May 11th -15th , 2020
Class 32 "BIOCHEMISTRY OF THE LIVER"

Tasks:

I. **Prepare at least 6-pages outline of the class, containing answers to the questions listed in the training guidelines.**

THEORETICAL PART

1. Role of the liver in carbohydrate, lipid, amino acid and protein metabolism.
2. Detoxification functions of the liver.
3. Heme synthesis, reactions.
4. Degradation of heme. Bilirubin metabolism, scheme.
5. Disorders in bilirubin metabolism: jaundice, its types.
6. Biochemical mechanisms of hepatic failure and hepatic coma.
7. Biochemical tests for diagnosis of liver disorders.

LITERATURE FOR TRAINING:

1. Harper's Illustrated Biochemistry / Robert K. Murray [et. al.]. – 28th ed. – New York [etc]: McGraw-Hill, Medical, 2009. – P. 141, 272-283, 609-615.
2. Harper's Illustrated Biochemistry / Robert K. Murray [et. al.]. – 29th ed. – New York [etc]: McGraw-Hill, Medical, 2012. – P. 161, 308-320, 676-682.
3. Biochemistry: manual for the medical faculty for international students (in English) / Н.Э. Петушок, А.А. Масловская, М.Н. Курбат. – Гродно: ГрГМУ, 2014. – P. 236-243.
4. Harper's Illustrated Biochemistry / Robert K. Murray [et. al.]. – 31st ed. – New York [etc]: McGraw-Hill, Medical, 2018. – P. 556-559, 305-315.
5. Harper's Illustrated Biochemistry / Robert K. Murray [et. al.]. – 30th ed. – New York [etc]: McGraw-Hill, Medical, 2015. – P. 583-588, 323-334.
6. Lecture "Biochemistry of the liver".

II. In the laboratory work **"DETERMINATION OF THE BILIRUBIN IN THE BLOOD SERUM"** you should write down values of extinctions, perform calculation, and make conclusion:

CLASS № 32
QUANTITATIVE DETERMINATION OF THE BILIRUBIN IN THE BLOOD SERUM

Bilirubin (formerly referred to as **haematoidin**) is the yellow breakdown product of normal haeme catabolism. Haeme is found in haemoglobin, a principal component of red blood cells. Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases. It is responsible for the yellow color of

bruises, the background straw-yellow color of urine (via its reduced breakdown product, urobilin – the more obvious but variable bright yellow color of urine is due to thiochrome, a breakdown product of thiamine), the brown color of feces (via its conversion to stercobilin), and the yellow discoloration in jaundice.

Metabolism

Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism. Bilirubin, when oxidized, reverts to become biliverdin once again. This cycle, in addition to the demonstration of the potent antioxidant activity of bilirubin, has led to the hypothesis that bilirubin's main physiologic role is as a cellular antioxidant.

HEME METABOLISM:

Unconjugated ("Indirect")

Erythrocytes (red blood cells) generated in the bone marrow are disposed of in the spleen when they get old or damaged. This releases hemoglobin, which is broken down to heme as the globin parts are turned into amino acids. The heme is then turned into unconjugated bilirubin in the reticuloendothelial cells of the spleen. This unconjugated bilirubin is not soluble in water, due to intramolecular hydrogen bonding. It is then bound to albumin and sent to the liver.

The measurement of direct bilirubin depends on its reaction with diazosulfanilic acid to create azobilirubin. However, unconjugated bilirubin also reacts slowly with diazosulfanilic acid, so that the measured indirect bilirubin is an underestimate of the true unconjugated concentration.

Conjugated ("Direct")

In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase, making it soluble in water: the conjugated version is also often called "direct" bilirubin. Much of it goes into the bile and thus out into the small intestine. Though most bile acid is resorbed in the terminal ileum to participate in enterohepatic circulation, conjugated bilirubin is not absorbed and instead passes into the colon.

There, colonic bacteria deconjugate and metabolize the bilirubin into colorless urobilinogen, which can be oxidized to form urobilin and stercobilin: these give stool its characteristic brown color. A trace (~1%) of the urobilinogen is resorbed into the enterohepatic circulation to be re-excreted in the bile: some of this is instead processed by the kidneys, coloring the urine yellow.

Although the terms direct and indirect bilirubin are used equivalently with conjugated and unconjugated bilirubin, this is not quantitatively correct, because the direct fraction includes both conjugated bilirubin and δ bilirubin (bilirubin covalently bound to albumin, which appears in serum when hepatic excretion of conjugated bilirubin is impaired in patients with hepatobiliary disease).[1]

Furthermore, direct bilirubin tends to overestimate conjugated bilirubin levels due to unconjugated bilirubin that has reacted with diazosulfanilic acid, leading to increased azobilirubin levels (and increased direct bilirubin).

PRINCIPLE OF THE METHOD:

Total bilirubin is now often measured by the 2,5-dichlorophenyldiazonium (DPD) method, and **direct bilirubin** is often measured by the method of Jendrassik and Grof. Diazo reagent interacts with direct bilirubin with formation of pink dye. **Indirect bilurubin** is insoluble in water and gives indirect reaction with diazo reagent, that is, the reaction produces a specoific colour after preliminary treatment.

RESULTS:

Extinction for total bilirubin E_1= 0.15

Extinction for direct bilirubin E_2= 0.07

Calculate concentrations of total, direct and indirect bilirubin using equations from laboratory work

CONCLUSION: **(your conclusion according to the results)**

DIAGNOSTIC IMPORTANCE.

Normal level of total bilirubin in blood serum 8,25 - 20,5 µl/l; conjugated bilirubin (direct)- 1,0 - 7,55 µl/l; unconjugated bilirubin (undirect) – 1,7 – 17,1 µl/l.

Increased level of total bilirubin in blood serum observed in different types of jaundices.

Hyperbilirubinemia results from a higher-than-normal level of bilirubin in the blood for adult this is any level above 17µmol/L and for newborns 340 µmol/L and critical hyperbilirubinemia 425 µmol/L.

Mild rises in bilirubin may be caused by the following:

- Hemolysis or increased breakdown of red blood cells
- Gilbert's syndrome – a genetic disorder of bilirubin metabolism that can result in mild jaundice, found in about 5% of the population
- Rotor syndrome - non-itching jaundice, with rise of bilirubin in the patient's serum, mainly of the conjugated type.

Moderate rise in bilirubin may be caused by:

- Pharmaceutical drugs (especially antipsychotic, some sex hormones, and a wide range of other drugs)
  Sulfonamides are contraindicated in infants less than 2 months old (exception when used with pyrimethamine in treating toxoplasmosis) as they increase unconjugated bilirubin leading to kernicterus.
- Hepatitis (levels may be moderate or high)
- Chemotherapy
- Biliary stricture (benign or malignant)

Very high] levels of bilirubin may be caused by:

- Neonatal hyperbilirubinaemia, where the newborn's liver is not able to properly process the bilirubin causing jaundice
- Unusually large bile duct obstruction, e.g. stone in common bile duct,

tumour obstructing common bile duct etc.

- Severe liver failure with cirrhosis (e.g. primary biliary cirrhosis)

Jaundice

Jaundice may be noticeable in the sclera (white) of the eyes at levels of about 2 to 3 mg/dL (34 to 51 μmol/L), and in the skin at higher levels. For conversion, 1 mg/dL = 17.1 μmol/L.

Jaundice is classified depending upon whether the bilirubin is free or conjugated to glucuronic acid into conjugated jaundice or unconjugated jaundice.

The notes and laboratory protocols will be revised by your teacher.

Please, report whether you have received this letter.