL of protein solution
3 mL of sodium hydroxide solution		
one-two drops of copper sulfate solution and mix		
✎ *Write your observations in the columns below:*		

Conclusion_____

_____
_____
_____
_____
_____
_____
_____

4.4. Ninhydrin colorimetric test

📖 In the pH range of 4-8 all α- amino acids react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent to give a purple colored product (diketohydrin). All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The imino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides and proteins also react positively when subjected to the ninhydrin reaction.

ninhydrin   amino acid

blue-purple ion

ninhydrin proline yellow product

33
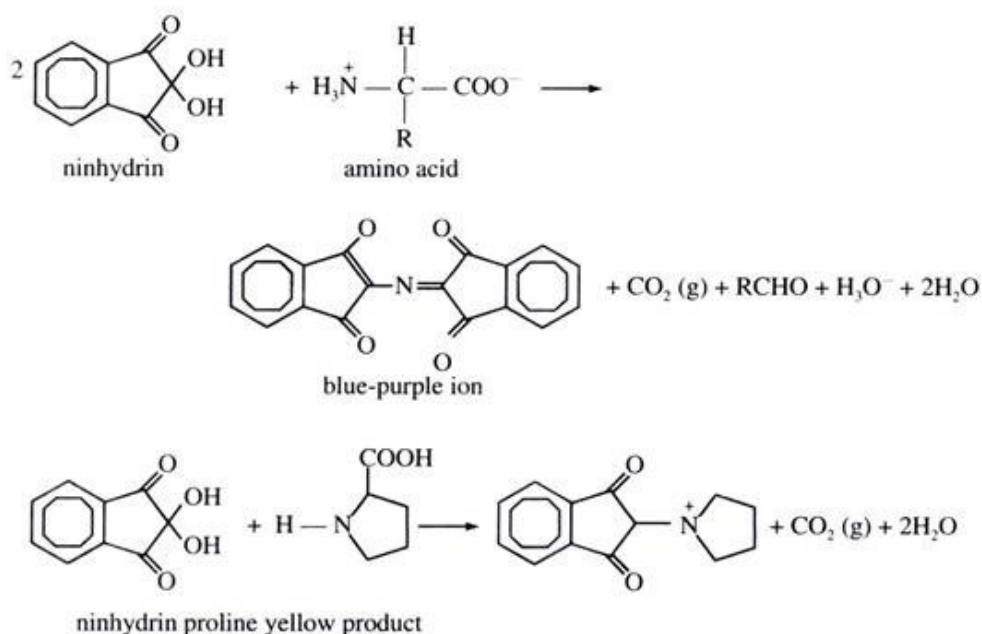
Reagents:
- 1% glycine and protein water solution,
- 0.1% ninhydrin solution in 95% alchohol solution.

Procedures:

Test tube 1	Test tube 2
Add to the test tube five drops of glycine solution	Add to the test tube five drops of protein solution
two drops of ninhydrin solution	
Held the test tubes in the hot water bath (at 70°C) and stir gently for 5 minutes	
✎ *Write your observations in the columns below:*	

Conclusion_____

_____

_____

_____

_____

_____

4.5. The formation of copper complex salt

Alpha-amino acids react with copper (II) carbonate under a heated condition to form blue colored copper complex salt. The reaction with the participation of glycine:
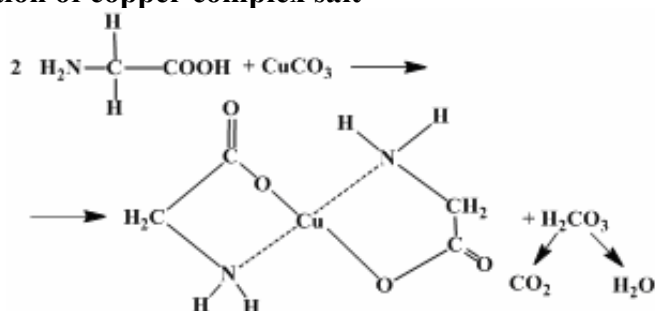
Reagents:

- 1% glycine and protein water solution,
- dry copper carbonate (II).

Procedures:

Test tube 1	Test tube 2
Add to the test tube 1 mL glycine solution	Add to the test tube 1 mL protein solution
Small amount of dry copper (II) carbonate with the help of spatula	
Heat the mixture in the flame of the burner until it boils	
✎ *Write your observations in the columns below:*	

Conclusion_____

_____

_____

_____

_____

Teacher's signature _____ (_____)

full name

5. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF PROTEINS

📖 BACKGROUND

PROTEINS – Proteins are very important molecules in our cells and are essential for all living organisms. By weight proteins are the major component of the dry weight of cells and are involved in virtually all cell functions.

Each protein within the body has a specific function, from cellular support to cell signalling and cellular locomotion. In total, there are seven types of proteins, including antibodies, enzymes, and some types of hormones, such as insulin.

PROTEIN FUNCTIONS

Type of Protein	Function	Examples
Structure	structural support	collagen in tendons and cartilage; keratin in hair and nails
Contractile	muscle movement	actin, myosin, tubulin and kinesin proteins
Transportation	movement of compounds	hemoglobin carries O_2; lipoproteins carry lipids
Storage	nutrient storage	ferritin stores iron is spleen and liver; casein stores proteins in milk
Hormone	chemical communication	insulin regulates blood sugar
Enzyme	catalysis of biological reactions	lactase breaks down lactose; trypsin breaks down proteins
Protection	recognition and destruction of foreign substances	immunoglobulins stimulate immune system

PROTEIN STRUCTURE TYPES

Some proteins contain only one polypeptide chain while others, such as hemoglobin, contain several polypeptide chains all twisted together. The sequence of amino acids in each polypeptide chain is unique to that protein; this is called the **primary structure**. If even one amino acid in the sequence is changed, that can potentially change the protein's ability to function. For example, sickle cell anemia is caused by a change in only one nucleotide in the DNA sequence that causes just one amino acid in one of the hemoglobin polypeptide molecules to be different. Because of this, the whole red blood cell ends up being deformed and unable to carry oxygen properly.

The primary structure is created through the linking of amino acids. This linking is accomplished by the formation of a **peptide bond**. This is a *dehydration reaction*. In other words the peptides combine and lose a water molecule.

from   https://study.com/academy/lesson/dipeptide-definition-formation-structure.html

NOTE:

✓ Peptides are always written in the N ⇒ C direction;
✓ Each peptide has ONLY ONE free amino group and ONE free carboxyl group; others are neutralized by formation of the peptide bond;
✓ Each peptide chain has an amine end and a carboxylic acid end and each amino acid is referred to as a **residue**. -- So, the ends are named, n-terminal residue and c-terminal residue.
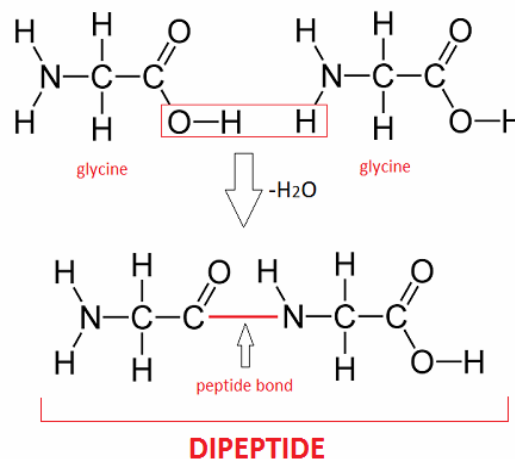
The two most common **secondary structures** are shown below, *alpha helix* and *beta sheet*:

from http://book.bionumbers.org/what-is-the-energy-of-a-hydrogen-bond/

✓ Rod-like structure (phone cord)
✓ Involves only one polypeptide chain
✓ Main chain atoms on the INSIDE**,** while R-group side chains on the OUTSIDE – stick out
✓ Stabilized by **HYDROGEN BONDS:** Carbonyl (C = O) of each amino acid is H-bonded to the amide (N-H) of the amino acid that is 4 amino acids further toward the C-terminus
✓ Helices can be right or left handed; But ALL PROTEINS ARE RIGHT HANDED

✓ Generally have rod-like shapes and are not so soluble in water. **β sheets** consist of **β strands** connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted, pleated sheet. A β-strand is a stretch of polypeptide chain typically 3 to 10 amino acids long with the peptide backbone almost **completely extended**
✓ Unlike the α-helix, β-sheets can involve one or more polypeptide chains – interchain or intrachain interactions
✓ R-groups stick UP and DOWN from β-sheets – alternating on either side of the strand; Usually small compact side chains like Gly, Ser, Ala
✓ Stabilized by Hydrogen bonds (**near perpendicular to direction of peptide backbone**): Carbonyl of each amino acid is H-bonded to the NH of another amino acid
✓ Adjacent chains can be **PARALLEL** or **ANTI-PARALLEL**

The tertiary structure is created by molecular interactions between the side groups of AAs:

1. *Hydrogen bonds*, in the most simple explanation, from between hydrogens attached to an oxygen or nitrogen and the lone pairs found on an oxygen or nitrogen.
2. *Disulfide bridges* form between cysteine and methionine amino acids.
3. *Salt bridges (Ionoc bonds)* are interactions between the ends of the Zwitter ion, the NH_3^+ and the COO^-.
4. *Hydrophobic interactions* are formed between those amino acids with hydrophobic R groups.

© 2011 Pearson Education. Inc.
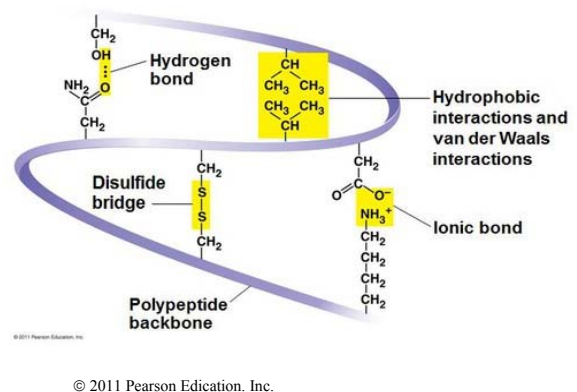
The **tertiary structure** gives the protein its function. If the tertiary structure is deformed the protein **will not** function. The primary structure is sequenced in a way as to form the tertiary structure. The side chains of the amino acids cause them to interact with the other parts of the chain. An egg white is a protein. When it comes out of the shell it is clear, when you cook the egg you destroy its tertiary structure and the protein unfolds and becomes white. This destroys the proteins secondary, tertiary and quaternary structures. The primary structure will normally stay intact if the food is cooked.
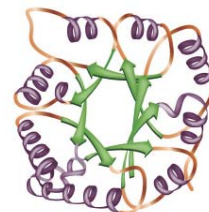
RIBBON DIAGRAMS
Cylinders or coils = α-helices
Arrows = β−strands
Ribbons = bends, loops and random

Some proteins have a **quaternary structure**. The quaternary structure occurs in proteins composed of more than one peptide chain. It means that tow or more proteins come together to form one large protein. For example, hemoglobin has a quaternary structure as it is composed of four subunits. Each subunit is a separate polypeptide chain.

DENATURATION OF PROTEIN

When a protein is destroyed it is said to be *denatured*. Remember that the purpose of a primary structure is so the secondary and tertiary structures will form. Certain conditions will cause the protein to unfold, leaving only the primary structure.

✓ *heat* – breaks hydrogen bonds by causing the atoms to vibrate too radically
✓ *UV light* – breaks hydrogen bonds by exciting bonding electrons
✓ *organic solvents* – breaks hydrogen bonds
✓ *strong acids and bases* – breaks hydrogen bonds and can hydrolyze the peptide bonds, breaking the primary structure
✓ *detergents* – disrupt hydrophobic interactions
✓ *heavy metal ions* – forms bonds to sulfur groups and can cause proteins to precipitate out of solution.

HOME PROJECT
Give an example of any diseases associated with defects in primary structure of proteins

_____
_____
_____
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_____
_____
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_____

PRACTICAL WORK 5

Equipment. Test tubes, test tube rack, graduated pipettes, glass rods.

5.1. Determination of the isoelectric point of gelatine by the degree of turbidity

📖 *Definition.* The determination of the isoelectric point (pI) of proteins is based on the ability of the proteins to precipitate out of solution at the pH that corresponds to their pI. At a pH above the pI, a protein carries a net negative charge, at a pH below pI – a net positive charge. At the isoelectric point a protein has no net charge and easily precipitates under the action of agents causing protein dehydration.

Reagents. 1% Gelatine solution, 0.1 and 1 M acetic acid solutions, 0.1 M sodium acetate solution, 96% ethyl alcohol, distilled water.

Procedures.

1. Add to six test tubes the appropriate amount of acetic acid solution, sodium acetate solution and gelatine solution in the proportions given in the table below.

# tube	Water	CH_3COOH (0.1 M)	CH_3COOH (1 M)	CH_3COONa (0.1 M)	1% gelatin solution	pH of the medium	Turbidity
1	3.7	0.3	-	2.0	2.0	5.6	
2	3.5	0.5	-	2.0	2.0	5.3	
3	3.0	1.0	-	2.0	2.0	5.0	
4	2.0	2.0	-	2.0	2.0	4.7	
5	-	4.0	-	2.0	2.0	4.4	
6	3.8	-	0.2	2.0	2.0	4.1	

2. Mix the contents of each test tube. Then to all the test tubes slowly add 2 mL of alcohol.

After 30 minutes, determine the isoelectric point, that is, find a test tube with a maximum degree of the turbidity of the solution. Use "+ / −" signs to describe the turbidity in the different test tubes.

3. You should observe the most precipitation in the test tube which has the pH close to the isoelectric point of gelatin.

✒ *Record the observations in the table above. Make conclusion.*

Conclusion_____
_____
_____
_____
_____
_____
_____
_____
_____

5.2. Precipitation of proteins by heating

📖 *Definition:* The best way to precipitate the proteins is boiling in the medium at the pH that corresponds to their isoelectric point. The addition of neutral salts (ammonium sulphate, sodium chloride, etc.) to the solution of the protein facilitates and accelerates precipitation during boiling in the result of the dehydration of protein particles.

Reagents. 1% ovalbumin solution, 3% acetic acid solution, saturated solution of sodium chloride, 10% sodium hydroxide solution

Procedures.

*In order to compare the dependence of precipitation of proteins on the concentration of hydrogen ions, add 5 mL of 1% ovalbumin solution **to five test tubes**.*

1. Neutral solution (check pH using indicator paper) of protein in **the first tube** is heated to a boiling, it becomes turbid and opalescence is observed due to the destruction of the hydration shell around the protein molecule and the increase of protein particles. But the protein micelles are charged and therefore remain in solution without precipitation.

2. Add to **the second test tube** 1 mL of 3% acetic acid solution until a weak acid reaction appears (check pH using indicator paper). Then heat it to boiling. The protein precipitates owing to sedimentation. *Under these conditions, the protein particles lose their charge, because the pH of the medium is close to the isoelectric point.*

3. Add to **the third test tube** 3 mL of 3% acetic acid solution to create an acidic medium (check pH using indicator paper). During boiling of the solution, the precipitate is not formed, *since the protein molecules acquire a positive charge, which increases their resistance.*

4. Add to **the fourth test** tube 5 mL of 3% acetic acid solution, 2 mL of saturated sodium chloride solution (check pH using indicator paper) and heat the mixture. A white precipitate falls *because the protein loses its charge due to interaction with sodium chloride.*

5. Add to **the fifth test tube** 2 mL of 10% sodium hydroxide solution to create an alkaline medium (check pH using indicator paper). The precipitate is not formed during the boiling of the mixture, *because the alkaline medium increases the negative charge of the protein.*

🖋 *Record the results of the observations in the table and make the conclusion about the mechanisms of precipitation of proteins by heating and about the effect of the pH of the solution*

# test tube **(pH)**	Protein charge	Observation
1.		
2.		
3.		
4.		
5.		

Conclusion_____

_____
_____
_____
_____
_____
_____

5.3. The study of protein precipitation under the action of different agents

📖 *Definition. Mineral* and some *organic acids* precipitate proteins due to the denaturation and dehydration of protein molecules, as well as the formation of insoluble salts between the acid anions and the positively charged protein particles. *Organic solvents* precipitate proteins due to denaturation and dehydration of their molecules, as well as the formation of complex salts between proteins and acids. *Salts of heavy metals* (copper, mercury, zinc, silver, lead) precipitate proteins forming the complex compounds with thiol groups of proteins.

Reagents. 1% ovalbumin solution, concentrated nitric acid, 10% trichloroacetic acid solution, 20% sulfosalicylic acid solution, 96% ethyl alcohol, saturated solution of sodium chloride, 0.1% copper sulfate solution, 1% lead acetate solution..

A. Precipitations of proteins by mineral acids.

Procedures:
1. Add to the test tube 3 mL of concentrated nitric acid.
2. Then, cautiously introduce down the side of the tube 3 mL of 1% ovalbumin solution. The fluids in the tube mustn't be stirred. Note the appearance of a protein precipitate at the zone of contact between the two fluids.
3. Now mix the contents of the tube thoroughly by careful shaking. Is protein precipitated by concentrated HNO_3?

✎ *Record the observations* _____

B. Precipitations of proteins by organic acids.

Procedures: Add to the two test tubes:

Test tube 1	Test tube 2
5 mL of 1% ovalbumin solution	
1 mL of 10% trichloroacetic acid solution	1 mL of 20% sulfosalicylic acid solution
✎ *What do you observe?*	

C. Precipitation of proteins by organic solvents

Procedures.

1. Add to test tubes 3 mL of 1% ovalbumin solution. Then add to the test tube 3 mL of 96% ethyl alcohol solution.
2. Add 1 mL of saturated sodium chloride solution to the tube.

✎ *Recorde the observations* _____

D. Precipitation of proteins by heavy metal ions

Procedure: Add to the two test tubes:

Test tube 1	Test tube 2
Add to two test tubes 3 mL of 1% ovalbumin solution	
two or three drops of 0.1% copper sulfate solution	two or three drops of 1% lead acetate solution
✎ *What do you observe?*	

Conclusion (about the mechanism of protein precipitation by different agents)

_____
_____
_____

Teacher's signature _____ (_____)

<div align="center">full name</div>

6. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF MONOSACCHARIDES

📖 **BACKGROUND** Carbohydrates represent a major energy source for a wide variety of organisms. Carbohydrates include simple unit structures, ***monosaccharides*** or simple sugars, and polymers or polysaccharides made up of repeating monosaccharide units. In other words, carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis

CLASSES OF CARBOHYDRATES

✓ **Monosaccharides** contain a single polyhydroxy aldehyde or ketone unit (e.g., glucose, fructose).
✓ **Disaccharides** consist of two monosaccharide units linked together by a covalent bond (e.g., sucrose). One H_2O is eliminated when sugars are linked together to form disaccharides or higher polymers.
✓ **Oligosaccharides** contain from 3 to 10 monosaccharide units (e.g., raffinose).
✓ **Polysaccharides** are very long chains of hundreds or thousands of monosaccharide units, which may be either in straight or branched chains (e.g., cellulose, glycogen, starch).

STEREOISOMERS

✓ Everyone monosaccharide (except dihydroxyacetone) has at least one **CHIRAL** center - carbon with 4 different groups bonded to it.
✓ A sugar with **n** chiral centers can exist in 2^n different forms.
✓ Glyceraldehyde, the simplest carbohydrate, contains one chiral center and has two isomeric forms that are mirror images of each other. These forms are stereoisomers of each other and are called the **D and L form.**
✓ **Almost ALL sugars are D-**isomers.

L-isomer   D-isomer
Glyceraldehyde
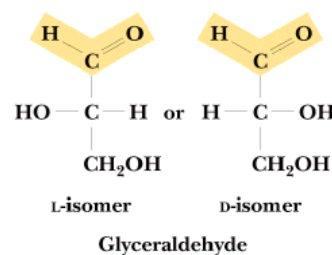
> • ***Isomers*** = same chemical formula, different structure
> • ***Enantiomers*** = isomers that are mirror images (D and L)
> • ***Diastereomers*** = isomers that are not mirror images
> • ***Epimers*** = isomers that differ at only one Carbon
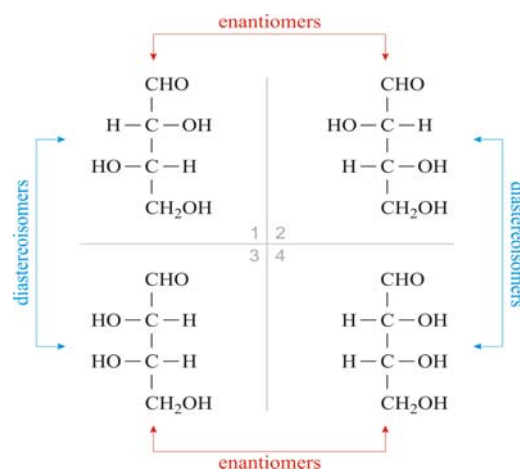
STRUCTURE AND NOMENCLATURE

With few exceptions (e.g., deoxyribose), monosaccharide have this chemical formula: $C_x(H_2O)_y$, where conventionally

$x \geq 3$. **Monosaccharides can be classified by the number x** of carbon atoms they contain: triose (3); tetrose (4),:pentose (5); hexose (6) and so on.

There are two possible structures, glyceraldehyde and dihydroxyacetone, representing the simplest members of the aldehyde based sugars or ***aldoses*** and the ketone based sugars or ***ketoses***. Normal numbering conventions put the aldehyde group at C-1. Natural ketoses have the carbonyl group at C-2.

D-glyceraldehyde   Dihydroxyacetone
Aldose family   **Ketose** family
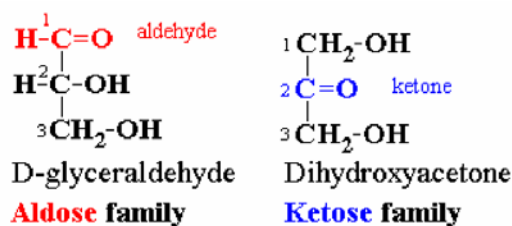
These is the **Fischer Projections** for D-glucose:
- ✓ Carbons are numbered from the top;
- ✓ Most oxidized C (one with the most number of bonds to O goes at top (C1);
- ✓ Last carbon will ALWAYS be part of a CH_2OH group (Not CHIRAL)
- ✓ Stereochemistry of the last CHIRAL carbon (2nd to last C in chain) determines the stereochemistry of the sugar:
  - • If –OH is to the RIGHT $\Rightarrow$ D-isomer
  - • If –OH is to the LEFT $\Rightarrow$ L-isomer
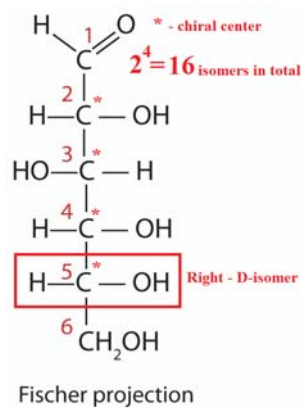
HAWORTH PROJECTIONS

Carbonyl reacting with hydroxyl group $\Rightarrow$ addition product called *hemiacetal*.

If C1 carbonyl group (aldehyde) interacts with alcohol on C5, a six membered ring (*pyranose*) is formed, but if C1 interacts with alcohol on C4, a five membered ring (*furanose*) is formed.

Reaction called an **ALDOL CONDENSATION**

The C1 carbonyl carbon becomes a new chiral center – a new C1 hydroxyl:
- **New C1 hydroxyl = anomeric carbon**
- Hydroxyl group is either above or below the ring – **two forms α (alpha) and β (beta)**
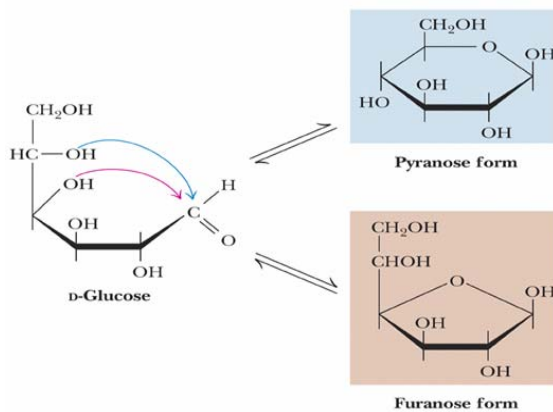
Going from
FISCHER PROJECTIONS to HAWORTH:

- Numbering remains the same for the carbons
- **If –OH is on the right $\Rightarrow$ points DOWN in Haworth**
- **If –OH is on the left $\Rightarrow$ points UP in Haworth**
- Terminal CH_2OH **ALWAYS** points **UP** relative to anomeric carbon in D sugars
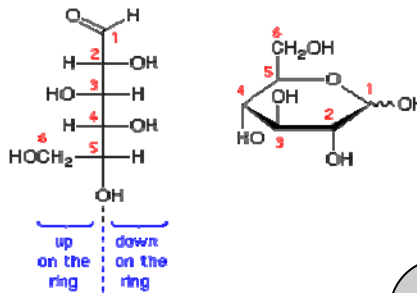
HOME PROJECT
What do you know about the *MOST IMPORTANT FUNCTIONS OF MONOSACCHARIDES?*

PHYSICAL PROPERTIES OF MONOSACCHARIDES

- ✓ Most monosaccharides have a sweet taste (fructose is sweetest; 73% sweeter than sucrose).
- ✓ They are solids at room temperature.
- ✓ They are extremely soluble in water: Despite their high molecular weights, the presence of large numbers of OH groups make the monosaccharides much more water soluble than most molecules of similar MW.

CHEMICAL PROPERTISE OF MONOSACAHARIDES

Reduction of Simple Sugars

C=O of aldoses or ketoses can be reduced to C-OH by $NaBH_4$ or H_2/Ni. Name the sugar alcohol by adding *-itol* to the root name of the sugar. Reduction of D-glucose produces D-glucitol, commonly called D-sorbitol. Reduction of D-fructose produces a mixture of D-glucitol and D-mannitol.
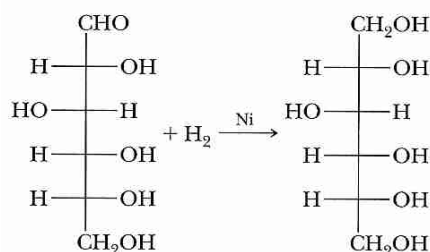
Oxidation by Bromine

Bromine water oxidizes the aldehyde group of an aldose to a carboxylic acid. Bromine in water is used for this oxidation because it does not oxidize the alcohol groups of the sugar and it does not oxidize ketoses.
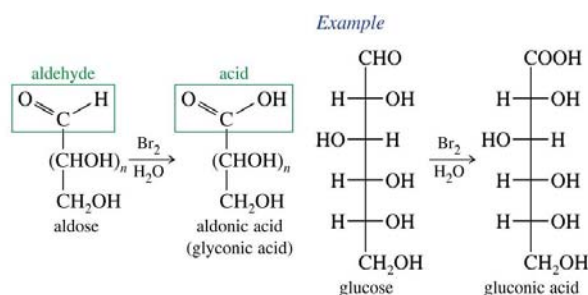
Nitric Acid Oxidation

Nitric acid is a stronger oxidizing agent than bromine, oxidizing both the aldehyde group and the terminal —CH_2OH group of an aldose to a carboxylic acid.
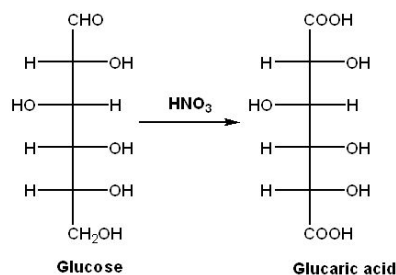
Oxidation carried out by an enzyme

Enzyme class = Dehydrogenases
Sugar gets *oxidized* and the cofactor gets *reduced*.
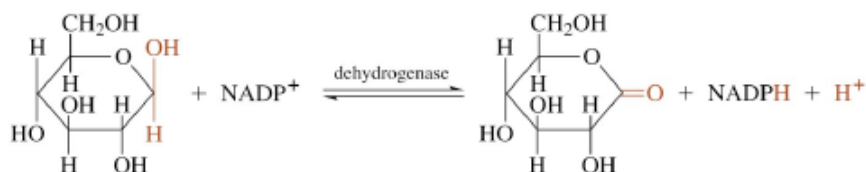
Esterification

Esters are formed by the reaction of alcohols (-OH) with acids. One of the most important biological esters is the *PHOSPHATE ESTER*. Performed in cells by transfer of a phosphoryl group from ATP to a carbohydrate hydroxyl – Catalyzed by KINASES
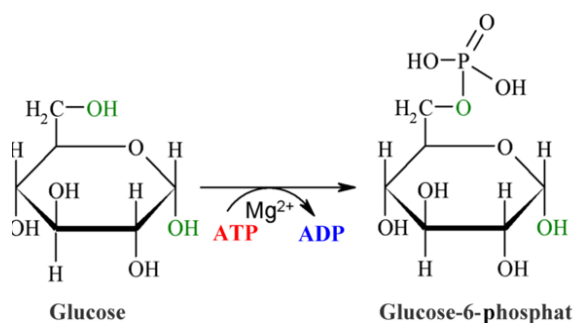
📖 The oxidoreductive properties of carbohydrates (sugars) deserve special attention in their detection. If the oxygen of the aldehyde group of sugar is not bound with any other structure, it shows reducing properties. It, for example, can reduce metal cation such as Cu^{2+} into Cu^{+}, Ag^{+} into Ag^{0}. They can easily oxidize to appropriate aldonic acids at the expense of the reduction of the oxidizing agent into an alkaline environment and much slower – in neutral and especially in an acidic environment. The aldehyde group oxidizes to the carboxyl group.

6.1. Trommer's test

📖 *Definition:* This test reveal reducing properties of carbohydrates. It is based on the reduction of heavy metal ions (the reduction by heating of copper (II) hydroxide in copper (I) oxide) and simultaneous oxidation of carbonyl group of aldehydes or monosaccharides to carboxyl group. Ketoses also give positive results in this test because in alkaline environment of the test they isomerize to aldoses.
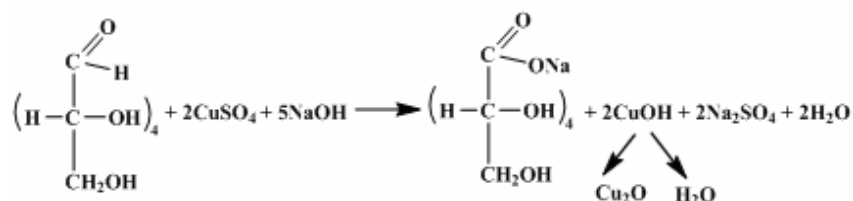
Reagents. Glucose solution, NaOH, $CuSO_4$ (all 5 %).

Equipment. Glass rods, tubes, pipettes graduated, droppers, test tube rack, burner.

Procedures:

1. To test tube add 3 ml of glucose,
2. 1 ml of NaOH, 5 drops of $CuSO_4$ and mix.
3. Carefully heat the test tubes in a boiling water bath to boil.

🖊 *Recorde the observations in each step (what's happened after boil)*

6.2. Fehling's test

📖 This test is based on the same reaction of Trommer's test, but in Fehling's reagent, the ion Cu^{2+} exists as a complex compound with tartrates, therefore, with excess copper it does not precipitate from the solution as Cu_2O.

Reagents. 5 % glucose solution, Fehling reagent is composed of two separate solutions: Fehling I is solution of $CuSO_4$ in diluted H_2SO_4, and Fehling II is sodium potassium tartrate in diluted NaOH.
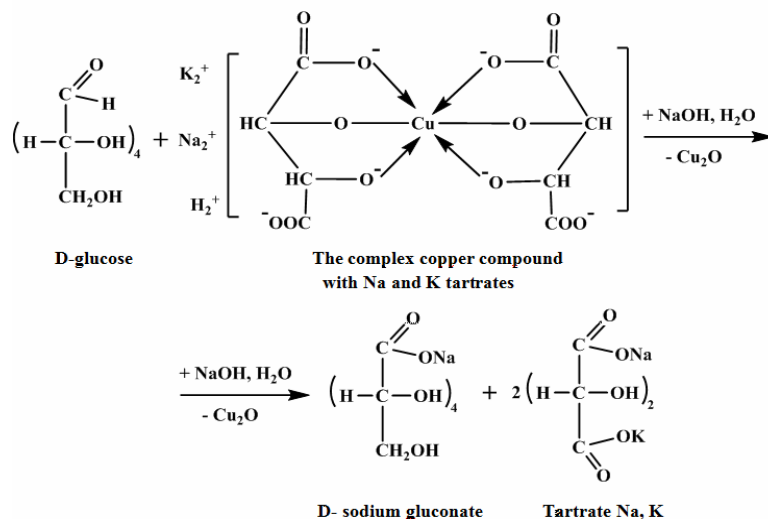
Equal volumes of the first and second solutions are mixed before work.

Equipment. Glass rods, tubes, pipettes graduated, test tube rack, burner.

Procedure:

1. To test tube add 1 ml of 5 % glucose solution and 1 ml of Fehling's reagents I and II.
2. Mix and heat the tube in the flame to boil.

🖊 *What's happened after boiling_____*

6.3. Barfed's test

📖 The reaction with copper acetate differs from all previous ones in that the oxidation of reducing sugars proceeds not in an alkaline but in a close to neutral medium, in which the reducing disaccharides, in contrast to monosaccharides, do not practically oxidize. This reducing test is performed to distinguish reducing monosaccharides from reducing disaccharides (e.g. lactose).

Glucose and other hexoses react with copper acetate to form Cu_2O:

$$\left(\begin{array}{c} C\!\!\nearrow^{O}_{\searrow H} \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array}\right)_4 + 2Cu(CH_3COO)_2 \xrightarrow[-4CH_3COOH]{+2H_2O} \left(\begin{array}{c} C\!\!\nearrow^{O}_{\searrow OH} \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array}\right)_4 + Cu_2O\downarrow$$
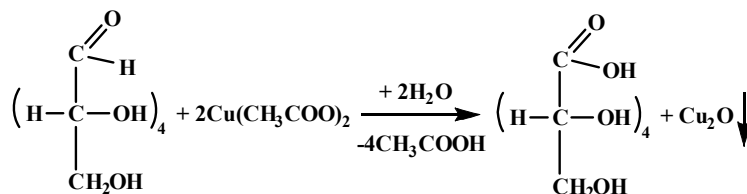
Materials. 5 % glucose solution, Barfoed's reagent (dissolve 13.3 g of copper acetate in 200 ml of hot H_2O at a temperature of 70 °C, filter the mixture and add 1.9 ml of glacial acetic to the filtrate).

Equipment. Glass rods, tubes, pipettes graduated, test tube rack, burner.

Procedure.

1. To the test tube add 1 ml of 5 % glucose solution
2. Add 1 ml of Barfoed's reagent, mix and heat the tube in the flame to boil.

✎ *What's happened after boiling*_____

_____

Conclusion_____

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6.4. Seliwanoff's test for ketoses

📖 Ketoses (e.g. fructose) when heated and in the presence of concentrated HCl, undergo dehydration more easily than aldohexoses. Hydroxymethylene-furfural creates cherry-red complex with resorcine (1,3-dihydroxybenzene).

Materials. The crystalline resorcinol, 5 % fructose solution, 5 % glucose solution, 25 % HCl.

β-D-fructose   hydroxymethylfurfural   Product of condensation

Equipment. Glass sticks, test tubes, graduated pipettes, a test tube rack, a water bath, a spatula.

Procedure. Add to the two test tubes:

Test tube 1	Test tube 2
5 ml of 5% fructose solution	5 ml of 5% glucose solution.
Add to the both test tubes 1 ml of 25% HCl and a few crystals of resorcinol	
Heat the tubes in a water bath to 80 ° C and observe the changes for 8-10 minutes	
✎ *What do you observe?*	

Conclusion_____

_____

_____

Teacher's signature _____ (_____)

full name

7. STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF DI- AND POLYSACCHARIDES

📖 **BACKGROUND**

DISACCHARIDES

Disaccharide sugars are formed by a condensation reaction between two monosaccharides. The covalent linkage is called an ***O-glycosidic bond*** and represents the formation of an acetal from a hemiacetal and an alcohol. The glycosidic bond in the disaccharide maltose is called an *α1-4 linkage* because the anomeric carbon is in the α conformation. The glucose molecule on the right remains the hemiacetal structure at its C-1 anomeric carbon and can convert to the aldehyde open chain form in a reaction involving the reduction of Cu^{2+} to form Cu^{+}. Using this functional definition (the reduction of Cu^{2+}), the glucose on the right is designated as the *reducing end* of the disaccharide molecule because it can participate in a reduction reaction. In contrast, the glucose on the left represents the *nonreducing end* because the C-1 carbon is part of the α1-4 linkage and cannot form the open chain structure in a Cu^{2+} reduction reaction. *Since maltose contains one reducing end it is called a* ***reducing sugar***.

modified from http://www.medbiochemistry.com/maltose/maltose3/
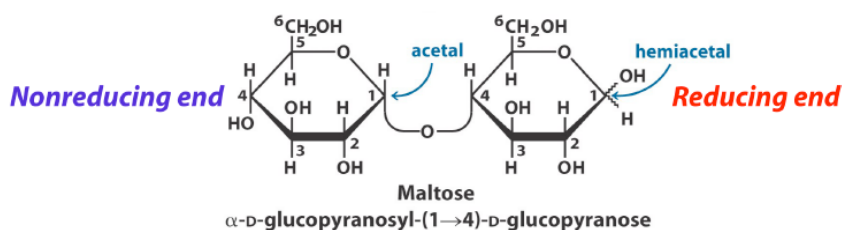
Disaccharides can contain different monosaccharide units connected through α or β glycosidic bonds involving ring carbons. Disaccharide are named by first listing the nonreducing monosaccharide on the left, followed by the glycosidic linkage between the two monosaccharides, and then the monosaccharide on the right. With this shorthand nomenclature, maltose can be described as a *Glc(α1-4)Glc* disaccharide in which the abbreviation "Glc" is used for glucose.

Figure shows the structures of three common disaccharides found in nature:

1) **lactose**, also called milk sugar, which contains a β1-4 glycosidic bond linking a galactose (Gal) to a glucose to form Gal(β1-4)Glc;

2) **sucrose**, made in plants and used as table sugar in its crystalline form, contains fructose (Fru) linked to glucose through the two anomeric carbons to form Glu(α1-β2)Fru;

3) **trehalose**, a glucose disaccharide made in insects, contains a glycosidic bond between the two anomeric carbons to form the disaccharide Glc(α1-α1)Glc.

It is important to note that of these three disaccharides, only lactose is a reducing sugar, because, like maltose, it contains a free anomeric carbon that can interconvert the hemiacetal to an aldehyde. Both sucrose and trehalose are ***nonreducing sugars*** because they lack a reducing end.
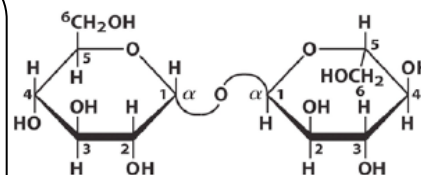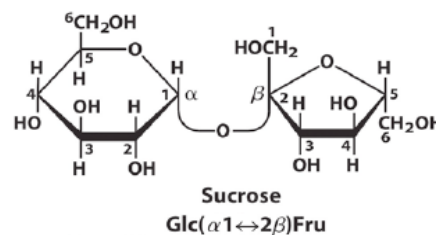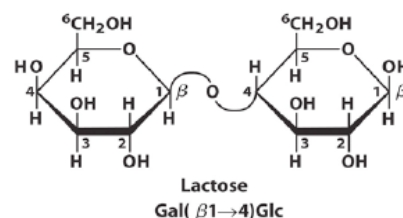
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!!! 5% of people from Scandinavia and 90% of Asian adults suffer from **lactose intolerance** – deficiency in enzyme lactase.
- For those with the deficiency:
✓ Lactose accumulates in small intestine;
✓ Degraded by intestinal bacteria producing CO2, hydrogen gas, and organic acids;
✓ Presence of excess undigested lactose is harmful as well
Treatment:
✓ Avoid products containing lactose (dairy products)
✓ Use commercial products to hydrolyze lactose before consumption
✓ Add enzyme called β-galactosidase (e.g. lactaid milk)

POLYSACCHARIDES

TWO MAIN FUNCTIONS: 1) energy storage; 2) structure

STORAGE POLYSACCHARIDES:

1) STARCH
- Found in chloroplasts of plant cells – especially abundant in potatoes, corn and wheat
- Mixture of 2 types of **GLUCOSE POLYMERS**
• **Amylose:**
   ✓ Linear, unbranched chain of α(1→4) D-glucose molecules
   ✓ Disaccharide repeating unit = **MALTOSE**
   ✓ Each amylase has 2 ends: *non-reducing end* (glucose molecule with free –OH on C4) and *reducing end* (glucose molecule with free –OH on C1 – anomeric carbon)
   ✓ Forms a coiled, relatively compact helical structure (~6 glucoses/turn)

One turn of the helix has SIX glucose units

AMYLOSE
α(1 → 4) glucose

Glucose = hexagons
Forms large left-handed helix
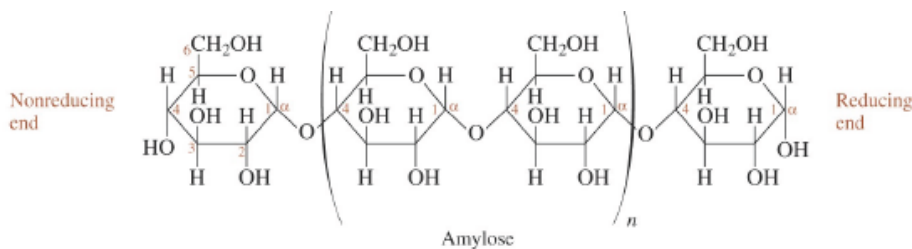
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Figure 7-20a Concepts in Biochemistry, 3/e
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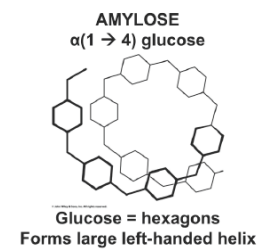
• **Amylopectin:**
   ✓ Main backbone is amylose (linear) with D-glucose molecules in α(1→4) linkage
   ✓ Also has **BRANCHES**: Connect to backbone and to each other by α(1→6) linkages
   ✓ **Branch points every 25-30 glucoses**
   ✓ Has **ONE** reducing end and many non-reducing ends

α(1→6) linkage between two glucose units

α(1→4) linkage between two glucose units
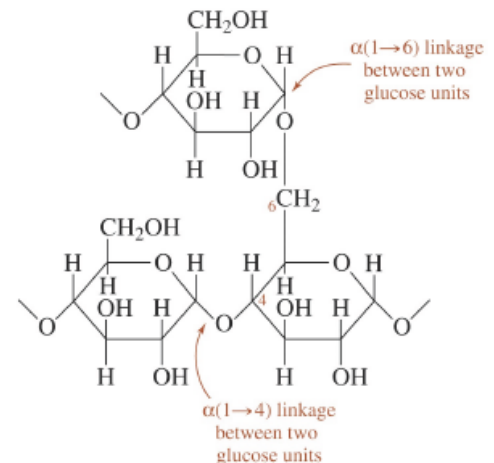
Figure 7-21 Concepts in Biochemistry, 3/e
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2) GLYCOGEN
- Animal carbohydrate storage
- Stored in liver and muscle as granules or particles
- Branched glucose polysaccharide
   ✓ Chains of glucose units
   ✓ Similar in structure to **amylopectin**
   ✓ Backbone linked by α-**1,4 bond** (like amylose)

   ✓ Have α-**1,6 branches every 8-10 residues** (like amylopectin with more branches)
   ✓ Has one reducing end and many non-reducing ends

Reducing end

α(1 → 6) branch points

Nonreducing ends

α(1 → 4) backbone

Amylopectin or glycogen
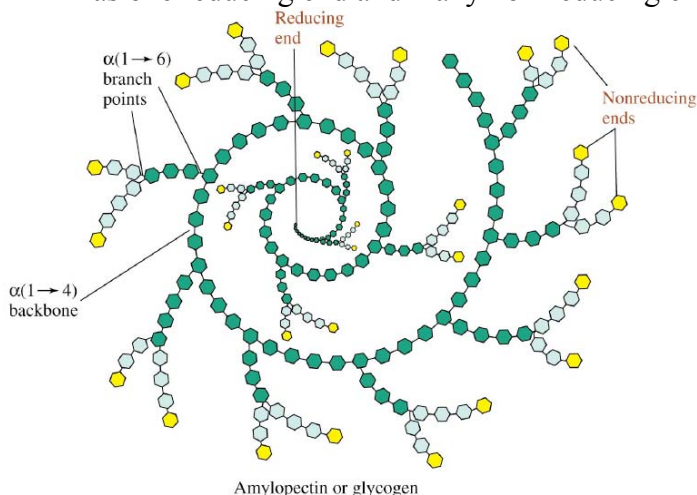
Figure 7-20b Concepts in Biochemistry, 3/e
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SIGNIFICANCE OF BRANCHING
• Branched structure allows several sites for simultaneous synthesis and degradation;
• Branching speeds up degradation: enzyme **glycogen phosphorylase** cleaves one glucose as a time from a NONreducing end of glycogen; each end can be attacked separately by the enzyme at the same time;
• Makes glycogen is an **efficient** way to store glucose;
− Structure makes it compact and coiled
– Each glucose is readily accessible

STRUCTURAL POLYSACCHARIDES:

1) CELLULOSE is the most abundant polysaccharide on earth, a homopolymeric molecule consisting of thousands of repeating glucose units connected by β1-4 glycosidic bonds. Cellulose provides plants with a rigid cell wall consisting of layers of cellulose fibers that are held together by hydrogen bonds. Figure shows the structure of the repeating Glc(β1-4)Glc unit in cellulose, called *cellobiose*, and a diagram depicting hydrogen bonding within and between cellulose strands. Most animals lack the enzyme **cellulase** which is required to hydrolyze the β1-4 glycosidic bonds in cellulose.

from *https://en.wikipedia.org/wiki/Cellulose*

Therefore, plant material high in cellulose fiber is considered "roughage" in the diet because it passes through the digestive system without being degraded. *Some animals have evolved symbiotic relationships with microorganisms that inhabit their digestive tracts and secrete cellulase.* Ruminating herbivores (plant eating organisms) such as cows and goats have an unusual stomach that permits them to regurgitate their food and thereby maximize mechanical and enzymatic breakdown of cellulose with the help of bacteria. Termites and moths also depend on the help of symbiotic microorganisms to help digest cellulose in their diets which can unfortunately include houses and sweaters.
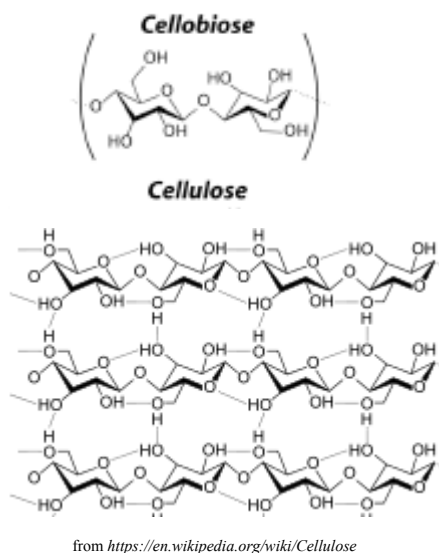
2) CHITIN is another abundant linear polysaccharide in nature, the structural component of invertebrate exoskeletons found in insects and crustaceans. Chitin consists of repeating **N-acetylglucosamine** units (NAG, also abbreviated GlcNAc) linked by a β1-4 glycosidic bond. The only difference between glucose and N-acetylglucosamine is the replacement of the C-2 hydroxyl with an acetylated amino group. Chitin, like cellulose, provides the organism with an excellent biomaterial for building a strong body frame by virtue of hydrogen bonding contacts within and between polysaccharide strands. Moreover, because of the β1-4 glycosidic bond, chitin can

from *https://glossary.periodni.com/glossary.php?en=chitin*

only be used as a source of carbohydrate fuel by microorganisms that contain the enzyme **chitinase**.
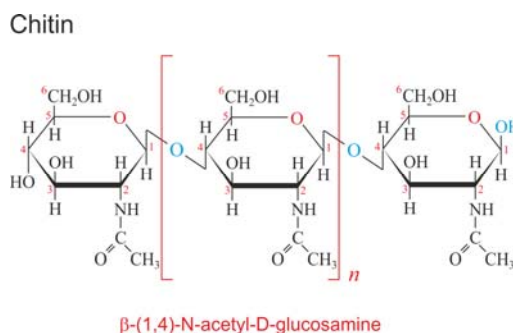
GLYCOPROTEINS
– Oligosaccharides can also be attached to proteins
– Through glycosidic linkages to **serine, threonine or asparagines**
• **O-glycosidic linkages to Ser or Thr**
• **N-glycosidic linkages to Asn or Gln**

FUNCTIONS OF OLIGOSACCHARIDES ON PROTEINS:
✓ Influence structure, folding and stability of protein
✓ May determine the lifetime of a protein (mark protein for age)
✓ Serve as markers to identify a cell type
✓ When glycosylated proteins are at the cell surface:
- Can **modulate cell-cell interactions (**Changes in carbohydrate content may influence contact inhibition of cells;
- Can **modulate cell – molecule interactions** (e.g. hormone w/receptor);
- Can serve as **antigenic determinants** (how antibody recognizes the protein) on proteins (The difference between blood types is due to glycosylation of red blood cell proteins.
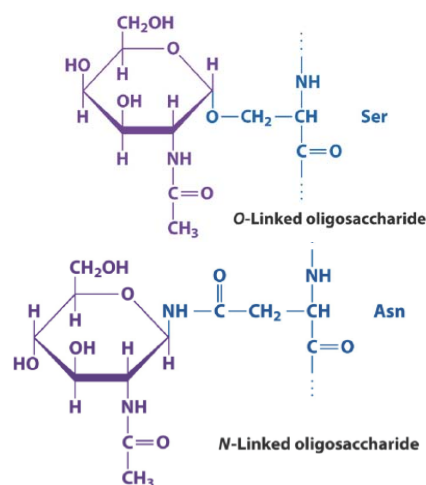
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PRACTICAL WORK 7

7.1. Detection of maltose and lactose

📖 *Definition.* Due to the presence of a free aldehyde group in the lactose molecule (in the glucose residue) and maltose (in the second glucose residue), these disaccharides have reducing properties and can participate in the reduction reactions, for example, maltose and lactose give a positive Trommer's test.

Equipment: Glass rods, tubes, pipettes graduated, droppers, test tube rack, burner.

Reagents: 0.5 % lactose, sucrose and maltose solutions, 0.5 % NaOH, 0.5 % $CuSO_4$.

Procedures: Add to tree test tubes:

Test tube 1	Test tube 2	Test tube 3
2 ml of 0.5% maltose solution	2 ml of 0.5% lactose solution	2 ml of 0.5% sucrose solution
1 ml of 0.5% NaOH		
5 drops of 0.5% $CuSO_4$		
Carefully heat the test tubes in the flame of burner		
✎ *What do you observe*?		

Conclusion_____

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7.2. Detection of sucrose

📖 *Definition.* To identify disaccharides that do not recover, one uses methods based on hydrolysis of disaccharides to monosaccharides ($C_{12}H_{22}O_{11}$ + H_2O → 2 $C_6H_{12}O_6$ (glucose + fructose)) followed by their detection by reactions Trommer's test, Seliwanoff's test, Fehling's test etc..

Equipment: Glass rods, tubes, pipettes graduated, droppers, test tube rack, burner.

Reagents: 5% sucrose solution, concentrated and 25% HCI, 5% NaOH, 5% $CuSO_4$, the crystalline resorcinol.

Procedures:

A. Obtaining of the sucrose hydrolyzate

1. Add to the test tube 7 ml of 5% sucrose solution and 5 drops of concentrated HCI;

2. Place the tube into a boiling water bath and heat it for 15 minutes.

3. After cooling, add to the tube 5% NaOH until a neutral pH appears.

B. Trommer's test

Add to two test tubes:

Test tube 1	Test tube 2 (control)
3 ml of HYDROLYSATE (from step A)	3 ml of sucrose solution
1 ml 5% NaOH and 5 drops of 5% $CuSO_4$	
Carefully heat the test tubes in the flame of burner to boil.	
✐ *What do you observe*?	

C. Seliwanoff's test for the detection of fructose

Add to two test tubes:

Test tube 1	Test tube 2 (control)
3 ml of HYDROLYSATE (from step A)	3 ml of sucrose solution
1 ml of 25% HCl and a few crystals of resorcinol	
Heat the tubes in a water bath to 80 ^{o}C for 5-10 min	
✐ *What do you observe*?	

Conclusion_____
_____
_____
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7.3. Detection of polysaccharides

A. Reactions of glucans identification

📖 *Definition:* Reserve polysaccharides of plant and animal tissues, such as starch and glycogen, react with iodine to form unstable complex adsorption compounds of blue (starch) or red-brown (glycogen) color, that discolour when heating and again stain solutions upon cooling. Discoloration of glucans compounds solutions with iodine is also observed when alkali is added. The disappearance of color upon heating and addition of alkali is due to the fact that molecular iodine participates take part in the formation of complexes, and not in the formation of iodide ions.

Reagents: 0,1% starch solutions, Lugol solution (1 g of iodine and 2 g of potassium iodide dissolved in 15 ml of H_2O and then diluted with H_2O, to a volume of 300 ml), 10% NaOH.

Procedures:
1. Add to the test tube 2 ml of 0.1% starch solutions;
2. Add 1–2 drops of Lugol solution and mix carefully.
✐ *What do you observe*?_____
3. Transfer 1 ml of coloured solutions to the other test tube which containe 1 ml of 10 % NaOH
✐ *What do you observe*?_____
4. Heat in water bath colored mixture remaining in the tube.
✐ *What do you observe*?_____
Conclusion_____
_____
_____

B. Hydrolysis of starch

📖 *Definition:* When the starch solution with mineral acids is heated, hydrolysis of the starch proceeds according to scheme:

$$(C_6H_{10}O_5)_n \rightarrow (C_6H_{10}O_5)_x \rightarrow (C_6H_{10}O_5)_y \rightarrow (n-1)H_2O \rightarrow nC_6H_{12}O_6$$

| **Starch** | **Dextrin** | **Oligosaccharides** | **Glucose** |
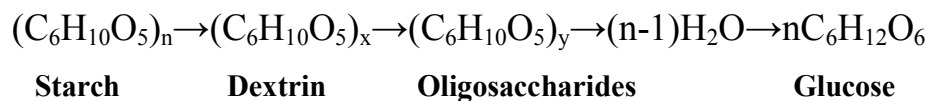
With complete hydrolysis of starch, D-glucose is formed, which can be detected by specific reactions.

Equipment: Glass rods, tubes, pipettes graduated, droppers, test tube rack, burner.

Reagents: 1% starch solution, concentrated HCI, 15% NaOH, 1% $CuSO_4$.

Procedures: Add to two test tubes:

Test tube 1	**Test tube 2**
5 ml of 1% starch solution	
5 drops of concentrated HCI	-
boil in a water bath for 15 minutes	-
After cooling in both solutions, conduct a ***Trommer's test:***	
Add 2 ml of 5% NaOH and 5 drops of 5% $CuSO_4$, mix carefully and heat on a water bath	
✐ *What do you observe?*	

Conclusion

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Teacher's signature _____ (_____)
full name

8. CLASSIFICATION, STRUCTURE AND BIOLOGICAL SIGNIFICANCE OF HETEROCYCLIC COMPOUNDS

📖 BACKGROUND

Heterocyclic compounds are the organic compounds contain one or more aromatic or non-aromatic rings with at least one atom (called **heteroatom**) in the ring being an element other than carbon (C), most frequently oxygen (O), nitrogen (N), or sulfur (S). These compounds belong to different classes of molecules. Heterocyclic compounds include many of the biochemical material essential to life.

HETEROCYCLIC COMPOUNDS CLASSIFICATION

There are two main ways of heterocyclic compounds classifying:
- ✓ ·By ring size
- ✓ ·Aromatic/non aromatic

When classification based on the ring size is used, heterocyclic compounds can be divided into three-, four-, five- and six-membered ones. Seven-, eight- or more -membered heterocyclic compounds are less common.

Heteroatom	Nitrogen	Oxygen	Sulfur	Nitrogen	Oxygen	Sulfur
3-Atom Ring	Aziridine	Oxirane	Thiirane	Azirine	Oxirene	Thiirene
4-Atom Ring	Azetidine	Oxetane	Thietane	Azete	Oxete	Thiete
5-Atom Ring	Pyrrolidine	Oxolane	Thiolane	Pyrrole	Furan	Thiophene
6-Atom Ring	Piperidine	Oxane	Thiane	Pyridine	Pyran	Thiopyran

There is another classification of heterocycles:

1) Nonaromatic heterocyclic compounds – cyclic ethers, amines, and sulfides behave like their acyclic analogs with the same functional group and do not require special discussion.

For example:
- ✓ *tetrahydrofurane* and *1,4-dioxane* are typical ethers;
- ✓ *1,3,5-trioxane* behaves as an acetal;
- ✓ *pyrrolidine* and *piperidine* are typical secondary amines;
- ✓ the bicyclic compound *quinuclidine* is a tertiary amine.

tetrahydrofuran 1,4-dioxane 1,3,5-trioxane

pyrrolidine   piperidine   quinuclidine
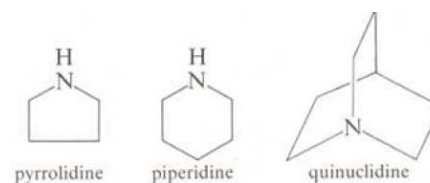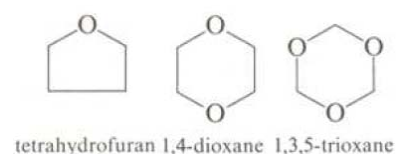
2) Aromatic heterocyclic compounds, which include such compounds as *pyridine*, where nitrogen replaces one of the CH groups in benzene, and *pyrrole*, in which the aromatic sextet is supplied by the four electrons of the two double bonds and the lone pair on nitrogen.

Other aromatic heterocycles contain more than one hetero atom, and still others contain fused aromatic rings. Examples which we will treat in more detail later include *oxazole, indole* and *purine*.

oxazole   indole   purine
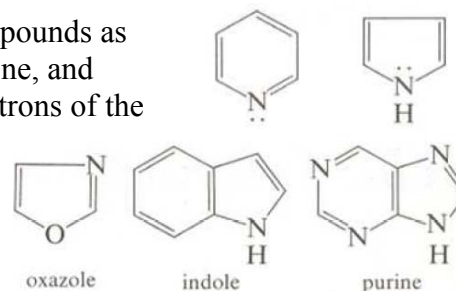
BIOLOGICAL SIGNIFICANCE OF HETEROCYCLIC COMPOUNDS

PYRROLE is not a basic compound! The unshared electron pair of nitrogen is not able to add proton.
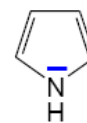
The most important pyrrole derivatives are cyclic tetrapyrroles (haem, chlorophyll) and linear tetrapyrroles (bile pigments).

Cyclic tetrapyrroles
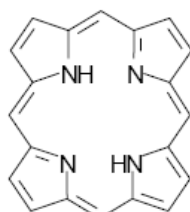
porphin
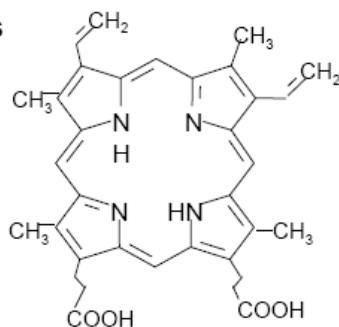
protoporphyrin IX
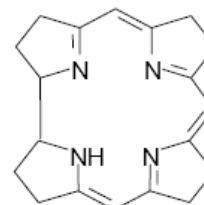
corrin

Linear tetrapyrroles
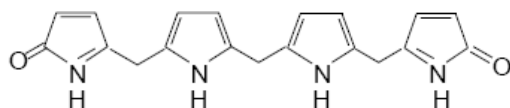
bilanes (with substituents at β-positions)
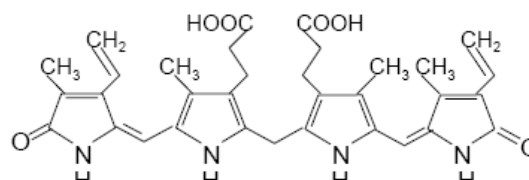
bilirubin
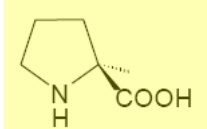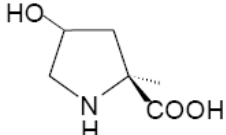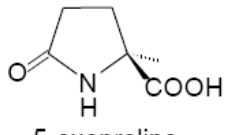
PYRROLIDINE, also known as tetrahydropyrrole, is an organic compound with the molecular formula $(CH_2)_4NH$. It is a cyclic secondary amine (pKB = 2.7), also classified as a saturated heterocycle.
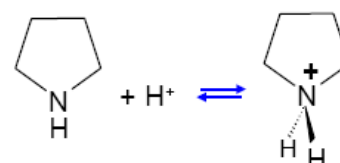
pyrrolidine + H⁺ ⇌ pyrrolidinium ion
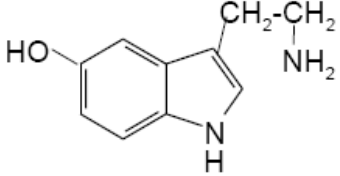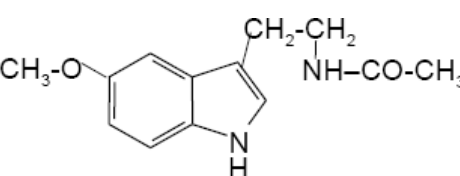
The most important pyrrolidine derivatives are:

A. Proline	**B. Hydroxyproline**	**C. 5-Oxoproline**
(pyrrolidine-2-carboxylic acid) is the only standard α-amino acid that has a secondary amino group:	(proline hydroxylated in position 4 or 3) is the characteristic constituent of collagen – the most abundant protein of connective tissue:	(lactam of glutamic acid) is formed in the group translocation of amino acids (γ-glutamyl cycle):
proline	4-hydroxyproline	5-oxoproline (pyroglutamate)

INDOLE is an aromatic heterocyclic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole is widely distributed in the natural environment and can be produced by a variety of bacteria. As an intercellular signal molecule, indole regulates various aspects of bacterial physiology, including spore formation, plasmid stability, resistance to drugs, biofilm formation, and virulence.
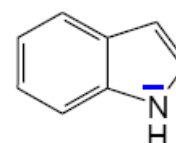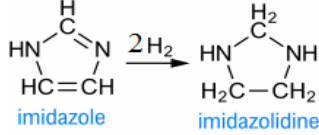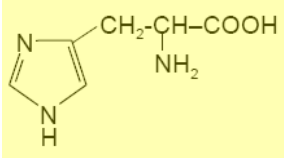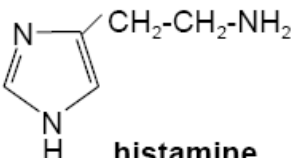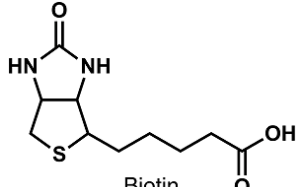
The most important indole derivatives are:

A. Tryptophan	B. Serotonin	C. Melatonin
(2-amino 3-(3-indolyl)-propanoic acid) is a standard amino acid from which biologically active compounds are derived.	(5-hydroxytryptamine) is a neurotransmitter in the central nervous system; it also acts as a vasoconstrictor released from blood platelets.	(*N*-acetyl-5-methoxytryptamine) is released from the pineal gland and has a role in the control of diurnal biorhythms.
tryptophan	serotonin	melatonin

IMIDAZOLE is a weak base, one of the nitrogen atoms can add proton. It is an aromatic heterocycle, classified as a diazole, and having non-adjacent nitrogen atoms. Many natural products, especially alkaloids, contain the imidazole ring. This ring system is present in important biological building blocks, such as histidine and the related hormone histamine. Many drugs contain an imidazole ring, such as certain antifungal drugs, the nitroimidazole series of antibiotics and the sedative midazolam.

imidazole + H⁺ ⇌ imidazolium cation
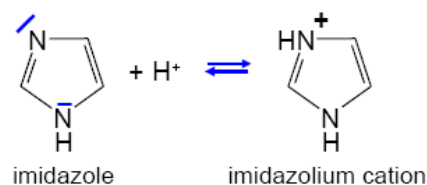
The most important imidazole derivatives are:

A. Histidine	B. Histamine	C. Imidazolidine
(2-amino-3-(4-imidazolyl)-propanoic acid) is an essential amino acid. The side chains of histidyl residues are responsible for the buffering ability of proteins at physiological pH values, because only the imidazolyl groups ($pK_B \approx 8.0$) can take part in protolytic reactions under those conditions.	originates by decarboxylation of histidine and acts as a vasodilator in inflammations and allergic reactions. It also causes contraction of smooth muscles of the bronchial stem and the acidic stomach secretion.	Classified as a diamine, it is formally derived by the addition of four hydrogen atoms to imidazole: imidazole → imidazolidine Imidazolidine occurs in the structure of creatinine and biotin.
histidine	histamine	Biotin

THIAZOLE is a heterocyclic compound that contains both sulfur and nitrogen. Thiazoles are structurally similar to imidazoles, with the thiazole sulfur replaced by nitrogen.

The thiazole ring occurs in:
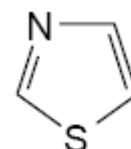
A. Thiamin (vitamin B1)
that also contains a pyrimidine ring.
Thiamin diphosphate is the coenzyme that transports "active aldehyde" groups in the course of oxidative decarboxylations of pyruvate and other α-ketocarboxylic acids.
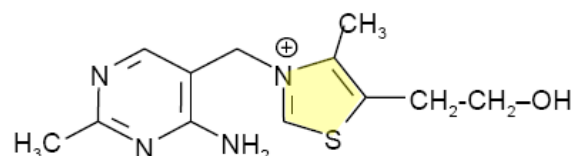
B. Thiazolidine

The fully saturated **thiazolidine** (tetrahydrothiazole) ring appears in the structure of antibiotics **penicillins**, the effect of which is caused by the unusual β-lactam ring.

N-acylaminopenicillanic acid
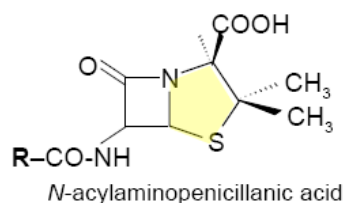
THIOLANE is completely hydrogenated thiophene.
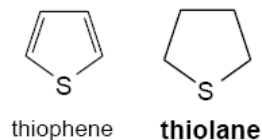
This ring structure appears (fused with imidazolidone) in **biotin**:

thiophene   **thiolane**
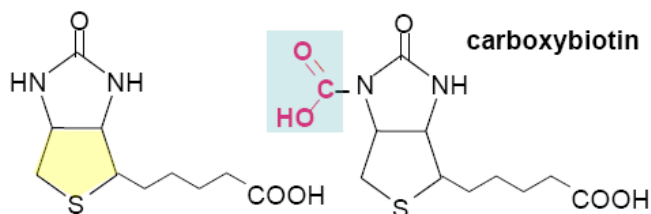
carboxybiotin

In the cells, biotin may bind CO_2 and resulting *carboxybiotin* serves as a donor of carboxyl in important carboxylations (e.g., biosynthesis of fatty acids, synthesis of oxaloacetate from pyruvate).
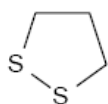
1,2-DITHIOLANE is a cyclic disulfide that can be easily reduced to acyclic alkan-1.4-dithiol.

It occurs as a part of **lipoic acid:**

lipoic acid (an **oxidant**)   dihydrolipoic acid (**transfer of acyls**)
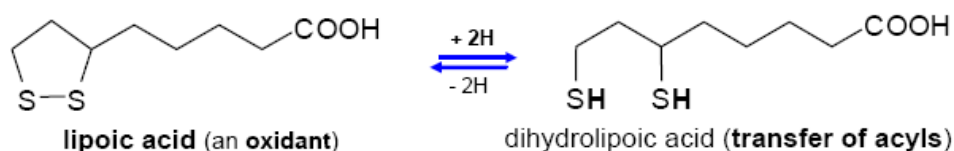
FURAN is a heterocyclic organic compound, consisting of a five-membered aromatic ring with four carbon atoms and one oxygen. It is toxic and may be carcinogenic in humans. Furan derivatives are rather rare in natural compounds. On the contrary, **TETRAHYDROFURAN** ring is quite common in cyclic forms of sugars.

furan   **tetrahydrofuran**   D-ribofuranose
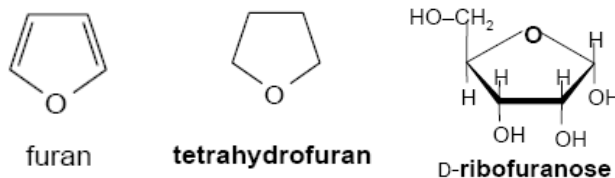
PYRAN, or **oxine**, is a six-membered heterocyclic, non-aromatic ring, consisting of five carbon atoms and one oxygen atom and containing two double bonds. There are two isomers of pyran that differ by the location of the double bonds. In **2*H*-pyran**, the saturated carbon is at position 2, whereas, in **4*H*-pyran**, the saturated carbon is at position 4.

2*H*-pyran   4*H*-pyran
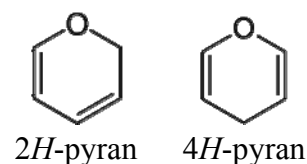
TETRAHYDROPYRAN ring occurs in cyclic forms of monosaccharides:

tetrahydropyran   D-ribopyranose
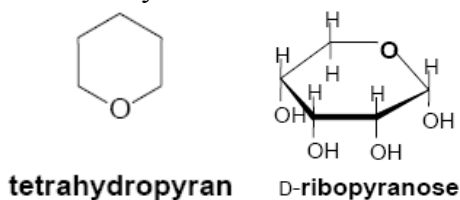
Many plant substances comprise the pyran ring that is usually fused condensed with the benzene ring. Those structures appear, e.g., in **flavonoids**, in the active component of hemp **tetrahydrocannabinol** (THC), tocopherols and derivatives of coumarin.

chroman   chromone
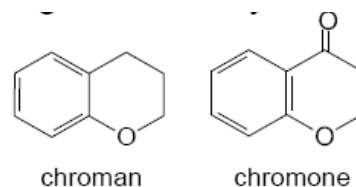
Tocopherols are important exogenous lipophilic antioxidants, which prevent oxidation of membrane lipids and lipoproteins by reactive oxygen species.

Derivatives of coumarin (warfarin, biscoumarins) prevent *in vivo* coagulation of blood. They act as antivitamins K by inhibition of biosynthesis of vitamin K-dependent coagulation factors. Coumarin appears in spoiled sweet-clover hay.


α-tocopherol (vitamin E)
(5,7,8-trimethyl derivative of the phenol tocol)
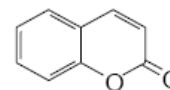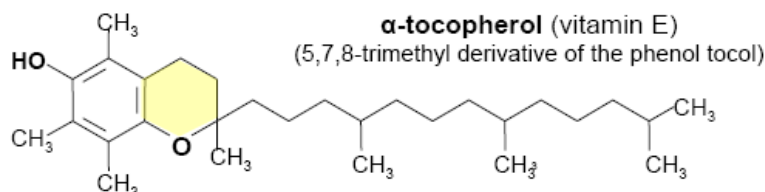
coumarin

PYRIDINE is a weak basic heterocyclic compound ($pK_B = 8.7$) with the chemical formula C_5H_5N. It is structurally related to benzene, with one methine group (=CH−) replaced by a nitrogen atom. The pyridine ring occurs in many important compounds.

pyridine   + H$^+$ pyridinium H
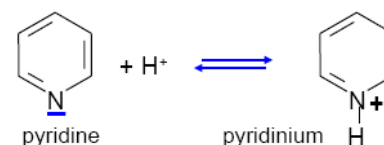
The most important derivatives of pyridine are two essential factors:

1) Nicotinic acid and **nicotinamide** is called **niacin** or pellagra-preventing factor (PPF); they are included in the group of vitamins B.

nicotinic acid
(pyridine-3-carboxylic acid)

nicotinamide

alkaloid nicotine
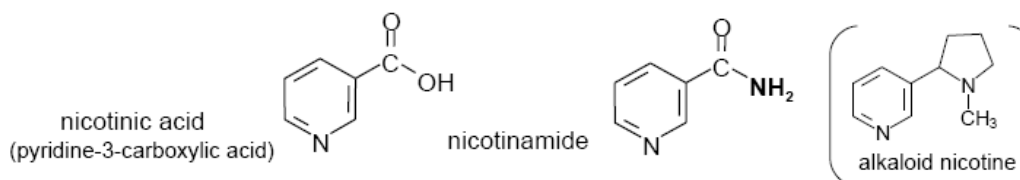
NAD$^+$ (nicotinamide adenine dinucleotide) and **NADP$_+$** (NAD phosphate) are **coenzymes of dehydrogenases**. The active group of NAD$^+$ (as well as of similar NADP$^+$) is nicotinamide: It acts as an **oxidant** that takes off **two atoms of hydrogen** from the substrate. One atom plus one electron (**hydride anion H$^-$**) is added to the *para*-position of the pyridinium ring, the remaining H$^+$ binds to the enzyme:

anhydride bond   adenine

H$^+$+ H$^\ominus$

Oxidized form NAD$^+$
(aromatic ring, positive charge)

Reduced form NADH + H$^+$
(quinoid ring, no charge)

+ H$^+$
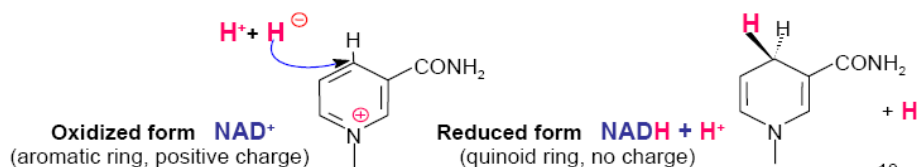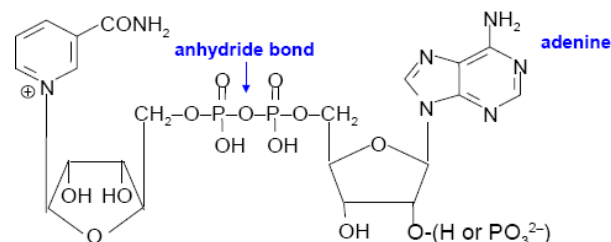
2) Vitamin B6 is the group name for three related derivatives pyridoxine, pyridoxal, and pyridoxamine.

pyridoxine   pyridoxal   pyridoxamine
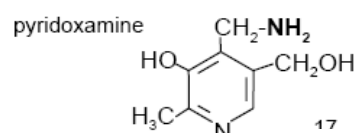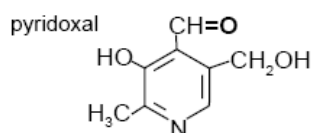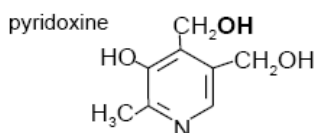
Pyridoxal phosphate is the prosthetic group in many enzymes taking significant part in α-amino acid metabolism namely. They catalyze, e.g., **transamination** and **decarboxylation of amino acids.** The aldehyde group of pyridoxal coenzyme binds to α-amino group of an amino acid forming an **aldimine intermediate** (Schiff base).

pyridoxal phosphate
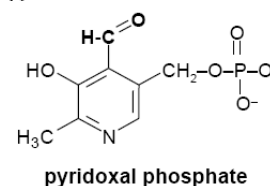
PYRIMIDINE is an aromatic heterocyclic organic compound similar to pyridine. One of the three diazines (six-membered heterocyclics with two nitrogen atoms in the ring), it has the nitrogen atoms at positions 1 and 3 in the ring.
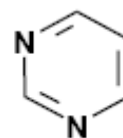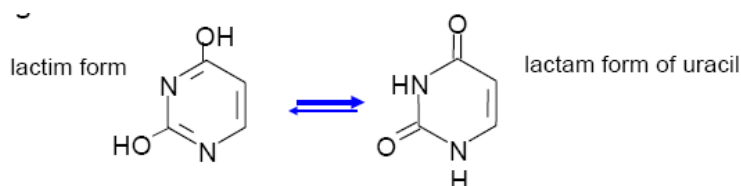
*Three pyrimidine bases are components of nucleic acids:*

cytosine
4-amino-2-hydroxypyrimidine

uracil
2,4-dihydroxypyrimidine

thymine
5-methyluracil
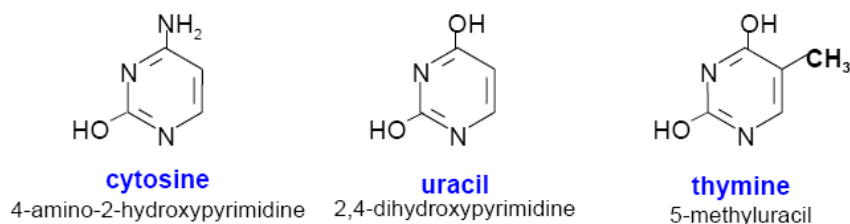
They exist in two tautomeric forms. Only the lactam forms can be ribosylated to give nucleosides:

lactim form

lactam form of uracil

PURINE (pyrimidinoimidazole) is a heterocyclic aromatic compound that consists of a pyrimidine ring fused to an imidazole ring.
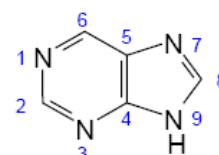
atypical numbering!

There are many naturally occurring purines.

They include the nucleobases adenine (**2**) and guanine (**3**). In DNA, these bases form hydrogen bonds with their complementary pyrimidines, thymine and cytosine, respectively. This is called **complementary base pairing**. In RNA, the complement of adenine is uracil, instead of thymine.

Other notable purines are hypoxanthine (**4**), xanthine (**5**), theobromine (**6**), caffeine (**7**), uric acid (**8**) and isoguanine (**9**).

| purine 1 | adenine 2 | guanine 3 | hypoxanthine 4 | xanthine 5 |

| theobromine 6 | caffeine 7 | uric acid 8 | isoguanine 9 |
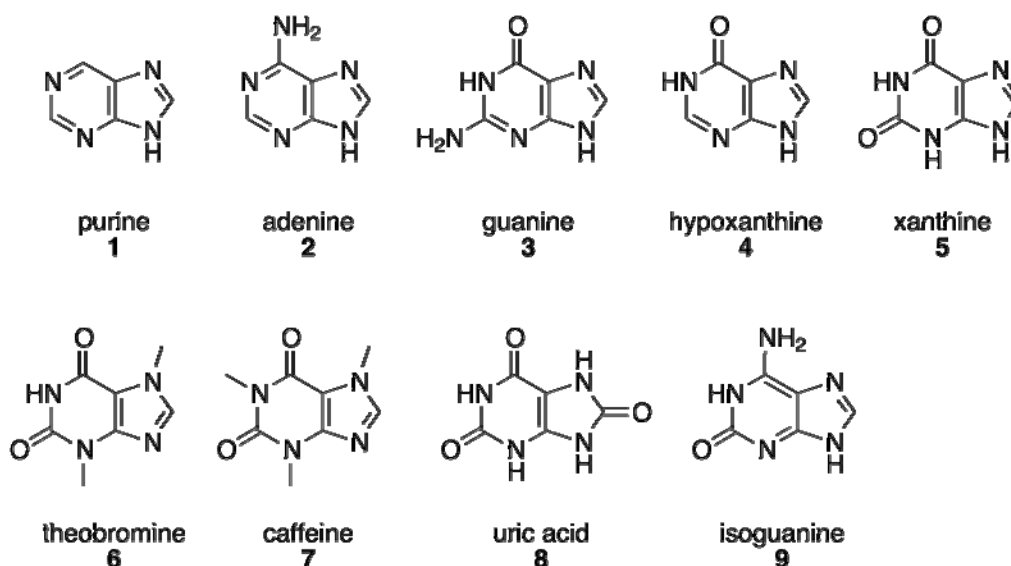
PTERIDINE is an aromatic chemical compound composed of used pyrimidine and pyrazine rings. Pteridine is a precursor in the synthesis of *dihydrofolic acid* in many microorganisms. The enzyme dihydropteroate synthetase converts pteridine and 4-aminobenzoic acid to dihydrofolic acid in the presence of glutamate. The enzyme dihydropteroate synthetase is inhibited by sulfonamide antibiotics.
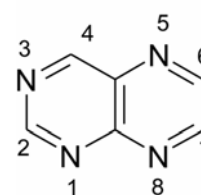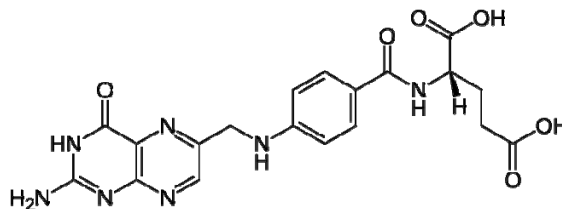

Pteridine

*Three important coenzymes are derived from pteridine*:

1) Tetrahydrobiopterin (BH$_4$) acts as the **reducing coenzyme** in certain hydroxylations catalyzed by monooxygenases. It supplies two atoms of hydrogen and is oxidized to the quinoid form of **dihydropterin**:

tetrahydrobiopterin (**BH$_4$**)
reduced form

dihydrobiopterin (**qBH$_2$**)
oxidized (quinoid) form
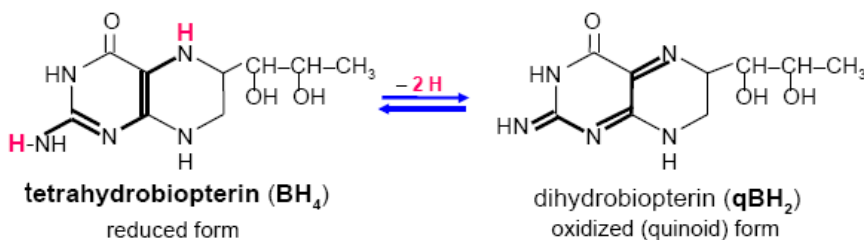
2) Folic acid (**folate**) is *N*-**pteroylglutamic acid**; the number of attached glutamyl residues may be higher. Although synthesizing the pteridine ring, mammals are unable to conjugate it with *p*-aminobenzoic acid and glutamate. They have to obtain folate from their diets or from microorganisms in their intestinal tracts.

3) By condensation with benzene ring, pteridine gives **benzopteridine**, the 2,4-dioxoderivative of which is **isoalloxazine**.

benzo[*g*]pteridine
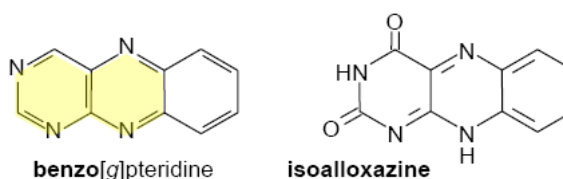
isoalloxazine

Riboflavin (vitamin B$_2$) is 6,7-dimethyl-9-(1-ribityl) isoalloxazine.
Ribityl is the remainder of sugar alcohol ribitol.
Riboflavin is the only coloured hydrophilic vitamin (intensive yellow).
It is a part of **coenzymes FAD and FMN.**
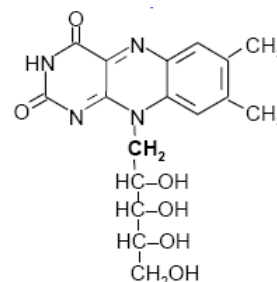
FAD (flavin adenine dinucleotide) and **FMN** (flavin mononucleotide)
Flavin prosthetic groups act as **oxidants** in certain types of reactions catalyzed by dehydrogenases or oxidases.

coenzyme FAD
(oxidized form)

oxidized form
FAD or **FMN**

reduced form
FADH$_2$ or **FMNH$_2$**
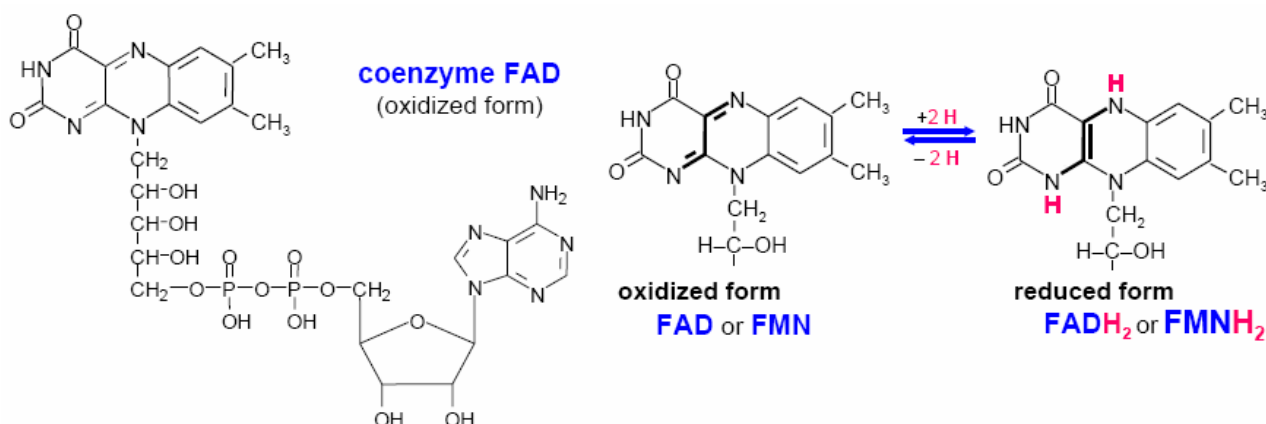
PRACTICAL WORK 8

Equipment: test tubes, pipettes graduated, droppers, filter paper, Bunsen burners, water bath.

8.1. Indigo carmine reactions

📖 *Definition.* The indigo-carmine method is a simple, rapid, accurate colorimetric procedure for determining small amounts of dissolved oxygen in water (0 to 50 ppm). A solution of reduced indigo-carmine dye added to a water sample is oxidized by the presence of any dissolved oxygen and changes color. The change in color of the dye is directly proportional to the amount of dissolved oxygen present in the sample.

Reagents: 0.5% glucose solution, 0.5% solution of indigo carmine, concentrated HNO_3, 5% Na_2CO_3.

A. Relation to oxidants.
Procedure:

  Mix into the test tube 1 ml of 0.5 % indigo carmine solution and 0.5 ml of concentrated HNO_3

✏ *What do you observe?*_____

_____

B. Relation to reductants.
Procedure:

1. Add to the test tube 0.5 ml of 0.5% indigo carmine solution and 0.5 ml of 0.5% glucose solution;
2. Mix it with 0.5 ml of 5% Na_2CO_3 and dilute with 1 ml of H_2O;
3. Heat the resulting solution carefully without shaking in a tilted tube.

✏ *What do you observe?*_____

_____

4. Then vigorously shake the test tube.

✏ *What do you observe?*_____

_____

8.2. Qualitative reactions to analgin
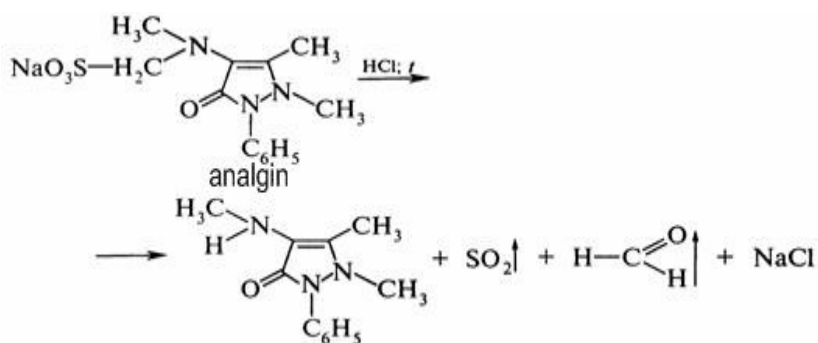
Reagents: analgin, 1% $FeCl_3$, 10% HCl.

A. *Procedure:*

1. In the test tube dissolve several crystals of analgin in 2 ml of distilled H_2O
2. Add 0.5 ml of 1% $FeCl_3$.

✏ *What do you observe?*_____

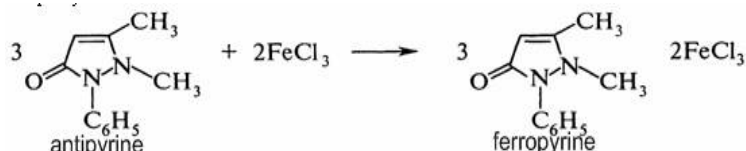_____

B. *Procedure:*

1. Add to the test tube several crystals of analgin, 1 ml of distilled H_2O and 1 ml of 10% HCl
2. Heat test tube for 2 min in a water bath

✎ *What do you observe?*_____

_____

3. Add to the test tube with cooled solution 0.5 ml of 1% $FeCl_3$.

✎ *What do you observe?*_____

_____

8.3. Color reaction to antipyrine with ferric (III) chloride
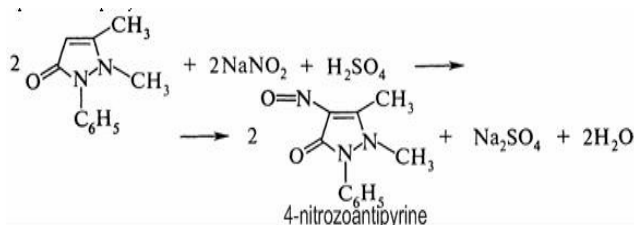

Reagents: antipyrine, 1% $FeCl_3$

Procedures:
   1. Add to the test tube several crystals of antipyrine
   2. 1 ml of H_2O
   3. 0.5 ml of 1% $FeCl_3$.

✎ *What do you observe?*_____

_____

8.4. Nitrosation of antipyrine

Reagents: antipyrine, 10% H_2SO_4, 5% $NaNO_2$

Procedures:

Add to the test tube several crystals of antipyrine, 1 ml of H_2O, 0.5 ml of 10% H_2SO_4 and 0.5 ml of 5% $NaNO_2$

✎ *What do you observe?*_____

_____

8.5. Qualitative reaction to neophylline with cobalt (II) chloride
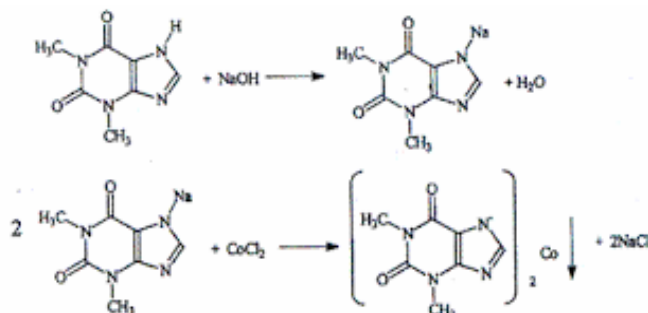
Reagents: neophylline, 10% NaOH, 5% CoCl$_2$.

Procedure:

1. Add to the test tube several crystals of neophylline, 0.5 ml of 10% NaOH and vigorously shake for 2-3 min.

2. Add 0.5 ml of 5% CoCl$_2$.

✎ *What do you observe?*_____

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Conclusion_____

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Teacher's signature _____ (_____)

<div align="right">*full name*</div>

9. STRUCTURE AND BIOCHEMICAL FUNCTIONS OF NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS

📖 BACKGROUND

NUCLEOTIDES are organic molecules that serve as the monomer units for forming the nucleic acid polymers deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), both of which are essential biomolecules in all life-forms on Earth.

Nucleotides are composed of *three subunit molecules*: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. They are also known as phosphate nucleotides.

A **nucleoside** is a nitrogenous base and a 5-carbon sugar. Thus a nucleoside plus a phosphate group yields a nucleotide. So *nucleotides are phosphorylared nucleosides*.

from *https://biologywise.com/nucleoside-vs-nucleotide*
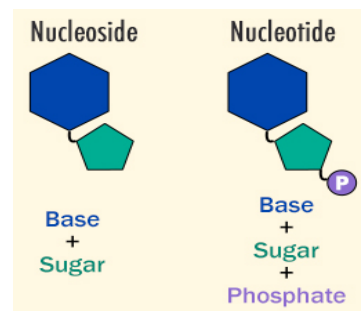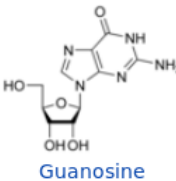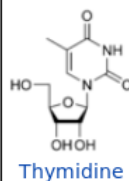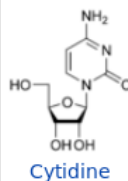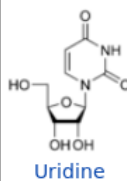
FUNCTIONS OF NUCLEOTIDES
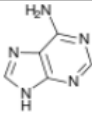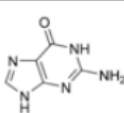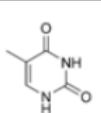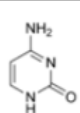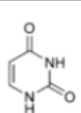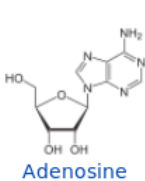
1. Play a central role in life-form metabolism at the fundamental, cellular level;

2. Carry packets of chemical energy in the form of the nucleoside triphosphates (ATP, GTP, CTP and UTP) throughout the cell to the many cellular functions that demand energy;

3. Participate in cell signaling (cGMP and cAMP);

4. Incorporated into important cofactors of enzymatic reactions (coenzyme A, FAD, FMN, NAD, and $NADP^+$).

In experimental biochemistry, nucleotides can be radiolabeled with radionuclides to yield radionucleotides.
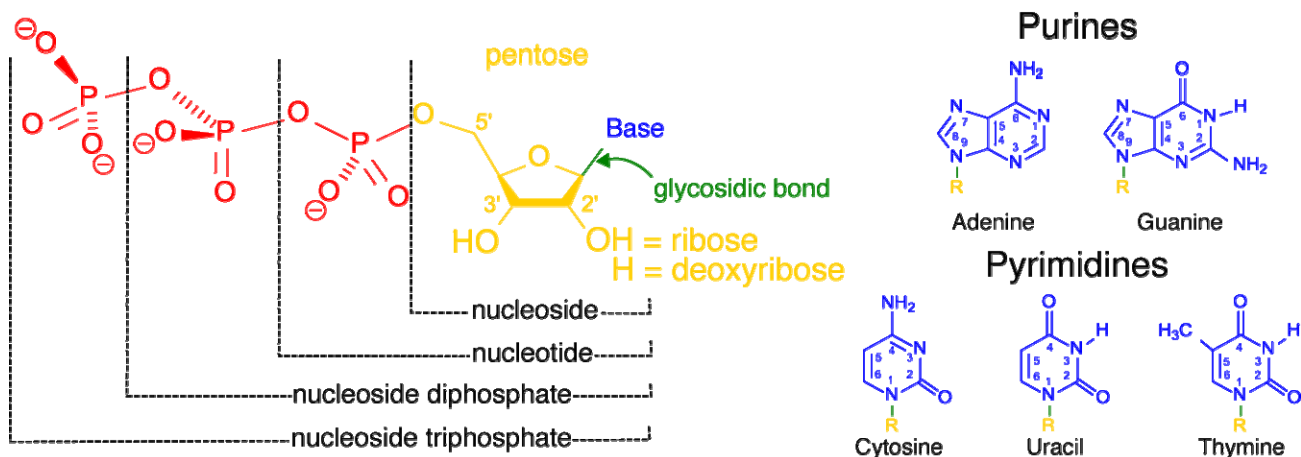
STRUCTURE OF NUCLEOTIDES

Nucleotides contain either a *purine* or a *pyrimidine* base (the nitrogenous base molecule, also known as a nucleobase) and are termed **ribonucleotides** if the sugar is *D-ribose*, or **deoxyribonucleotides** if the sugar is *2-deoxy-D-ribose* (deoxyribose). Both sugars are linked to the heterocycle by a **a-N-glycosidic bond,** almost always to the **N-1** of a pyrimidine or to **N-9** of a purine. Table below lists the major purines and pyrimidines and their nucleoside derivatives. Single-letter abbreviations are used to identify adenine (A), guanine (G),cytosine (C), thymine (T), and uracil (U), whether free or present in nucleosides or nucleotides. The prefix "d" (deoxy) indicates that the sugar is 2′-deoxy-D-ribose (eg, in dATP)

The conjugated double bonds of purine and pyrimidine derivatives absorb ultraviolet light. All the common nucleotides absorb light at a wavelength close to 260 nm. The mutagenic effect of ultraviolet light is due to its absorption by nucleotides in DNA that results in chemical modifications.

from *https://biology.stackexchange.com/questions/3864/dna-as-an-acid*

Individual *phosphate molecules* repetitively connect the sugar-ring molecules in two adjacent nucleotide monomers, thereby connecting the nucleotide monomers of a nucleic acid end-to-end into a long chain.

These chain-joins of sugar and phosphate molecules create a **'backbone'** strand for a *single-* or *double helix* (RNA or DNA).

In any *one strand*, the chemical orientation (directionality) of the chain-joins runs *from the 5'-end to the 3'-end* — referring to the five carbon sites on sugar molecules in adjacent nucleotides.

In a *double helix*, the two strands are oriented *in opposite directions*, which permits base pairing and complementarity between the base-pairs, all which is essential for replicating or transcribing the encoded information found in DNA.
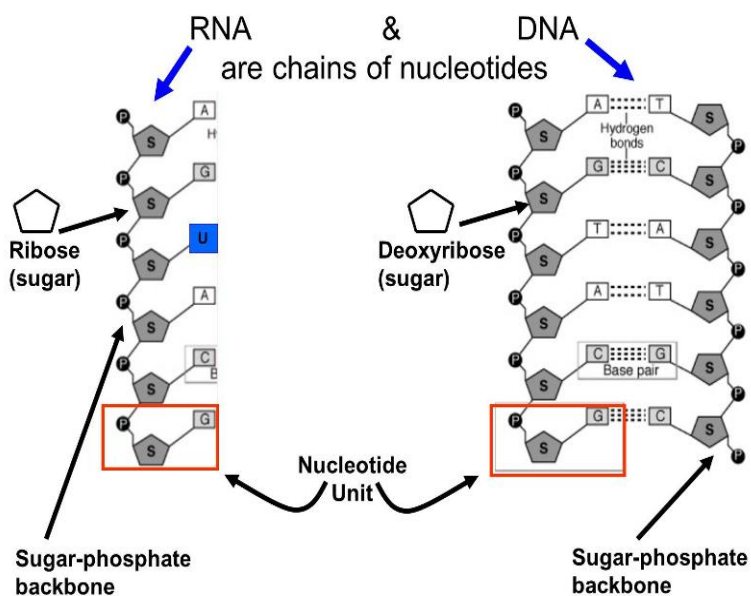
Unlike in nucleic acid nucleotides, singular **_cyclic nucleotides_** are formed when *the phosphate group is bound twice to the same sugar molecule,* i.e., at the corners of the sugar hydroxyl groups. These individual nucleotides function in cell metabolism rather than the nucleic acid structures of long-chain molecules.


3', 5'-Cyclic AMP (cAMP)   3', 5'-Cyclic GMP (cGMP)

63
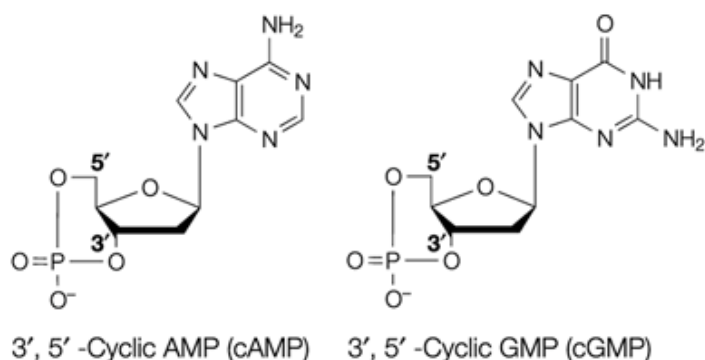
Nucleic acids are polymeric macromolecules assembled from nucleotides. The purine bases adenine and guanine and pyrimidine base cytosine occur in both DNA and RNA, while the pyrimidine bases thymine (in DNA) and uracil (in RNA) in just one.

Adenine forms a base pair with thymine with *two hydrogen bonds*, while guanine pairs with cytosine with *three hydrogen bonds*.

The **DNA** carries the information for building proteins. The sequence of nucleotides in a DNA molecule determines the sequence of amino acids in a polypeptide of a protein. When a cell divides, its DNA is copied and passed from one cell generation to the next generation. DNA is organized into chromosomes and found within the nucleus of our cells.

RNA is essential for the synthesis of proteins. Information contained within the genetic code is typically passed from DNA to RNA to the resulting proteins. There are several different types of RNA. **Messenger RNA (mRNA)** is the RNA transcript or RNA copy of the DNA message produced during *DNA transcription.* Messenger RNA is translated to form proteins. **Transfer RNA (tRNA)** has a three dimensional shape and is necessary for *the translation of mRNA* in protein synthesis. **Ribosomal RNA (rRNA)** is a component of ribosomes and is also involved in protein synthesis. **MicroRNAs (miRNAs)** are small RNAs that help to regulate gene expression.
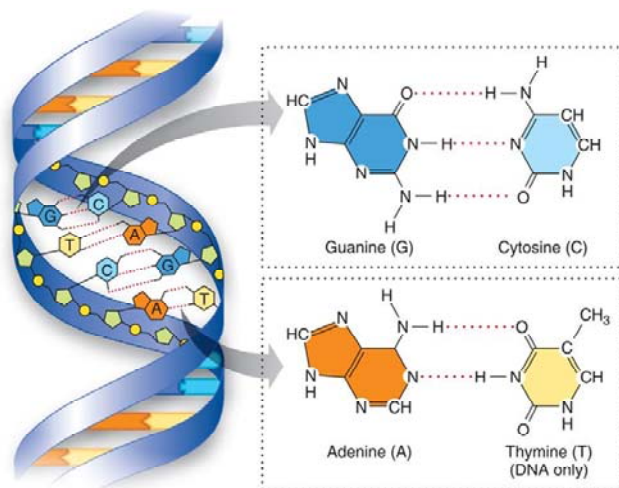
DNA structure with base pairs: G with C and A with T

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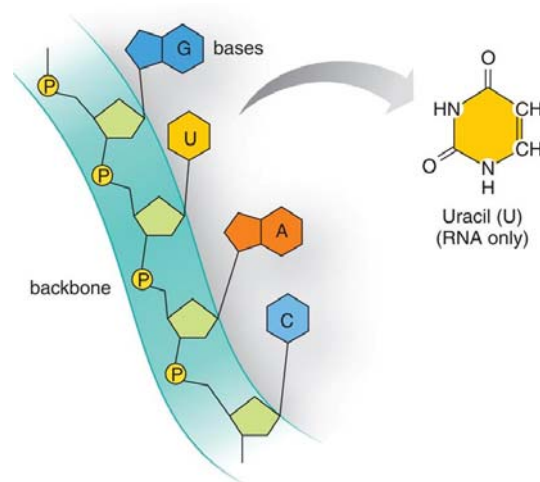
RNA structure with bases G, U, A, C

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HOME PROJECT
Give examples of synthetic nucleotide analogs applied in clinical medicine?

PRACTICAL WORK 9

9.1. Purification of nucleic acids from yeast and detection of nucleotide components

Equipment. Porcelain mortar with pestle, glass rods, centrifuge tubes, flask, funnels, centrifuge, tube rack with test tubes, pipettes, water bath.

Reagents. Dry yeast; 0.4 % i 0.02 M NaOH, 96 % ethanol, 5% H_2SO_4, 2 M NaOH, diphenylamine-containing reagent (1 g phenylamine dissolve in a mixture that consists of 100 ml of glacial acetic (CH_3COOH) and 2.75 mg of concentrated sulphuric acids (H_2SO_4); molybdenum reagent (7,5 g of ammonium molybdate dissolved in 100 ml of H_2O and add 100 ml of 32 % solution of nitric acid with a relative density of 1.2), 10 % NaOH; 1% $CuSO_4$.

A. Preparation of nucleic acids from yeast

📖 *Definition:* Ribonucleoproteins (RNP) and deoxyribonucleoproteins (DNP) can be isolated from animal tissues (or yeast) using alkali. The nucleoprotein may be precipitated from the concentrated salt solution by the addition of alcohol.

Procedures:

1. 10 g of baker's yeast carefully grind in a porcelain mortar for 10 min with 30 ml of 0.4 % NaOH, which is added in small portions. Heat the suspension in a boiling water bath for 15 minutes, stirring periodically. Nucleic acids transfer to the solution.

2. After cooling, centrifuge the mixture (10 min at 2000 *g)*. Nucleic acids remain in the supernatant. Insoluble components (precipitate) should be disposed.

3. To the thus obtained solution of nucleic acids, add three times the volume of ethanol and allow to stand for 15 minutes. Nucleic acids then precipitate from the solution in the form of sediment (pellet), which must be removed by centrifugation (10 min at 2000 *g)* and then dissolved in 5 ml of 0.02 M NaOH.

!!! The resulting preparation of nucleic acids - sodium nucleate - use for further experiments.

B. Detection of deoxyribose - test with diphenylamine

📖 Carbohydrates (sugars) in the composition of nucleic acids interact with the following compounds: orcin (reaction to the detection of ribose in ribonucleoproteins), diphenylamine, indole and Schiff's reagent - fuchsin-sulfuric acid (reaction to the detection of deoxyribose in deoxyribonucleoproteins).
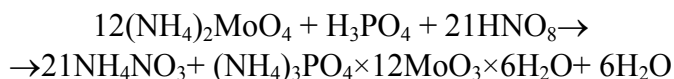
Procedures:

To 1 ml of sodium nucleate add 2 ml of a diphenylamine-containing reagent (in a mixture of acetic and concentrated sulphuric acids). Mix and put in a boiling water bath for 10 minutes.

✎ *What color do you observe?* _____

C. Detection of phosphate in nucleic acid

📖 *Definition.* The phosphoric acid residue in the nucleoproteins is detected by the reaction with a molybdenum reagent where a precipitate of the phosphoric salt of ammonium molybdate is formed:

$$12(NH_4)_2MoO_4 + H_3PO_4 + 21HNO_8 \rightarrow$$
$$\rightarrow 21NH_4NO_3 + (NH_4)_3PO_4 \times 12MoO_3 \times 6H_2O + 6H_2O$$

Procedure:

Add to the test tube 1 ml of sodium nucleate, 1 ml of molybdenum reagent mix thoroughly and boil the solution.

✎ *What color do you observe?* _____

D. Detection of proteins in nucleoproteins

📖 *Definition:* The presence of the protein component of nucleoprotein is determined by biuret reaction. The manifestation of color depends on the degree of hydrolysis of proteins, native protein in this reaction gives a blue-violet color and its breakdown products (peptides) - red-violet.

Procedure:

Add to the test tube 0.5 ml of sodium nucleate, 0.5 ml of 10 % NaOH and 2-3 drops of 1 % $CuSO_4$, mix thoroughly.

✎ *What colours do you observe?* _____

Conclution_____

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Teacher's signature _____ (_____)
<div align="center">*full name*</div>

RECOMMENDED LITERATURE

Basic

1. Lecture notes

2. Harvey R. A. Lippincott's Illustrated Reviews: Biochemistry / R. A. Harvey, D. R. Ferrier – 5th ed: Lippincott Williams & Wilkins, 2011. – 520 p.

3. Murray R. K. Harper's Illustrated Biochemistry / R. K. Murray, D. K. Granner, V. W. Rodwell. – 27th ed.: McGraw Hill, 2006. - 692 p.

4. Nelson D. Lehninger Principles of Biochemistry / D. Nelson, M. Cox. – 4th ed.: W.H. Freeman & Company, 2005. – 1216 p.

5. Moran L.A. Principles of Biochemistry / L.A. Moran, H.R. Horton, K.G. Scrimgeour, M.D. Perry. – 5th ed.: Pearson, 2012. – 824 p.

Additional

1. Koolman J. Color Atlas of Biochemistry: textbook / J. Koolman, K.-H. Roehm. – 2nd ed. – Stuttgart-New York : Thieme, 2005. – 467 p.

2. Lieberman M. Medical Biochemistry: textbook / M. Lieberman; A. Marks, C. Smith. – 2nd ed. – New York : Lippincott Williams & Wilkins, 2007. – 540 p.

3. Marks D. B. Biochemistry: The Chemical Reactions of Living Cells / D. B. Marks, D. Metzler - [2nd ed., vol. 1,2] – USA : Elsevier Academic Press, 1994.– 1974 p.

4. Marshall J. W. Clinical Chemistry : textbook / J. W. Marshall, S. K. Bangert. – Fifth edition. – China : Mosby, 2004. – 422 p.

5. Newsholme E. A. Functional Biochemistry in Health and Disease / E. A. Newsholme, T. R. Leech. - UK : John Wiley & Sons Ltd, 2010. – 543 p.

6. Smith C. Basic Medical Biochemistry: A Clinical Approach: textbook / C. Smith, A. Marks, M. Lieberman. – 2nd ed. - New York : Lippincott Williams & Wilkins, 2009. – 920 p.

Навчальне видання

БІОЛОГІЧНА ТА БІООРГАНІЧНА ХІМІЯ
Робочий зошит для іноземних студентів спеціалізації "Медицина"

Частина 1. Загальна біоорганічна хімія

(Англійською мовою)

Упорядники:

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Гребіник Дмитро Миколайович, **Компанець** Ірина Володимирівна, **Синельник** Тетяна Борисівна,
Савчук Олексій Миколайович, **Остапченко** Людмила Іванівна