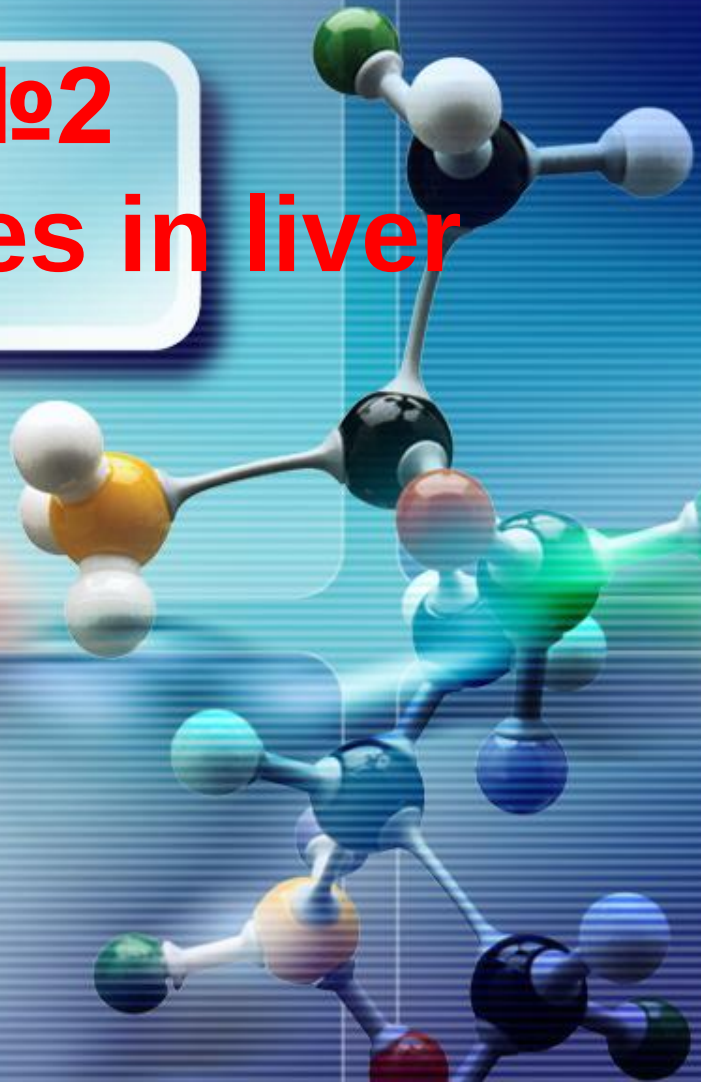
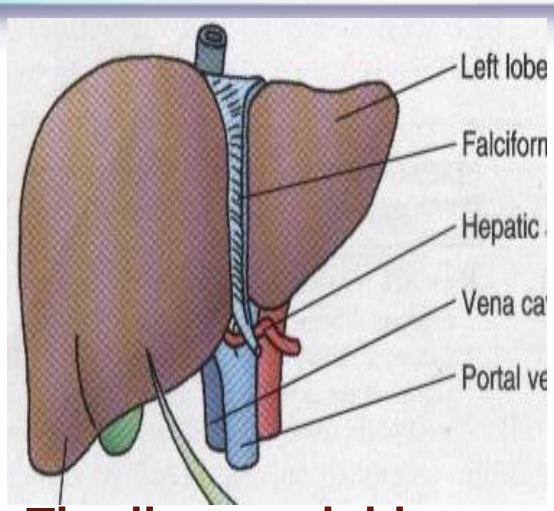
Federal state budgetary educational institution of higher education "Volgograd state
ical University" of the Ministry of health of the Russian Federation

Department of clinical laboratory diagnostics

LECTURE №2
Biochemical studies in liver diseases



The Role Of The Liver In Organism



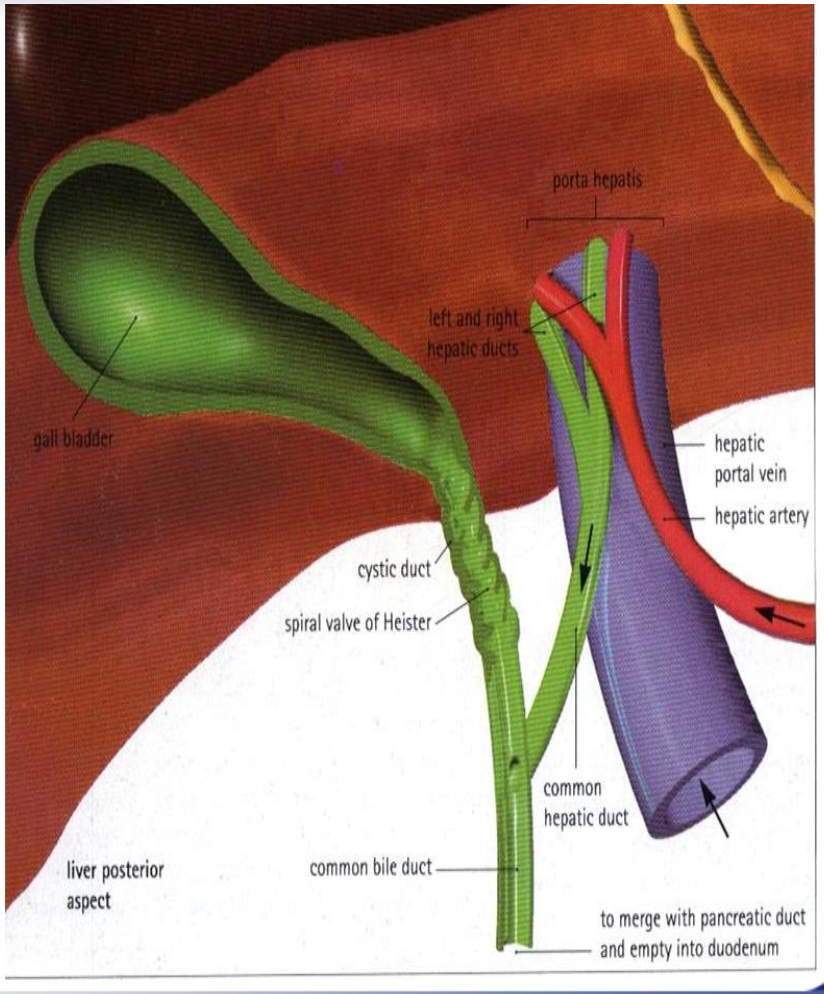
The liver is enveloped by peritoneum, which forms a simple squamous epithelium covering over the dense irregular connective tissue capsule (Glisson's capsule) of the gland. Glisson's capsule is attached to the liver except at the porta hepatis, where it enters the liver forming a conduit for the blood and lymph vessels and bile ducts.

The liver, weighing approximately 1500 g, is the largest gland in the body. It is located in the upper right-hand quadrant of the abdominal cavity, just inferior to the diaphragm. The liver is subdivided into four lobes – right, left, quadrate and caudate. the liver has both endocrine and exocrine functions; cells (hepatocytes) in the liver are responsible for the formation of the liver's exocrine secretion, bile, and its numerous endocrine products. In addition hepatocytes convert noxious substances into nontoxic material that are excreted in bile.

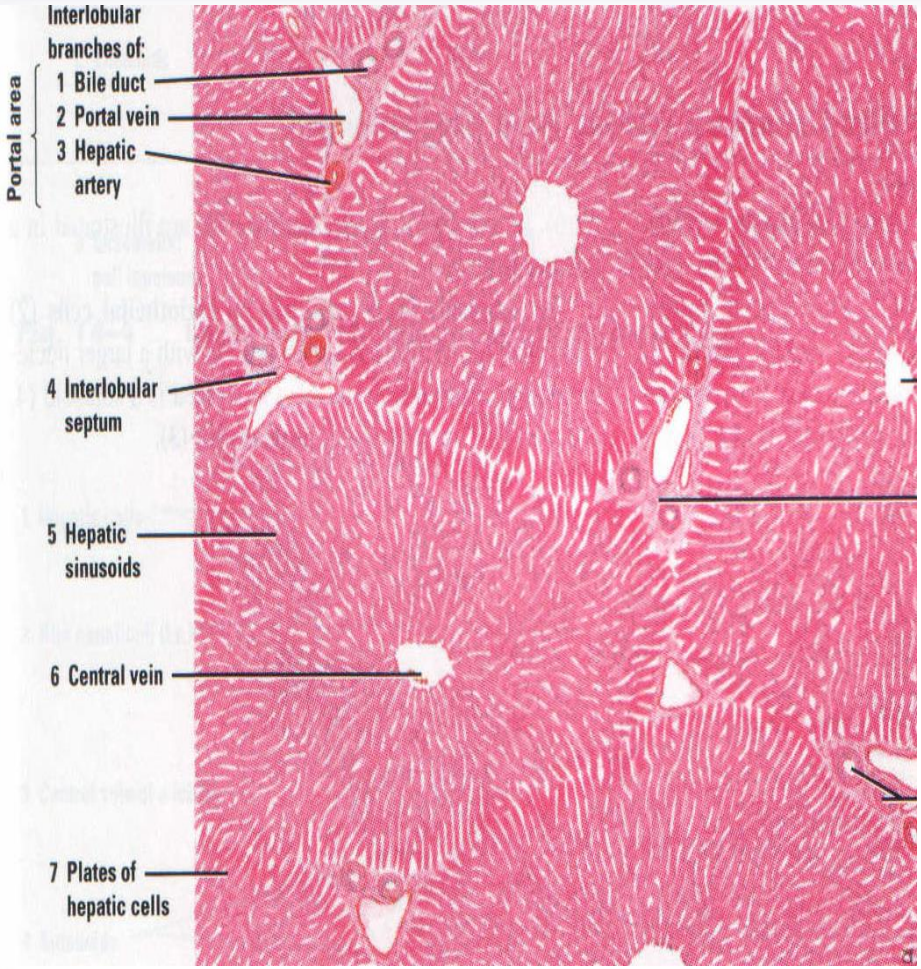
The inferior, concave aspect of the liver houses the porta hepatis, through which the portal vein and hepatic artery bring blood into the liver and through which the bile ducts drain bile from the liver.

The Role Of The Liver In Organism

The liver has a dual blood supply, receiving oxygenated blood from the left hepatic artery and the right hepatic artery (25%) and nutrient-rich blood via the portal vein (75%). Blood leaves the liver at the inferior aspect of the organ through the hepatic vein, which deliver their contents into the inferior vena cava. Bile also leaves liver at the porta hepatis, by way of the right and left hepatic ducts, to be delivered to the gallbladder for concentration and storage.



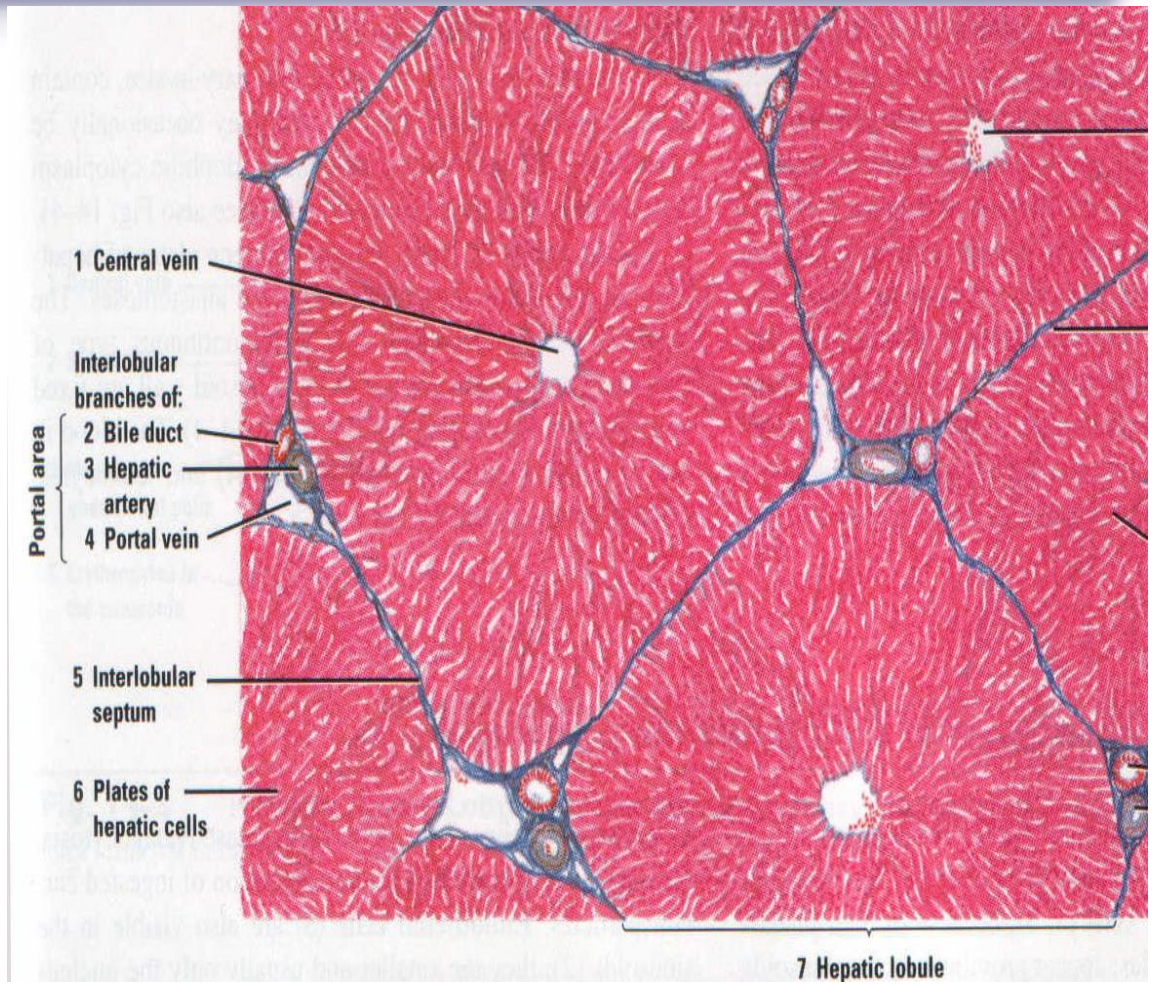
HUMAN LIVER, H & E



The liver is unusual, in that its connective tissue elements are sparse; thus, the bulk of the liver is composed of uniform parenchymal cells, the hepatocytes.

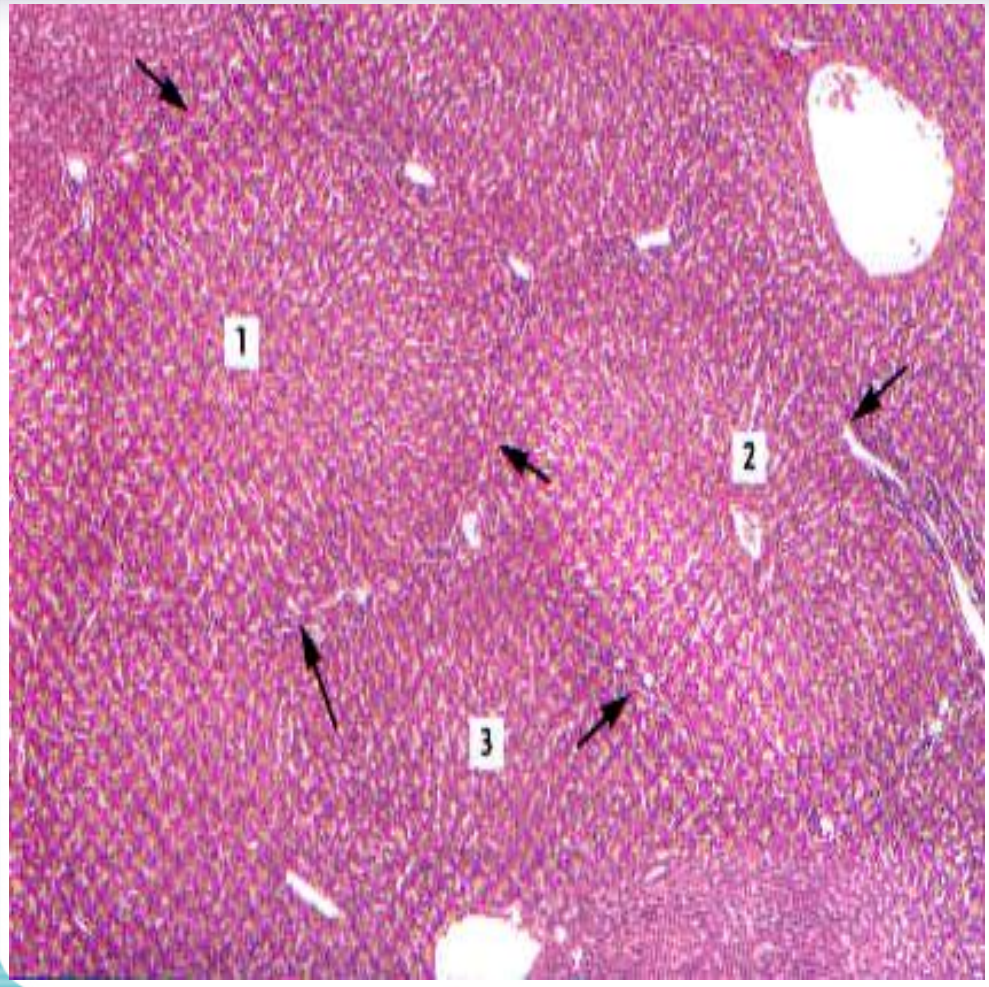
Hepatocytes are arranged in hexagon-shaped lobules (classical lobules) about 2 mm in length and 0.7 mm in diameter.

LIVER OF A PIG



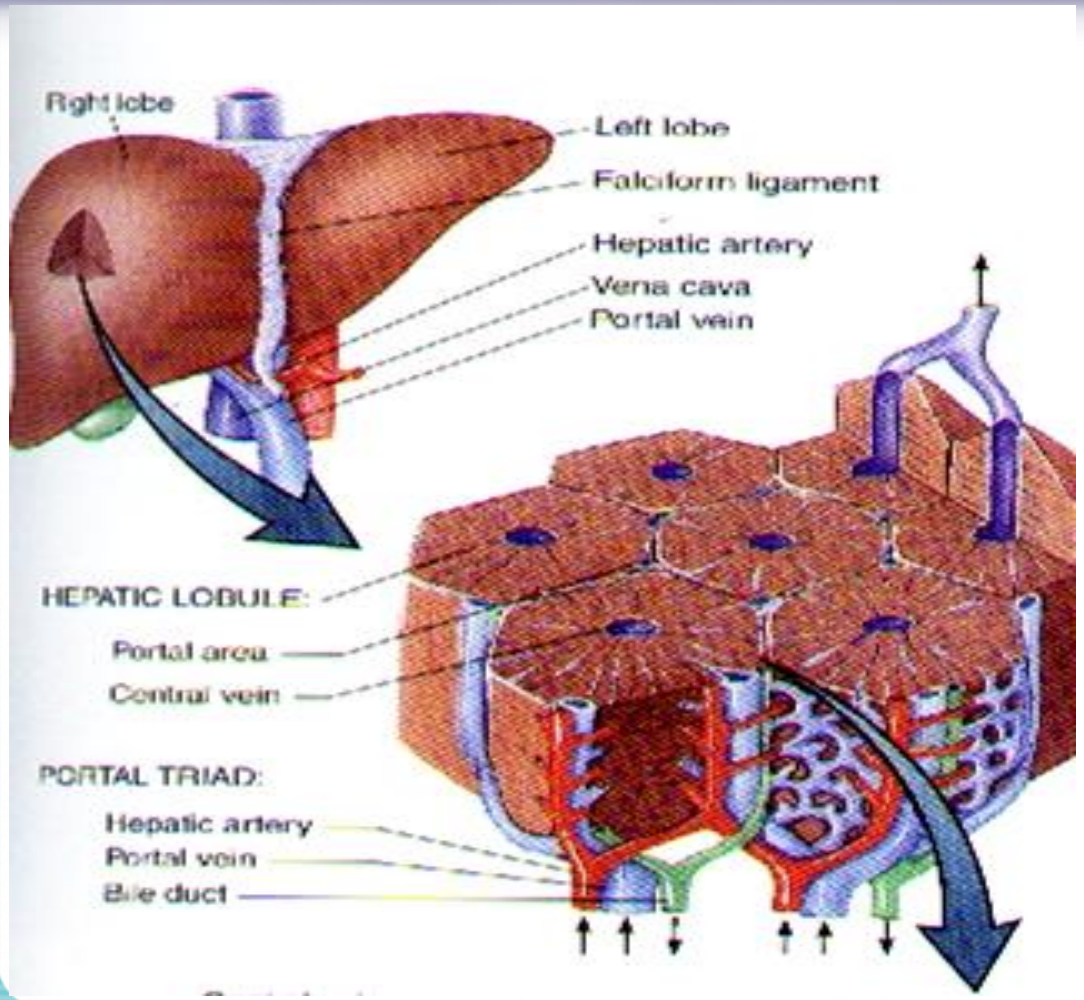
These lobules are clearly demarcated by slender connective tissue elements in animals, like pig and camel. The classical liver lobule was the first to be defined histologically because the connective tissue arrangement in the pig liver afforded an obvious rationale.

Liver, Human, H & E



However, because of the scarcity of connective tissue and the closely packed arrangements of the lobules in humans, the boundaries of the classical lobules can only be approximated. Where three classical lobules are in contact with each other, the connective tissue elements are increased, and these regions are known as portal areas housing triads.

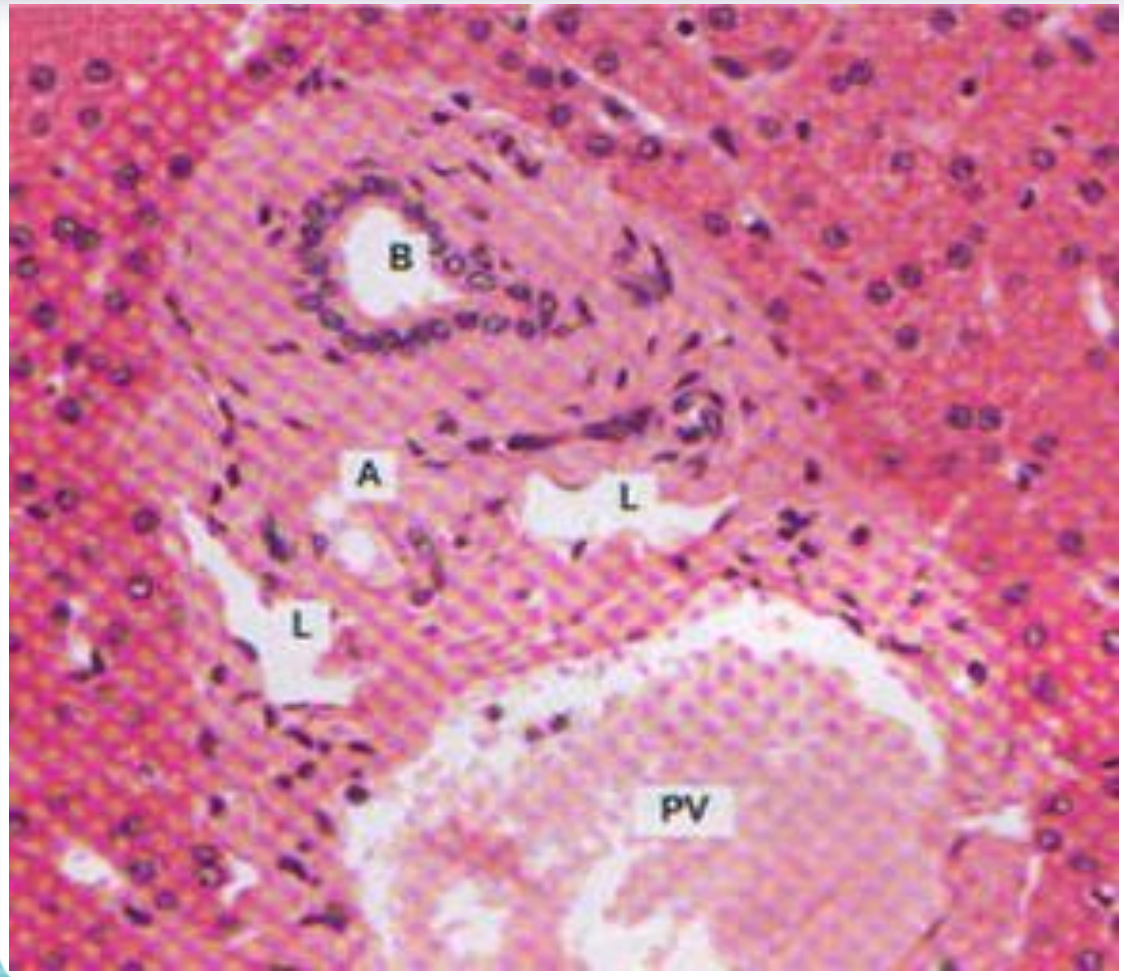
Portal triads



Portal triads include: interlobular artery (a branch of the hepatic artery) inter-lobular vein (a branch of the portal vein) and interlobular bile duct. These vessels and ducts follow the longitudinal axis of each lobule.

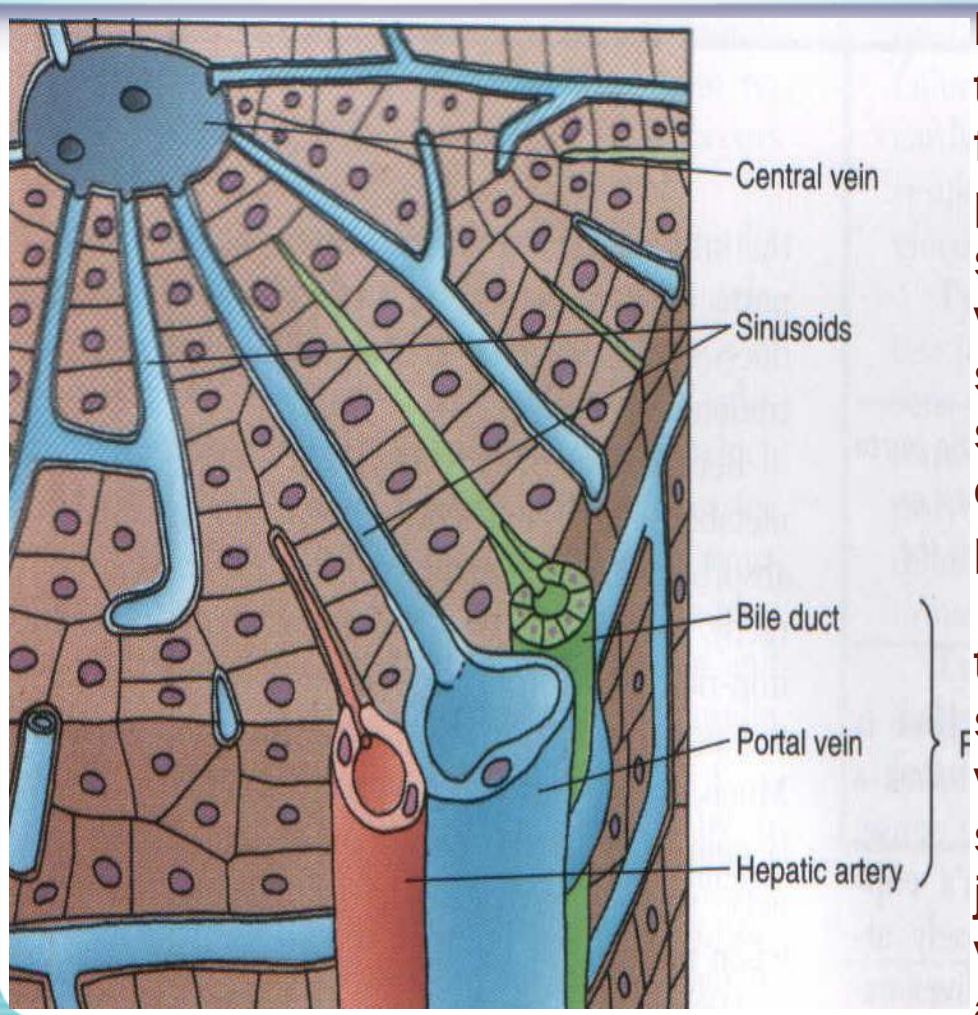
The central longitudinal axis of each classical lobule is occupied by the central vein, the initial branch of the hepatic vein.

PORTAL AREA OF THE LIVER



Portal areas house slender branches of the hepatic artery (A) with a regular lumen, tributaries of the relatively large portal vein (PV) with larger diameter and irregular lumen, interlobular bile ducts (B) recognized by their simple cuboidal epithelium), and thin-walled lymph vessels. All are contained within a fibrocollegenous supporting stroma.

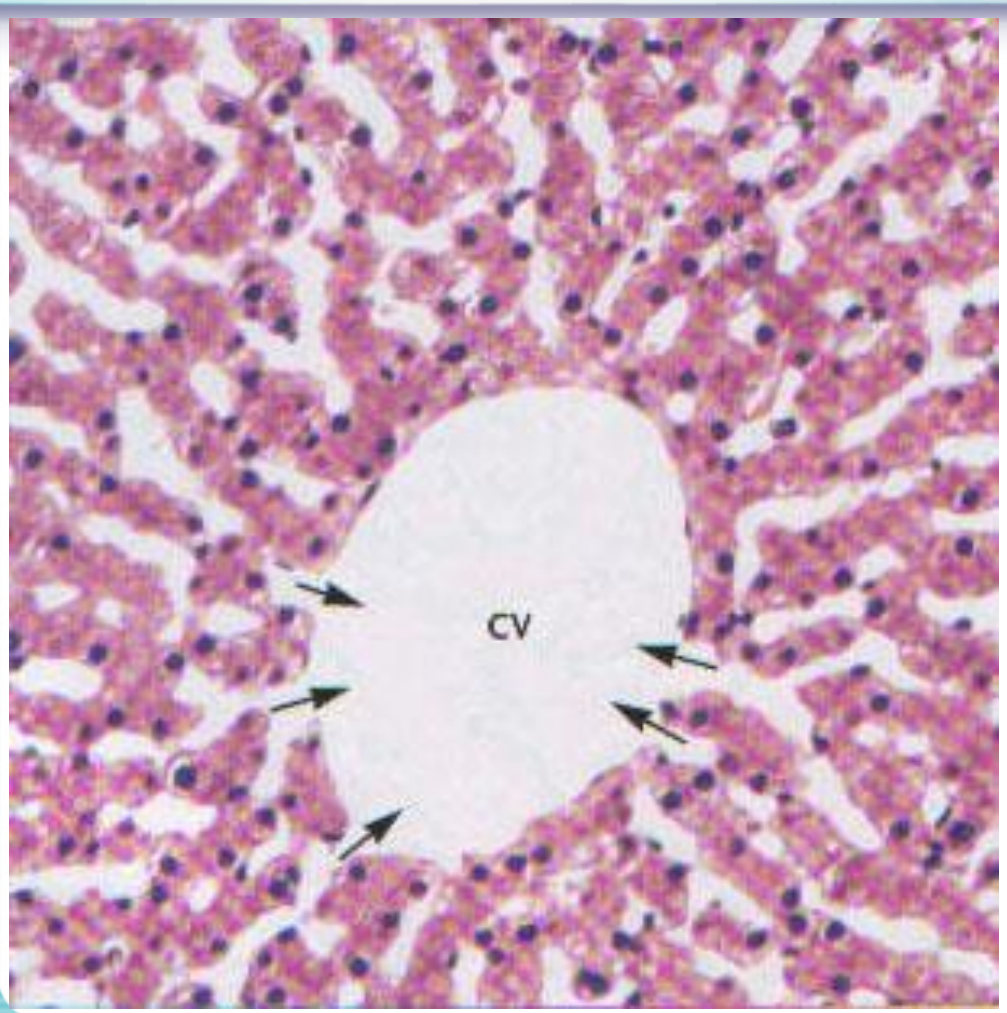
The Role Of The Liver In Protein Metabolism



Hepatocytes radiate, like spokes of the wheel, from the central vein, forming anastomosing fenestrated plates of liver cells, hepatocytes, separated from each other by large vascular spaces known as hepatic sinusoids. As blood enters the sinusoids, its flow slows considerably and it slowly percolates into the central vein.

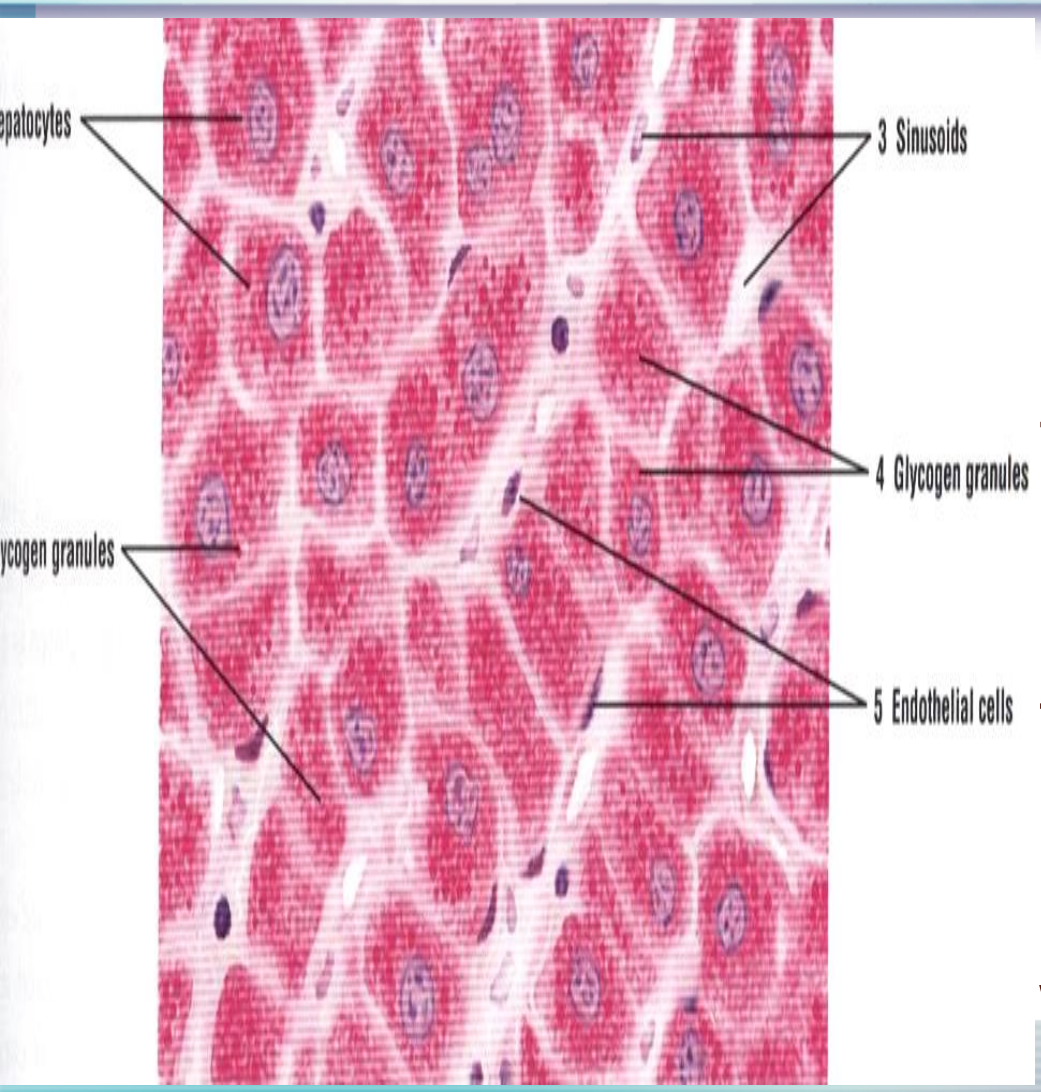
As the central vein leaves the lobule, it terminates in the sublobular vein. Numerous central veins deliver their blood into a single sublobar vein, the latter joining each other to form collecting veins, which in turn form the right and the left hepatic veins.

SINUSOIDS OF THE LIVER DRAINED BY THE CENTRAL VEIN



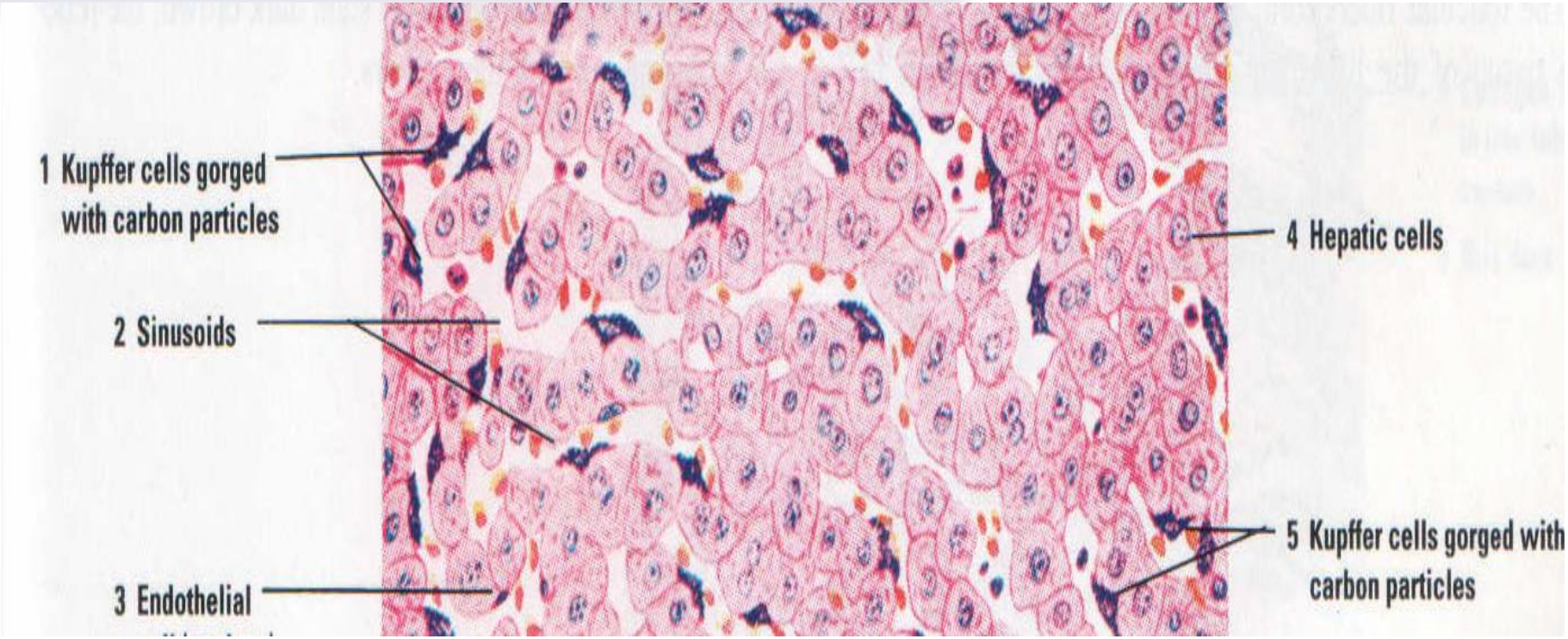
Hepatocytes (liver cells) are arranged as interconnecting flat plates between which are sinusoids containing blood supplied by the branches of the hepatic artery and portal vein. Anastomosing plates of hepatocytes, no more than two cells thick, radiate from the central vein (CV) towards the periphery of the classical lobule. See sinusoidal channels passing between interconnecting colums of hepatocytes on their way to the central terminal hepatic venule.

Glycogen particles in the liver cells. PAS & H



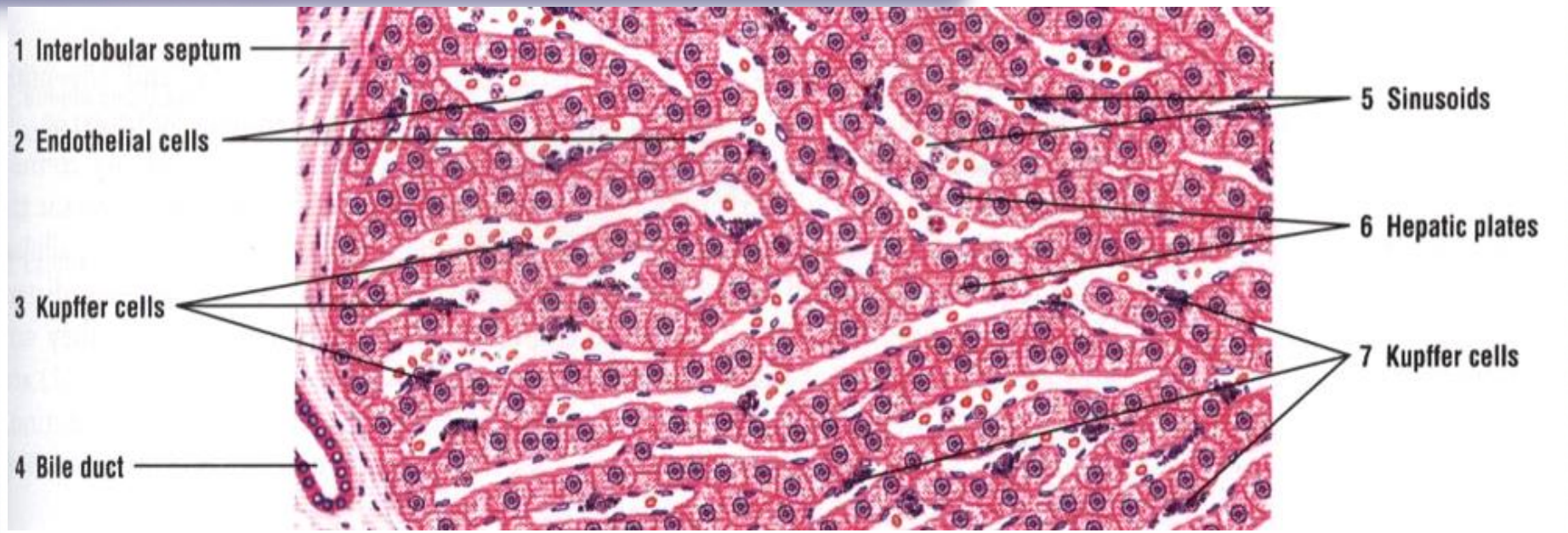
Sinusoids between the plates of hepatocytes are lined with sinusoidal lining cells. These endothelial cells contain large fenestrae. Often they do not make contacts with each other leaving gaps of up to 0.5 mcm between them. Thus particulate matter less than 0.5 mcm in diameter may leave the lumen of the sinusoids with relative ease.

Kupffer cells

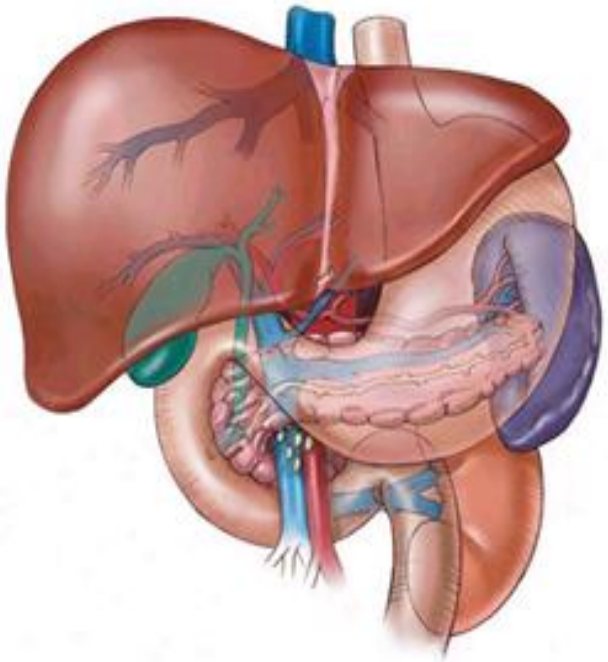


Resident macrophages, known as Kupffer cells, are associated with the sinusoidal lining cells in the sinusoids. Frequently phagosomes of Kupffer cells contain endocytosed particulate matter and cellular debris, especially defunct erythrocytes that are being destroyed by these cells.

Kupffer cells in the pig liver, (India ink preparation), H & E.



Kupffer cells belong to the body's system of mononuclear phagocytes. They derive from monocyte precursors, have Fc receptors as well as receptors for complement and thus can phagocytose foreign particulate matter. The significance of these cells is high as blood from the portal vein contains a number of microorganisms that enter the bloodstream from the lumen of the alimentary canal. These bacteria become opsonized in the lumen or mucosa of the gut or in the bloodstream. Kupffer cells recognize and endocytose at least 99% of these microorganisms. Kupffer cells also remove debris and defunct RBCs from the blood.



The liver plays an important role in the metabolism of proteins, carbohydrates and lipids.

Liver cells metabolize, excrete and detoxify exogenous and endogenous substances.

Liver functions

1. Meta-bolic
2. Depo-sited
3. Secre-ting
4. Detoxi-fying
5. Excre-ting
6. Homeo-static

Liver functions

1

Meta-bolic

Regulating the metabolism of proteins and amino acids, lipids, carbohydrates and biologically active substances (hormones, vitamins), minerals.

Liver functions

2

Depo-sited

Accumulation of carbohydrates, proteins, fats, hormones, vitamins and minerals

Liver functions

3

Secre-ting

Production of bile, which is an important way of removing a number of substances that are converted in the liver from the plasma; the liver is also involved in digestion by emulsifying fats

Liver functions

4

Detoxi-fying

Kupffer cells, hepatic macrophages, serve as a biological filter: they form slightly toxic ether sulfuric acids that later go to the intestine.

Liver functions

5

Excre-ting

Toxic compounds indole, skatole, tyramine bind to sulfuric and glucuronic acids in the liver

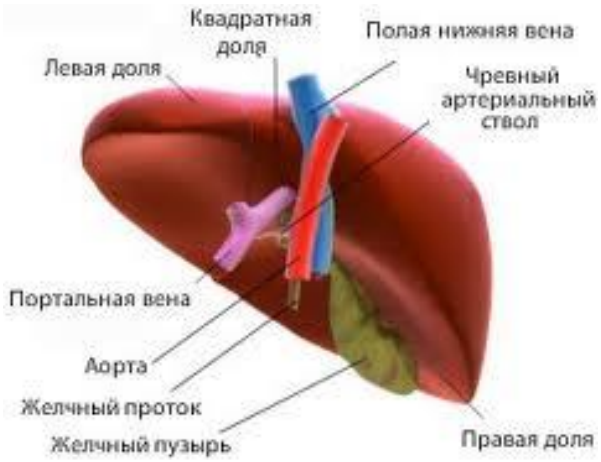
Liver functions

6

Homeo-static

The liver is involved in metabolic regulation, antigenic homeostasis.

Liver functions



The variety of hepatic functions is reflected in the abundance of laboratory test elaborated for assessment of the functional state of the liver. Various enzymatic proteins are found in the liver. Along with enzymes found in other organs, the liver contains specifically hepatic enzymes.

The Role Of The Liver In Protein Metabolism

❖- **Synthesis of specific plasma proteins: albumin, prothrombin, fibrinogen, kappa factor and proaccelerin;**

❖- **The formation of urea and uric acid;**

❖- **Synthesis of choline and creatine;**

❖- **Transamination and deamination of amino acids.**

The Role Of The Liver In Protein Metabolism

Pathological process in hepatocytes reduces their synthetic abilities dramatically thus causing the albumin content in the blood plasma to fall immediately, which could lead to a decrease in plasma oncotic pressure, the development of edema, and later, ascites

The Role Of The Liver In Protein Metabolism

Pathological process in hepatocytes

↓

Reduce their synthetic possibilities

↓

Reduction of albumin in the blood plasma

↓

Reduced oncotic pressure of blood plasma

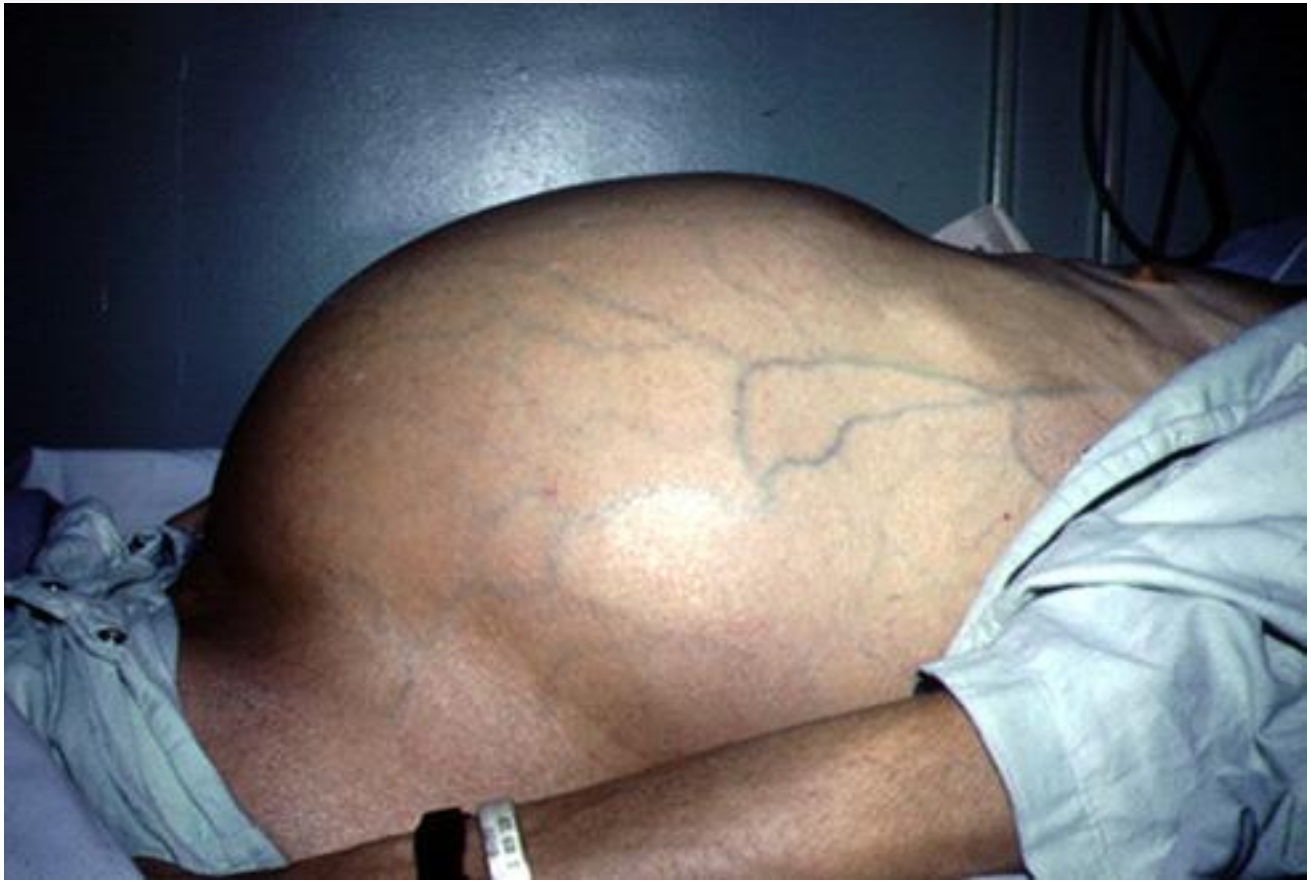
↓

Edema

↓

Ascites

The Role Of The Liver In Protein Metabolism



The Role Of The Liver In Protein Metabolism

The Role Of The Liver In Protein Metabolism

- In hepatic lesion the process of deamination of amino acids is disturbed, which leads to an increase in their concentration in the blood and urine.

- Deamination of amino acids is accompanied by formation of ammonia, which is poison for cells. Ammonia is neutralized through synthesis of urea. This process takes place almost exclusively in the liver, urea formation is one of the most important of its functions.

The Role Of The Liver In Protein Metabolism

✓Amino acids undergo transamination in the liver, it occurs in other organs, but the intensity of these enzymatic reactions in the liver is significant.

✓The activity of transaminases (ALT - alanine aminotransferase, AST - aspartate aminotransferase) is elevated in various destructive changes, such as myocardial infarction and hepatitis.

✓When there are necrotic changes in the cardiac muscle, the activity of AST increases in the blood dramatically, while in hepatitis an increase in ALT is noted.

The Role Of The Liver In Protein Metabolism

✓To diagnose liver diseases, studies of the enzyme spectrum of lactate dehydrogenase (LDH) are performed. Increased activity of the fifth fraction of lactate dehydrogenase (LDH-5) evidence of destructive processes in the liver tissue, and increased activity of LDH-1 - damage to the myocardium.

✓The mitochondries of hepatocytes is rich LDH-1 and LDH-2, whereas LDH-4 LDH-5 is mainly concentrated in the cytoplasmic fraction of hepatocytes.

The role of the liver in lipid metabolism

- *Synthesis of fatty acids*
- *Synthesis triglyceride*
- *Synthesis of plasma lipoproteins*
- *Synthesis of phospholipids, cholesterol and its esters*
- *Lipolysis of triglycerides*
- *Oxidation of fatty acids*
- *Formation of acetone (ketone) phone*

The role of the liver in lipid metabolism

- **Triglycerides synthesized in the liver either remain in the liver or are secreted into the blood as part of lipoproteins, mainly VLDL (VLDL) and are transported to the adipose tissue.**
- **Biosynthesis of cholesterol is governed by the negative feedback principle.**

Clinical and biochemical syndromes of liver damage:

1) Cytolytic syndrome

2) Mesenchymal-inflammatory syndrome

3) Cholestatic syndrome (cholestasis)

4) Syndrome of hepatocellular insufficiency

Clinical and biochemical syndromes of liver damage:

Syndrome of disrupted integrity of hepatocytes (or *cytolysis*, *cytolytic syndrome*).

AST, ALT, LDG4 and LDG3 *(up 5-20)*, (increasing)
Serum ferritin,
Serum iron, vitamin B12,
Bilirubin of the direct fraction;
All these indicators are increasing.

Morphological basis of this syndrome is acidophilic and hydropic degeneration and necrosis of hepatocytes with lesion and enhanced permeability of cell membranes.

Clinical and biochemical syndromes of liver damage:

Syndrome of disrupted integrity of hepatocytes (or *cytolysis*, *cytolytic syndrome*).

- 5% of healthy people may experience a slight elevation in liver enzymes without any signs of liver damage.
- ACT is located in cardiac and skeletal muscle, kidney, brain, pancreas and erythrocytes; ALT in skeletal and cardiac muscle (although in much smaller amounts ACT); LDH in virtually all cells and body fluids; isozymes LDG5 and LDG4 are more common to the liver.

Clinical and biochemical syndromes of liver damage:

Cholestasis syndrome
(excretory-biliary syndrome)

AF, gamma-GTF
Cholesterol,
Fraction of conjugated bilirubin,
Bile acids,
Phospholipids

<u>Morphological basis</u>: hyperplasia of smooth cytoplasmic network, accumulation of bile components in the hepatocyte, which are often accompanied by cytolysis of hepatocytes.

Clinical and biochemical syndromes of liver damage:

Syndrome of hepatocellular insufficiency
(synthetic failure)

Unconjugated fraction of Bilirubin

Serum total protein and especially albumin,
Transferrin, Cholesterol,
Clotting factors II, V, VII,
Cholinesterase,
Alpha-lipoproteins

<u>Morphological substrate</u> of the syndrome is pronounced dystrophic changes of hepatocytes and / or a significant decrease in the functioning of liver parenchyma as a result of necrotic changes.

Clinical and biochemical syndromes of liver damage:

Mesenchymal-inflammatory syndrome

Erythrocyte sedimentation rate
Immunoglobulin
C-reactive protein, seromucoid
Antibodies to subcellular fractions of hepatocytes,,

In morphological studies of the liver we see characteristic activation and proliferation of lymphoid cells and reticulohistiocytic cells, increased fibrogenesis, formation of active septa with hepatocytic necrosis, intrahepatic migration of white blood cells, and vasculitis.

JAUNDICE

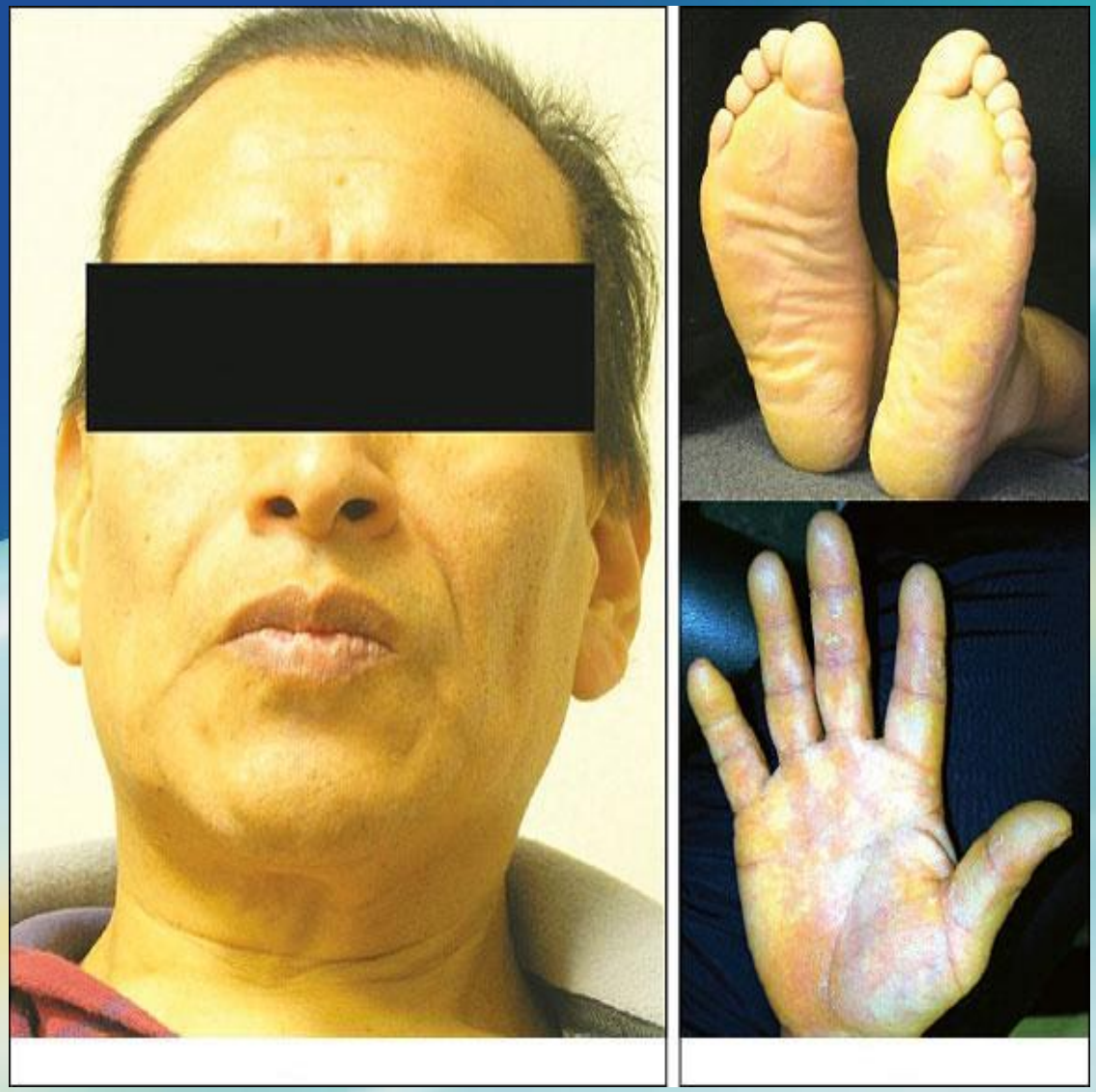
- **In any hepatic lesion jaundice may develop, which is often the first sign of liver disease.**
- **Jaundice is innatural yellow coloration of the skin or sclera. This is due to the presence of bilirubin in plasma in concentrations greater than 40 mmol / l. The normal concentration of bilirubin in the plasma is under 22 mmol / l.**

JAUNDICE

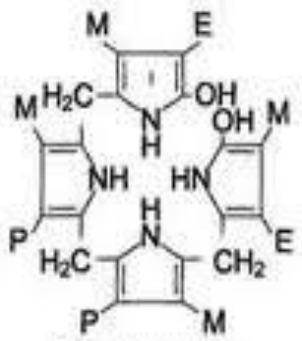
LOGO

http://medpynkt.ru/bolezni/jeltyxa.php&usg

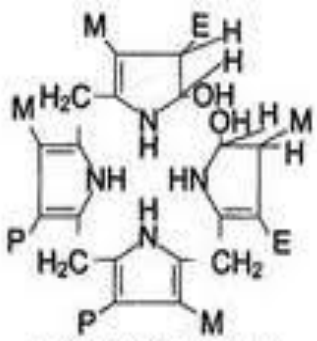
JAUNDICE



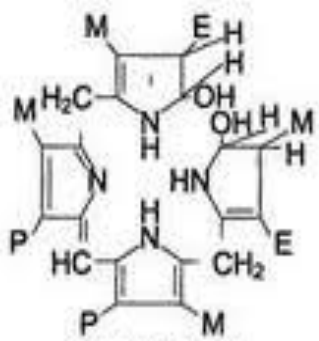
Bile pigments —are breakdown products of hemoglobin and other derivatives of porphyrin excreted in the bile, urine, feces.



Mesobilinogin



Stercobilinogin

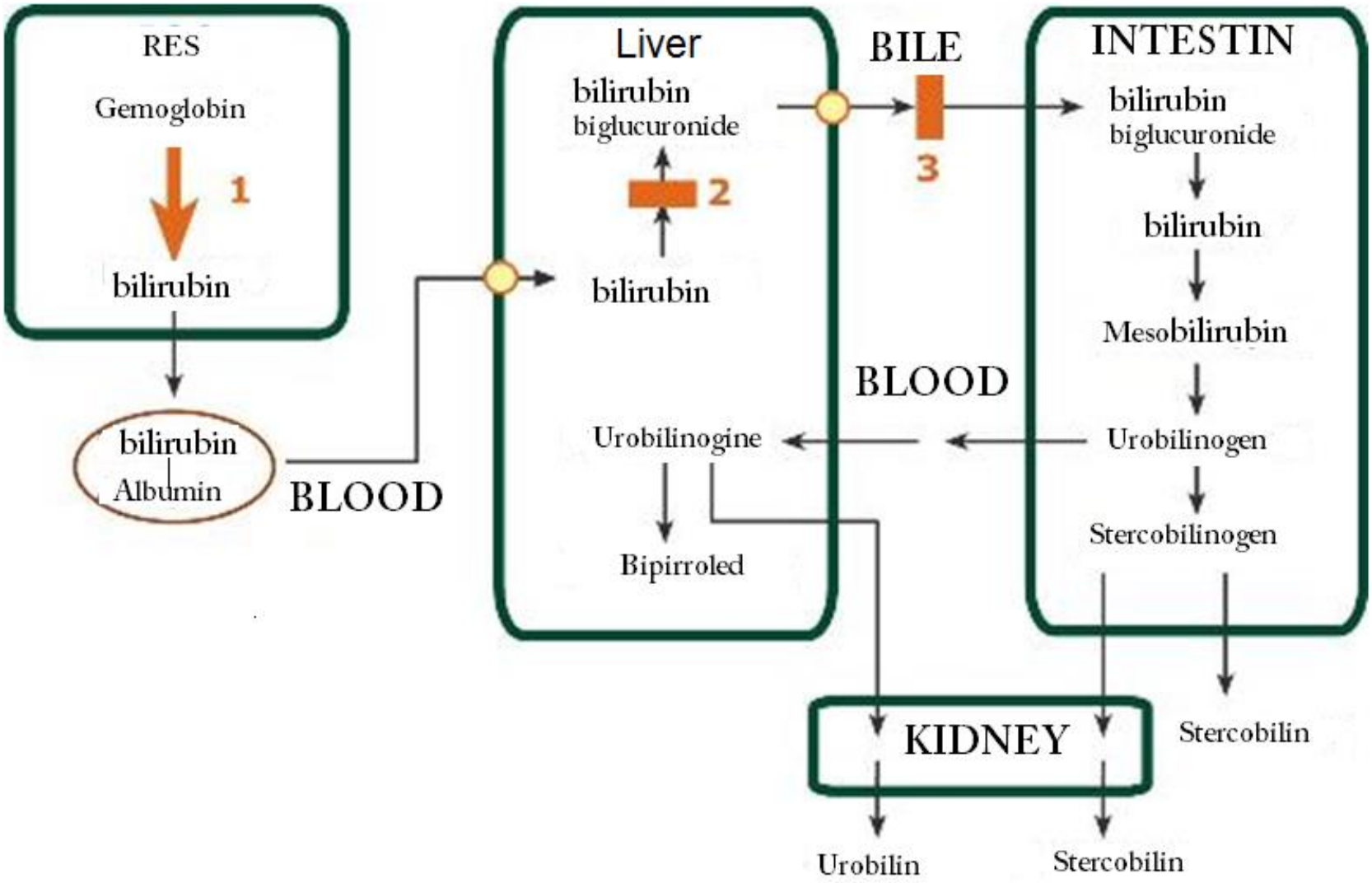


Stercobilin

Their greater portion is formed during the catabolism of hemoglobin in red blood cells upon disintegration of the cells of mononuclear phagocytes. Bile pigments are compounds containing four pyrrole groups linked by one-carbon bridges in the open, non-closed circuit (in contrast to the closed structure of the heme)

- **A small amount of bilirubin is formed in the cells of the mononuclear phagocyte system of the heme that was not used for the synthesis of hemoglobin.**
- **In the liver, bilirubin forms a pair of compounds, or conjugates, mainly with glucuronic acid, and to a lesser extent - with sulfuric acid.**
- **About 300 mg of bilirubin is produced in the body daily.**

Scheme of bilirubin production



Scheme of bilirubin production

Further metabolism of bilirubin is associated with its arrival in the biliary tract and intestines. In the inferior portions of the biliary passages and intestine, a gradual recovery of conjugated bilirubin to urobilinogen proceeds under the effect of microbial flora.

A portion of urobilinogen (mesobilinogen) absorbed in the intestine and the portal vein reenters the liver, where in norm conditions it undergoes an almost total destruction.

Another part of urobilinogen (stercobilinogen) is absorbed into the blood in the hemorrhoidal veins, getting into the bloodstream and is excreted by the kidneys in the urine in small quantities in the form of urobilin, which is often not detected by clinical laboratory methods.

Finally, the third part of stercobilin is converted to urobilinogen and excreted in the feces, giving it its characteristic dark brown color.

3 main causes of elevated bilirubin content in the blood

- The rate of synthesis of bilirubin is increased exceeding the excretory capacity of the liver *(*hemolytic, *up liver* jaundice*)*
- Inhibition of conjugation an/or excretory mechanisms in the liver: metabolize bilirubin is reduced (hepatic, or hepatocellular jaundice).
- Obstruction of the biliary system, which prevents the outflow of bile (cholestatic, obstructive jaundice)

Hemolytic jaundice

Mechanism: increased destruction of red blood cells - both mature cells and their precursors. Destruction of mature cells may be the result of hemolysis or effect recovery of blood after internal bleeding, such as soft tissue injuries.

Hemolytic jaundice

- Hyperbilirubinemia in hemolytic jaundice is caused by accumulation of unconjugated bilirubin, which is not excreted by the kidneys.
- This increases the flow of bilirubin from the liver into the intestine.
- A large amount of urobilinogen is produced, whose content in the urine increases.

Hepatocellular jaundice

- Inborn errors of bilirubin transport lead to jaundice due to imperfect absorption, decreased conjugation or impaired excretion of bilirubin.
- Generalized hepatocellular dysfunction may occur in hepatitis and decompensated liver cirrhosis.

Hepatocellular jaundice

- **Pathogenesis of jaundice in these cases is complicated, the contributing factors are disturbance of the uptake, intracellular transport, and reduced conjugation of bilirubin.**
- **In the presence of generalized dysfunction of the liver the uptake of bilirubin is reduced, and, therefore, its greater quantities are excreted by the kidneys.**

Cholestatic jaundice

Jaundice may be the effect of obstruction of an outflow of bile from the hepatocytes in the duodenum. It can be caused by lesions in the liver (intrahepatic cholestasis) or in the bile ducts and pancreas head (extrahepatic cholestasis).

Cholestatic jaundice

Jaundice is caused by a disturbance of excretion of conjugated bilirubin and its accumulation, which is filtered by the glomerules and appears in the urine.

However, bilirubin may not be detected in the urine, possibly because changes in the processes of conjugation lead to formation of less soluble bilirubin bound to albumin.

Newborn Jaundice

Hemolytic disease of the newborn

Causes. **Incompatibility of the mother's and fetal blood in the group or Rh factor.**

Accumulation of hydrophobic forms of bilirubin in the subcutaneous fat causes yellowness of the skin. However, the real danger is posed by accumulation of bilirubin in the gray matter of nervous tissue and stem nuclei with development of "kernicterus" (bilirubin encephalopathy).

Newborn Jaundice

Hemolytic disease of the newborn

Laboratory diagnosis. In the blood revealed severe anemia, reticulocytosis, erythro- and normoblastozis.

Hyperbilirubinemia due to indirect fraction from 100 to 342 mmol / l, in the future, and direct attached fraction.

Bilirubin level in the blood increases rapidly and to 3-5 day of life is maximized.

Physiological (transient) jaundice of the newborn

Causes: relative decrease in the activity of UDP-glucuronyl transferase in the first days of life associated with an increased degradation of fetal hemoglobin.

Nonhemolytic neonatal hyperbilirubinemia caused by breast milk.

It occurs in 1% of breastfed infants.

Causes. **Suppression of UDP-glucuronyl transferase activity, presumably breast milk estrogens.**

Laboratory diagnosis. **Increase in the concentration of free serum bilirubin at 140-240 mkmol/l.**

Changes in laboratory parameters in different types of liver disease

Test	Liver disease		
	Disease with necrosis of hepatocytes (viral, drug, autoimmune **hepatitis)**	Cholestatic disease (primary biliary **cirrhosis)**	Infiltrative process (**liver cancer**)
Amino-transferase	**Moderate or significant increase**	Normal or slight increase	Normal or slight increase
Alkaline phosphatase	Normal or slight increase	**Moderate or significant increase**	**Moderate or significant increase**
Total bilirubin	From normal to a significant increase	From normal to a significant increase	From normal to a slight increase

Changes in laboratory parameters in different types of liver disease

Test	Liver disease		
	Disease with necrosis of hepatocytes (viral, drug, autoimmune **hepatitis**)	Cholestatic disease (primary biliary **cirrhosis)**	Infiltrative process (**liver cancer**)
Prothrombin time	**Increased, not dependent on vitamin K**	**Increased, dependent on vitaminK**	Normal
Albumin	**Reduced in chronic**	Normal	Normal
Bile acids	From minor to significant increase	From minor to significant increase	Normal

Thank you for your attention!