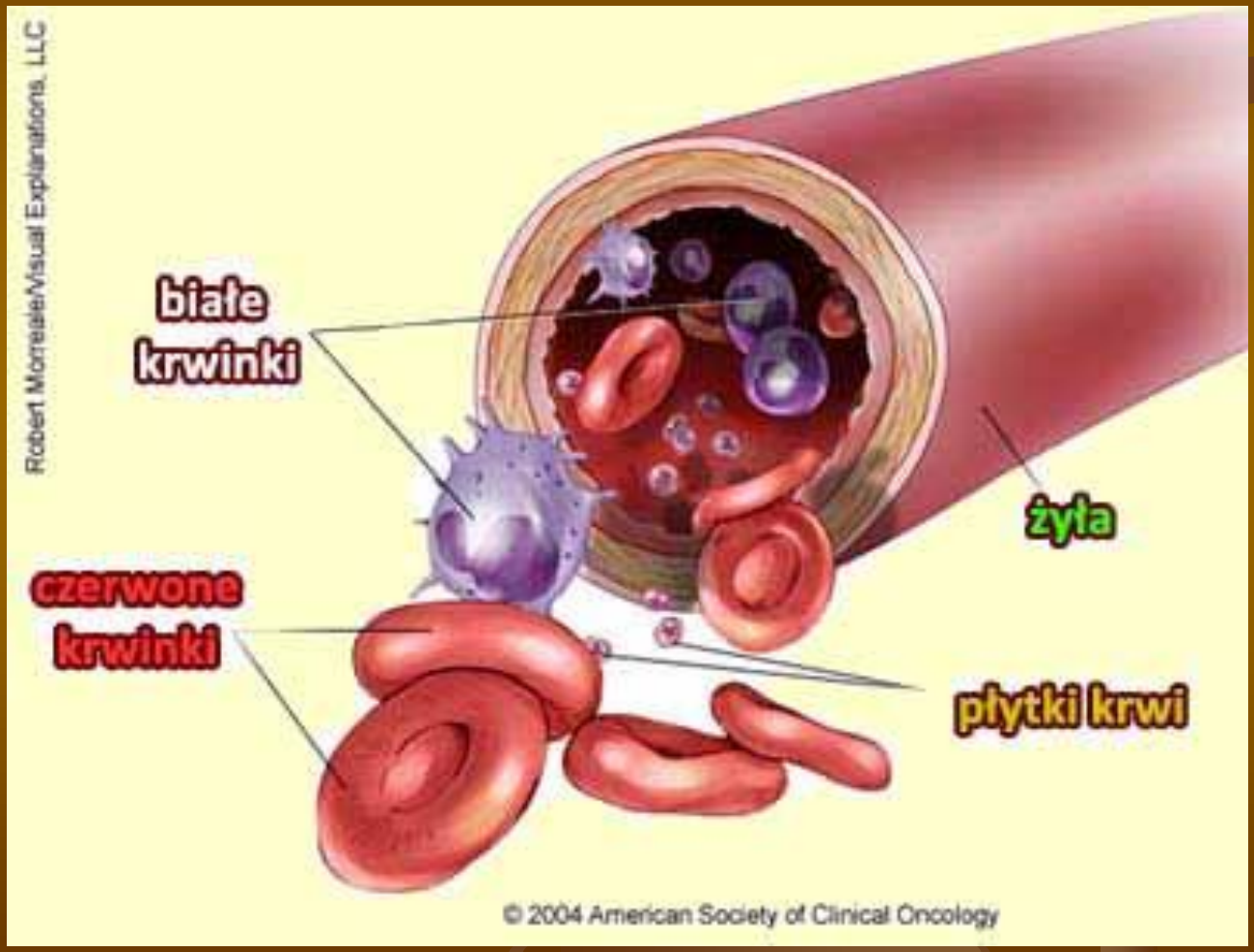
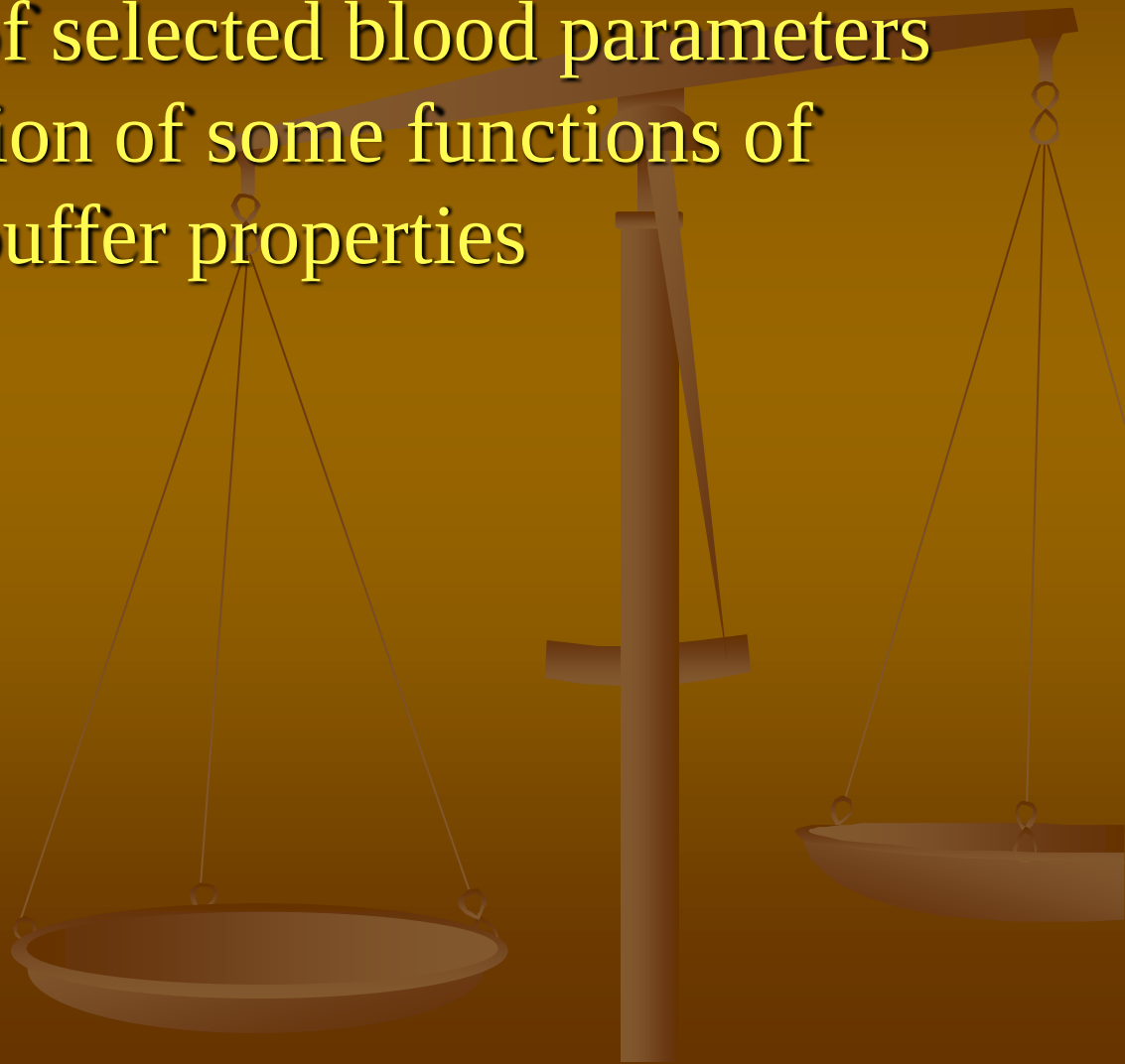
Body fluids - blood



The aim:

The identification of selected blood parameters and the examination of some functions of blood including buffer properties



Properties:

Liquid tissue containing plasma and morphotic elements like white blood cells, red blood cells and blood platellets.

Colour depends on the content of haemoglobin in erythrocytes.

Odour is species specific and depends on the content of volatile fatty acids

Specific gravity 1,04- 1,06g/cm^3

- pH 7,3-7,5 (in herbivores pH is more basic); pH of birds 7,56, pH of fish 7,52- 7,7

Functions:

- Transport – providing with oxygen, hormons, nutrients to tissues and removal of waste to kidneys
- Regulation – maintenance of constant osmotic pressue, acid-base balance and temperature
- Defence – fagocytosis, mechanisms of resistance

Erythrocytes:

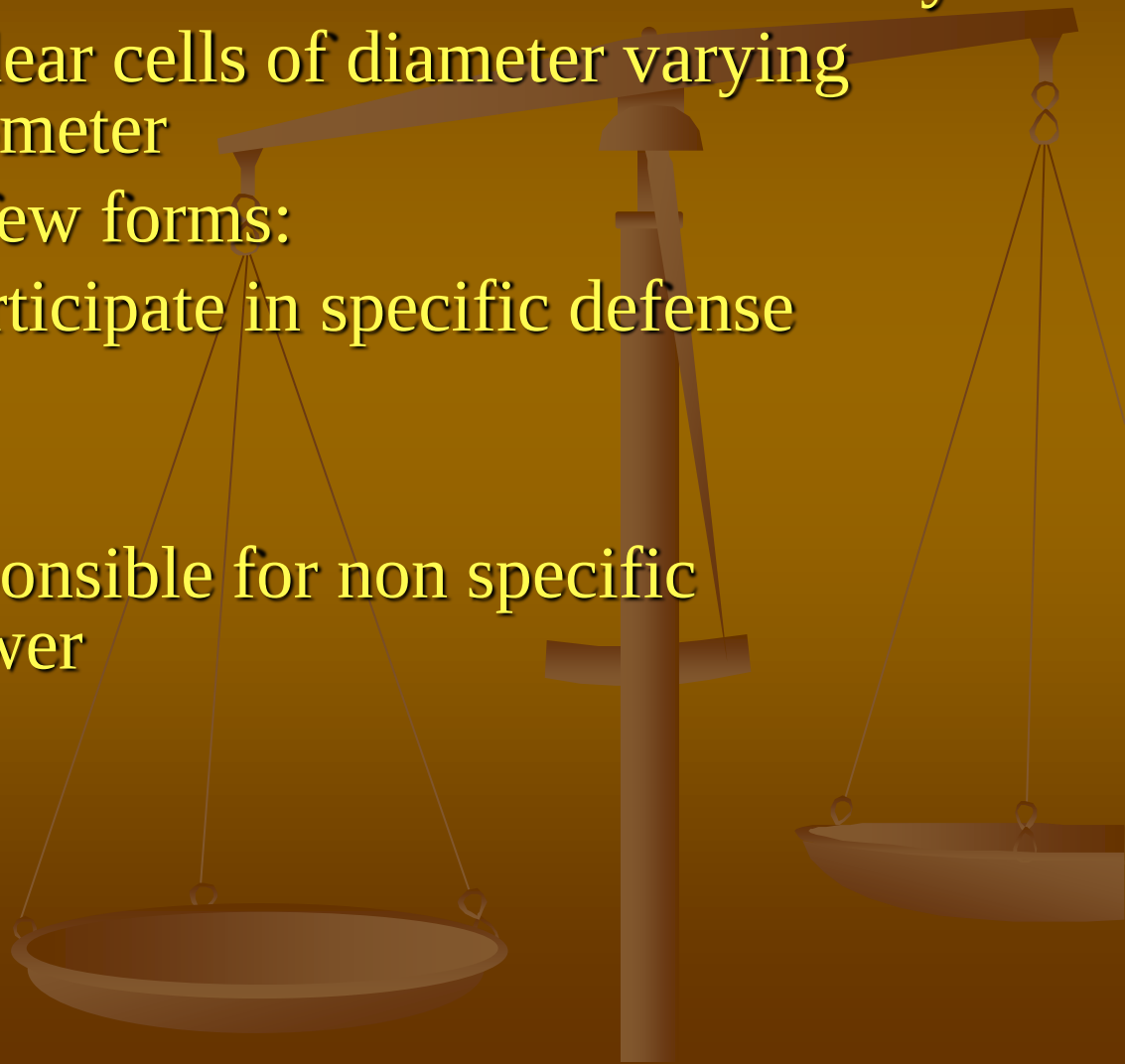
- Produced in bone marrow under the action of erythropoetin
- Deprived of cell organelles
- Possess lipid-protein membrane which determines osmosis, permeate water, anions, urea, glucose, does not permeate sodium and kalium what assures isotonic conditions (hypertonic – shrinking of erythrocytes, hypotonic- swelling of erythrocytes)

- The interior of erythrocyte is filled with haemoglobin which plays a role in gas exchange and maintenance of pH
- Iron in haemoglobin is maintained as divalent ion by ascorbic acid, glutathione and NADPH
- Glucose is energetic material for erythrocytes, it is trapped by insulin independent mechanism, 10% of glucose is oxidised in pentose-phosphate cycle

Leukocytes:

All leukocytes participate in defense functions of body

- Leukocytes are nuclear cells of diameter varying between 6-40 micrometer
- Leukocytes are in few forms:
- Agranulocytes – participate in specific defense
  - monocytes
  - limfocytes
- Granulocytes – responsible for non specific immunological answer
  - neutrophiles
  - basophiles
  - eosinophiles



Blood platellets:

- Their precursors are thrombocytes
- Participate in blood clothing
- Do not contain nucleus
- Express ability to adhere to each other and create clusters – clothing
- Secrete hormons - serotonin, histamin, derivatives of prostaglandins, catecholamins

Blood collection

Conditions of blood collection

- Blood – collected with sodium edetate ($EDTANa_2$, etylenodiaminotetraacetate)
- Plasma – collected with anticoagulants :

  - Ca^{2+}- binding - EDTA, citrate, oxalate – not useful for the determination of ions

  - heparin: plasma is not useful for the determination of lipids
- Serum – blood centrifuged after production of cloth – the content is changed – the lack of fibrinogen

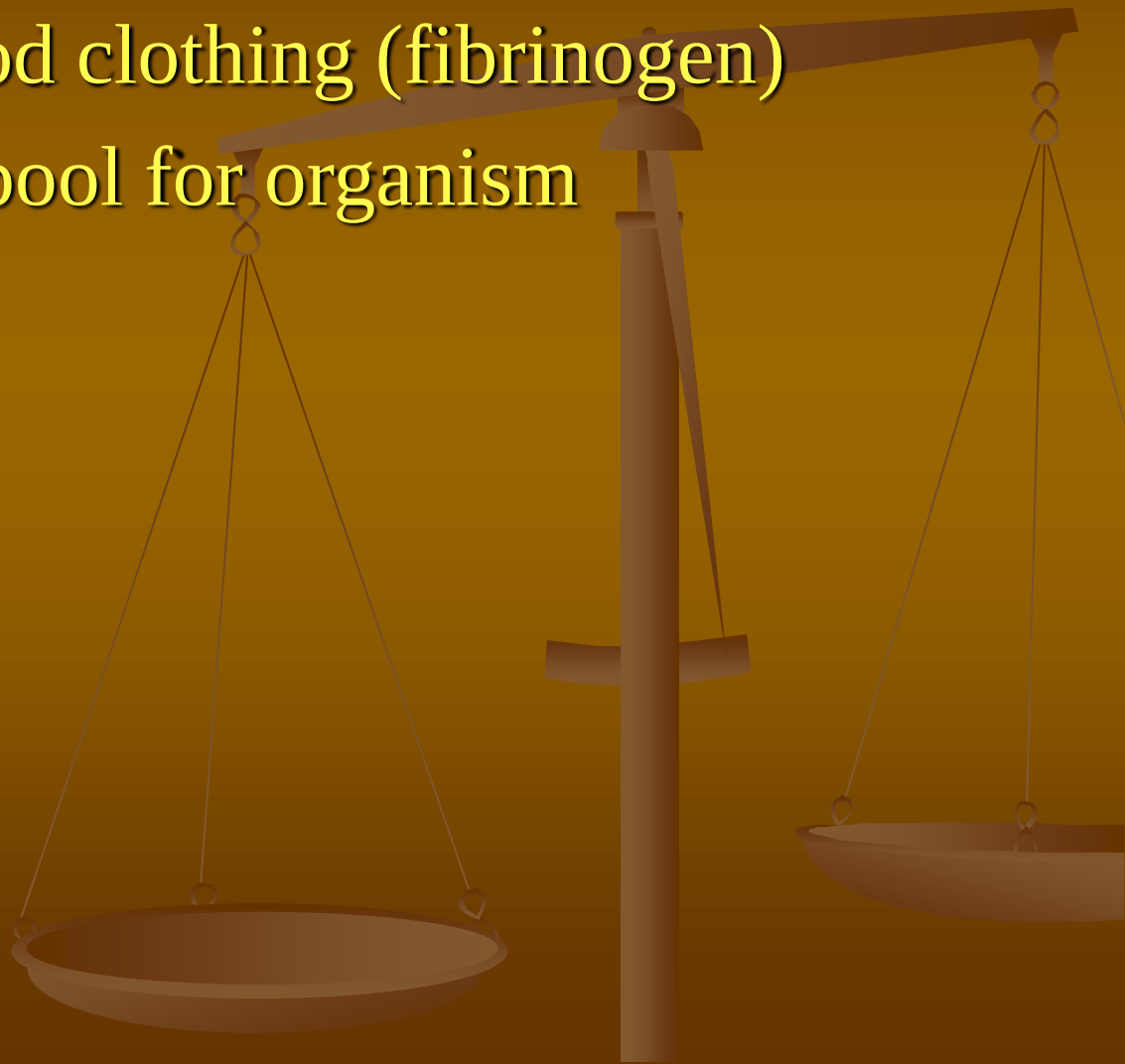
Contents:

- 90- 92% water
- 7% proteins (albumins, alfa1-globulins, alfa2-globulins, beta-globulins and gamma-globulins)
- 1-2% minerals
- Remaining: lipids, cholesterol, glucose and its metabolites, organic acids, non-protein nitrogen components

Functions of plasma proteins:

- Catalytic (enzymes)
- Immunological resistance (gamma-globulins)
- Buffers
- Osmotic pressure (albumins and prealbumins)
- Transport of hormones and compounds which are poorly soluble (cations of selected metals and other compounds)

- They are the source of kinins (alfa2-globulins)
- Participate in blood clothing (fibrinogen)
- They are protein pool for organism

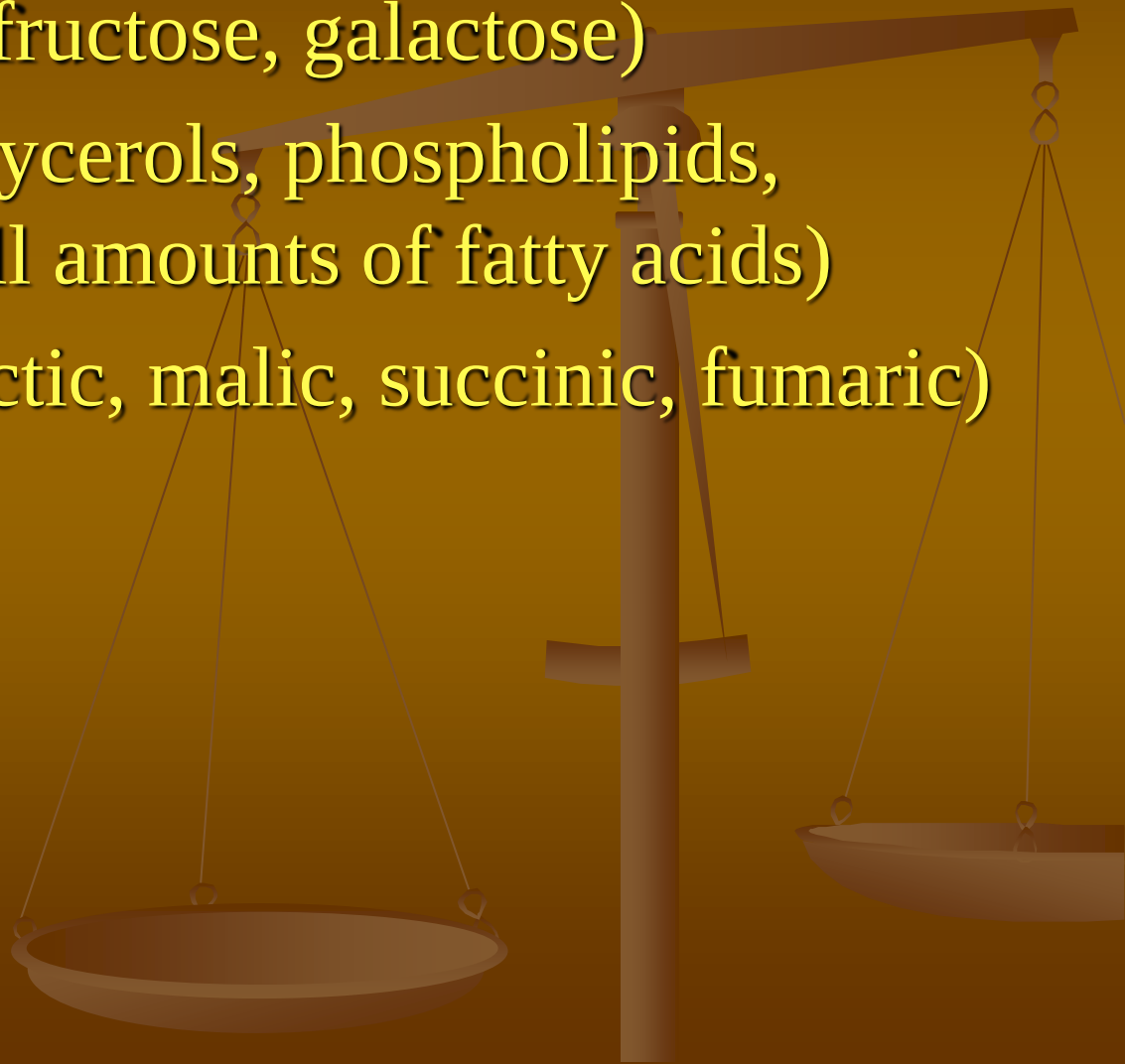


Non-protein nitrogen compounds:

- Urea
- Aminoacids
- Uric acid
- Alantoin
- Creatinin
- Bilirubin

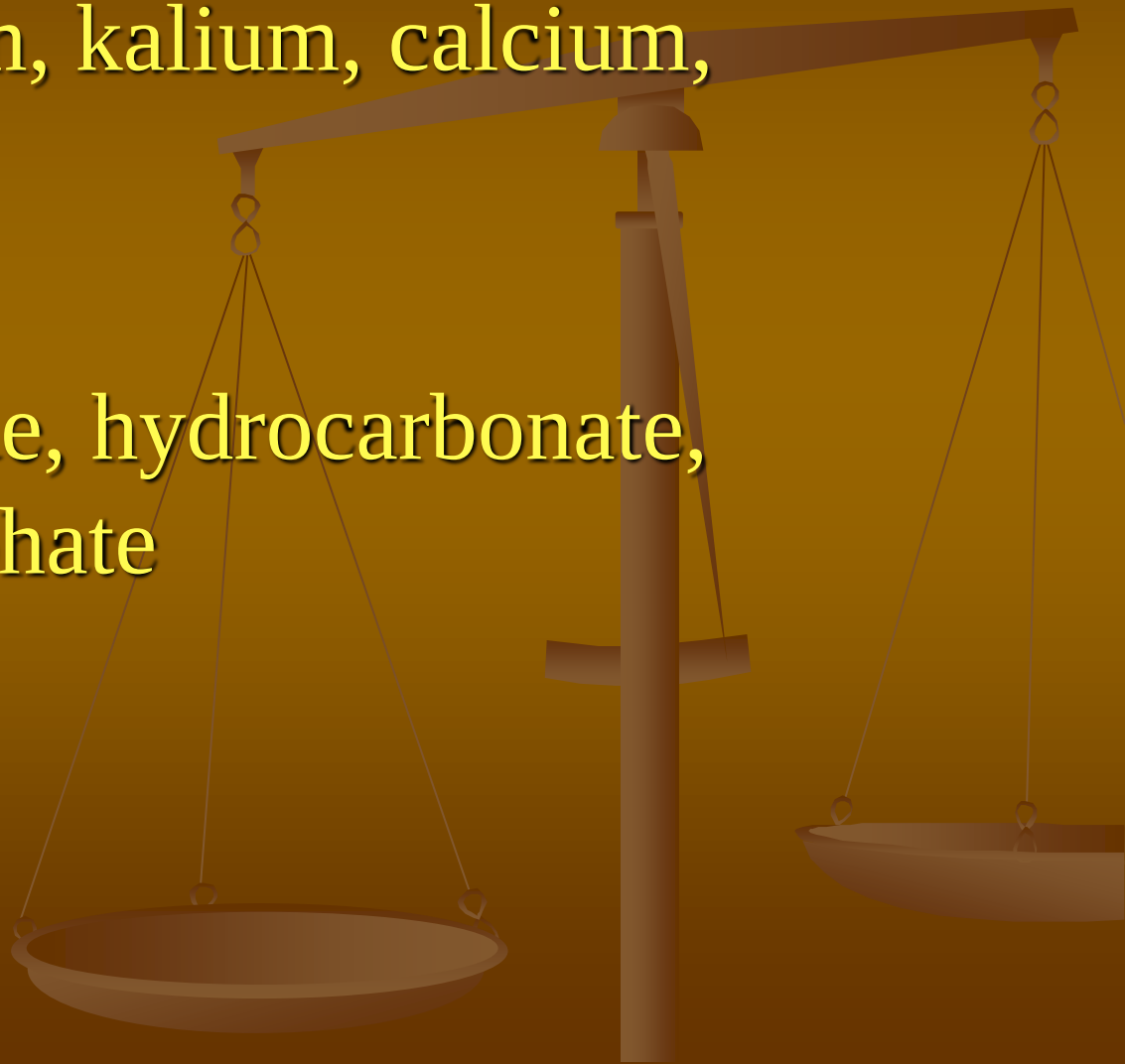
Non-nitrogen organic compounds:

- Sugars (glucose, fructose, galactose)
- Lipids (triacyloglycerols, phospholipids, sfingolipids, small amounts of fatty acids)
- Organic acids (lactic, malic, succinic, fumaric)



Mineral compounds:

- Cations: sodium, kalium, calcium, magnesium
- Anions: chloride, hydrocarbonate, phosphate, sulphate



Cholesterol

Cholesterol – organic compound which name originates from greek word „chole” - bile.

Tissues rich in cholesterol: brain tissue, adrenal glands, cell membranes. Free forms of cholesterol and esterified with lipids and lipoproteins are in plasma.

Acetyl-SCoA is the substrate for endogenous synthesis of cholesterol.

Cholesterol is the substrate for the synthesis of:

- Steroid hormons
- Bile acids
- 7-dehydrocholesterol (is converted into cholecalciferol – wit.D_3 in ultraviolet light)

Cholesterol in plasma is transported by lipoproteins. Core of lipoproteins are triacylglycerols and cholesterol esters surrounded by hydrophile layer of lipids with polar groups (phospholipids, free cholesterol) and proteins called apoproteins.

Lipoproteins are divided into:

- Chylomicrons
- VLDL
- HDL
- LDL

Chylomicrons are lipoproteins with the smallest density and the biggest diameter.

VLDL – are produced by liver. Their function is related to the transport of lipids from liver to peripheral tissues.

LDL – lipoprotein of low density named also as „bad cholesterol”. It is the main transporter of cholesterol from liver to other tissues (kidneys, muscles, adrenal cortex). This function is filled by depositing of free cholesterol on the surface of cell membranes or binding with membrane receptors. LDL in plasma is produced as the result of pathways: VLDL→IDL→LDL.

HDL – lipoprotein of high density named also as „good cholesterol”.

It is the main lipoprotein which transports cholesterol from blood to liver where liver cells absorb HDL via endocytosis.

This fraction purifies plasma from cholesterol which is immediately esterified via enzyme acyltransferase lecithin:cholesterol. This complex is strongly hydrophobic and does not participate in the structure of biological membranes.

Experiment 1. Detection of inorganic compounds in blood plasma

Protocol

Firstly, the plasma should be deproteinated. Take 2.5 cm^3 of plasma to centrifuge tube, add 2.5 cm^3 distilled water and 2.5 cm^3 5% TCA (trichloroacetic acid). Wait 15 min and then centrifuge the tube at 3 000 rpm for 10 min. Obtained deproteinated plasma filtrate can be used for the following tests:

Detection of Cl^- ions

Take 0.5 cm^3 of plasma filtrate to glass tube and add 2 drops of 0.2% $AgNO_3$ – as positive result white, caseous precipitate of AgCl will be formed.

Detection of SO_4^{-2} ions

Take 0.5 cm^3 of plasma filtrate to glass tube and add 2 drops of 5% $BaCl_2$ – as positive result white cloudiness of $BaSO_4$ will be formed.

Detection of HPO_4^{-2} ions

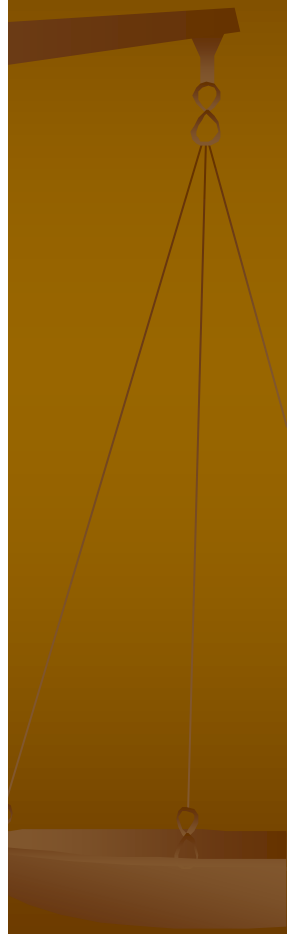
Take 0.5 cm^3 of plasma filtrate to glass tube, add 0.5 cm^3 ammonium molybdate solution and 0.5 cm^3 concentrated HNO_3. Heat it carefully over the burner to boil. As positive result yellow precipitate of ammonium phosphoro-molybdate will be formed.

Detection of Ca^{+2} ions

Take 0.5 cm^3 of plasma filtrate to glass tube, add 0.5 cm^3 ammonium oxalate $(NH_4)_2C_2O_4$ and add few drops of concentrated NH_4OH. As positive result white cloudiness of calcium oxalate (CaC_2O_4) will be formed.

Detection of iron ions

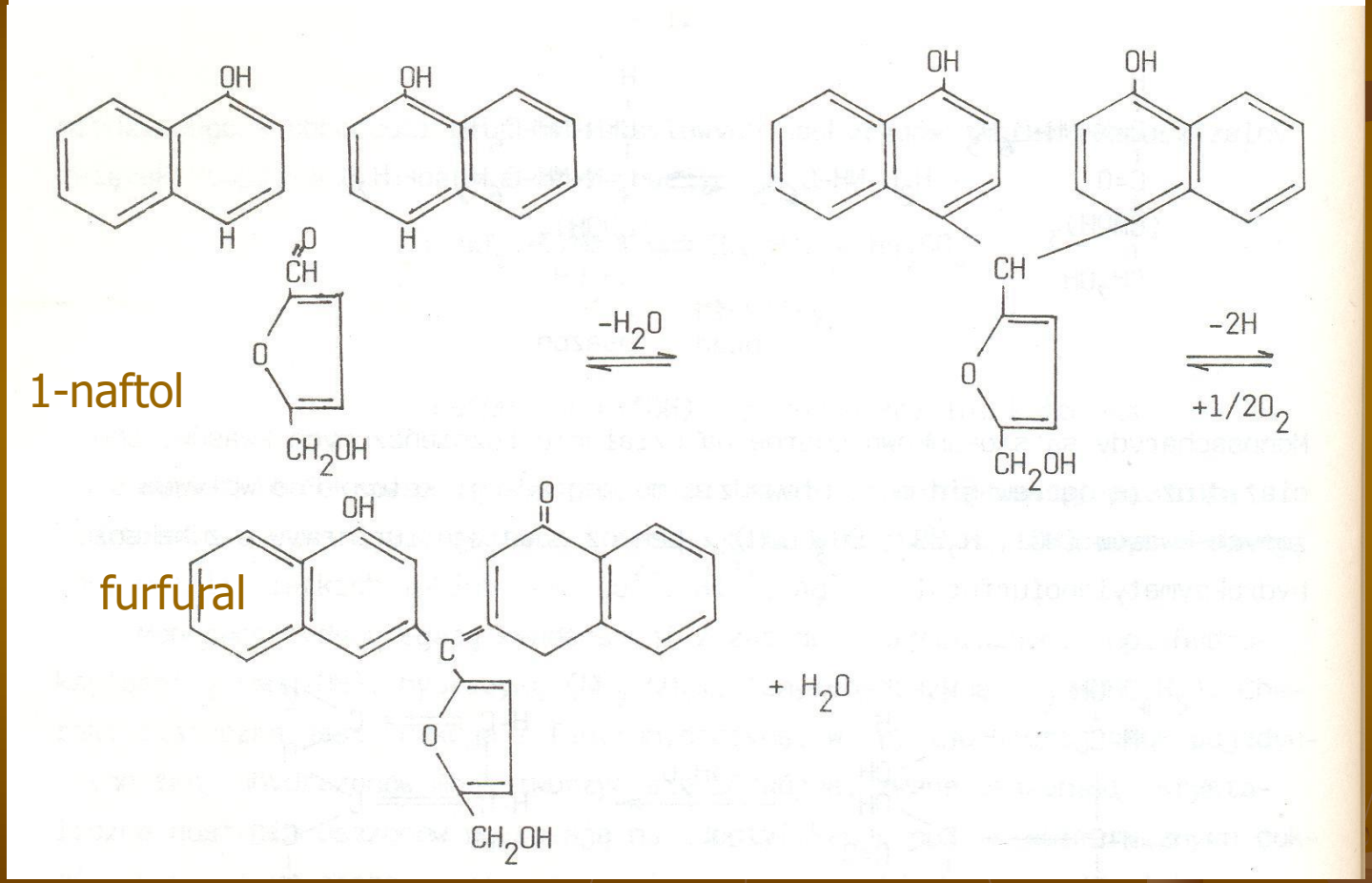
Take 0.5 cm^3 of plasma filtrate, add few drops of 20% sulpho-salicylic acid solution and add 0.5 cm^3 of concentrated NH_4OH. In alkaline environment, iron forms yellow complex with sulpho-salicylic acid.



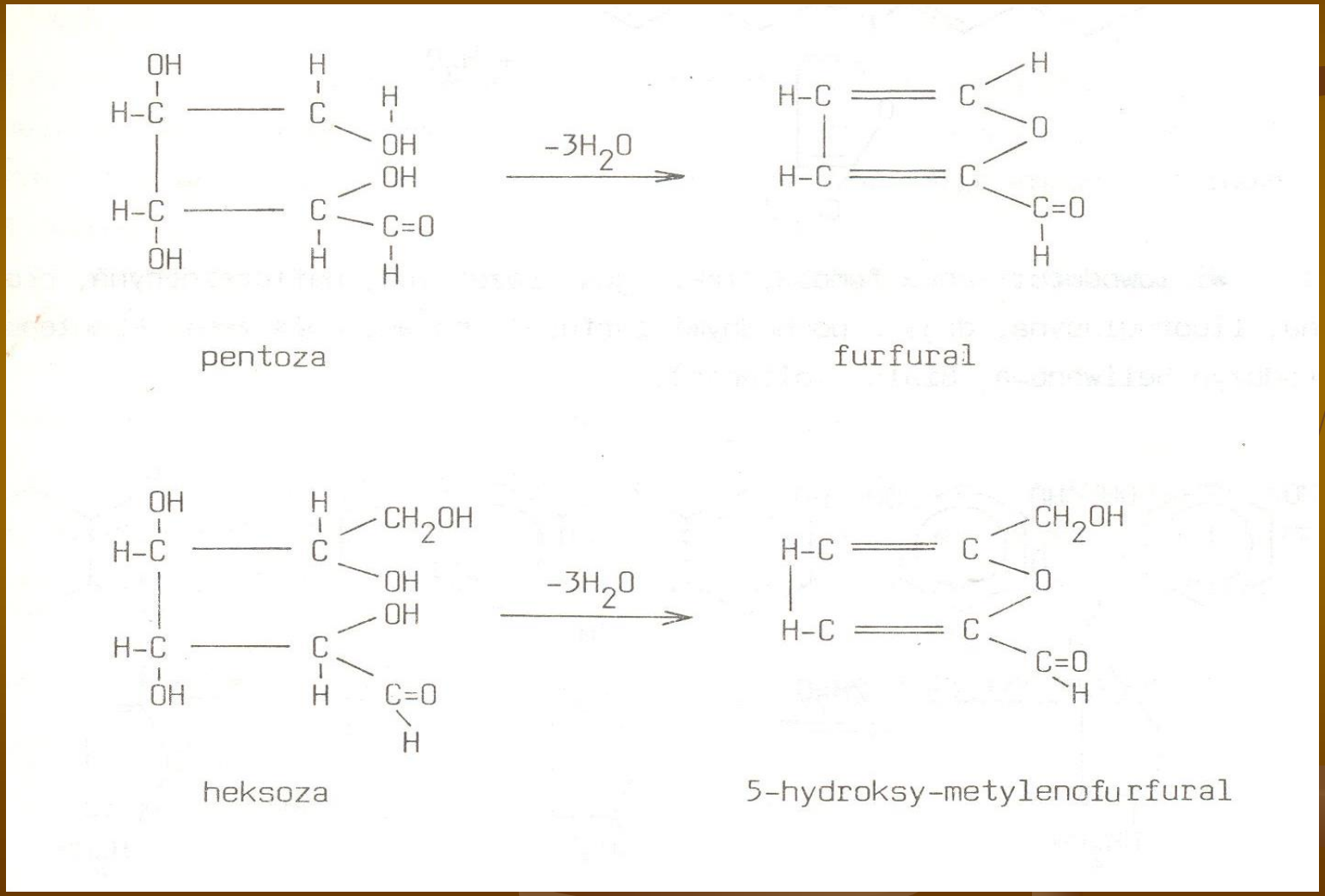
Experiment 2. Detection of organic compounds in blood plasma

Protocol

Take 0.5 cm^3 plasma and add 2 drops of Molisch reagent. Mix and add carefully 1 cm^3 of concentrated H_2SO_4. On the border of the two given reagents, red-violet roundel will be formed.



Production of furfural



Buffer-like properties of plasma are shown by the comparison of water solutions with and without addition of plasma to counteract the changes in pH after adding acid or base

Experiment 3. Demonstration of buffering properties of the blood

Protocol

Use 4 conical flask(Erlenmeyer flasks) marked as 1, 2, 3, and 4:

To flask 1 - add 10 cm^3 distilled H_2O and 3 drops of bromophenol blue

To flask 2 - add 9 cm^3 distilled H_2O, 1 cm^3 plasma, and 3 drops of bromophenol blue

To flask 3 - add 10 cm^3 distilled H_2O and 3 drops of phenolphthalein

To flask 4 - add 9 cm^3 distilled H_2O, 1 cm^3 plasma and 3 drops of phenolphthalein

Next, to flask 1 add a few drops of 0.1 mol/dm^3 HCl to obtain the change of tint (colour). Then, to flask 2, add as many drops of 0.1 mol/dm^3 HCl to obtain the same colour as in flask 1. Similarly, to flask 3 add a few drops of 0.1 $mol.dm^3$NaOH to have pink colour and as many NaOH solution to flask 4 to achieve the same, pink colour. Explain, why in flask 2 and 4 you can add so many acid or basic solution.

The influence of physical and chemical factors on stability of erythrocytes is estimated by the observation of erythrocytes in iso- hypo- and hypertonic solutions as well as in the presence of organic solvents.

Experiment 4. The influence of chemical and physical factors on the durability of erythrocytes

Protocol

Take 4 glass tubs and add to each 2 drops of whole blood. Then add:

to 1st – 5 cm^3 0.9% NaCl

to 2nd – 5 cm^3 distilled water

to 3rd – 5 cm^3 0.9% NaOH and a few drops of chloroform or ether

to 4th – 5 cm^3 2% NaCl

Mix vigorously each tube and observe what will happen to the erythrocytes. Explain the results achieved in each sample.


Now, lets start the determinations ...
BLOOD