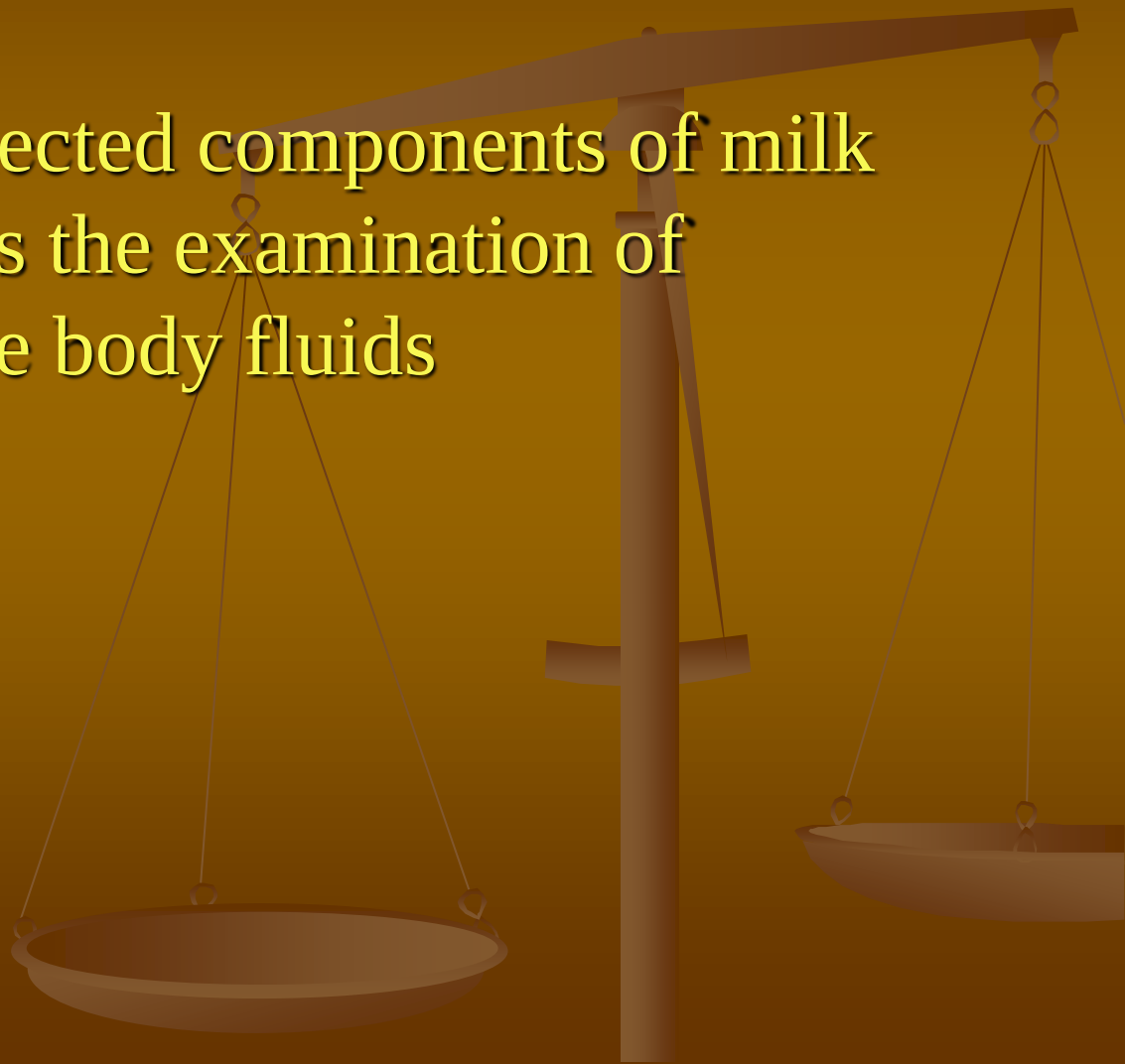
# Body fluids
# milk, bile

# Milk

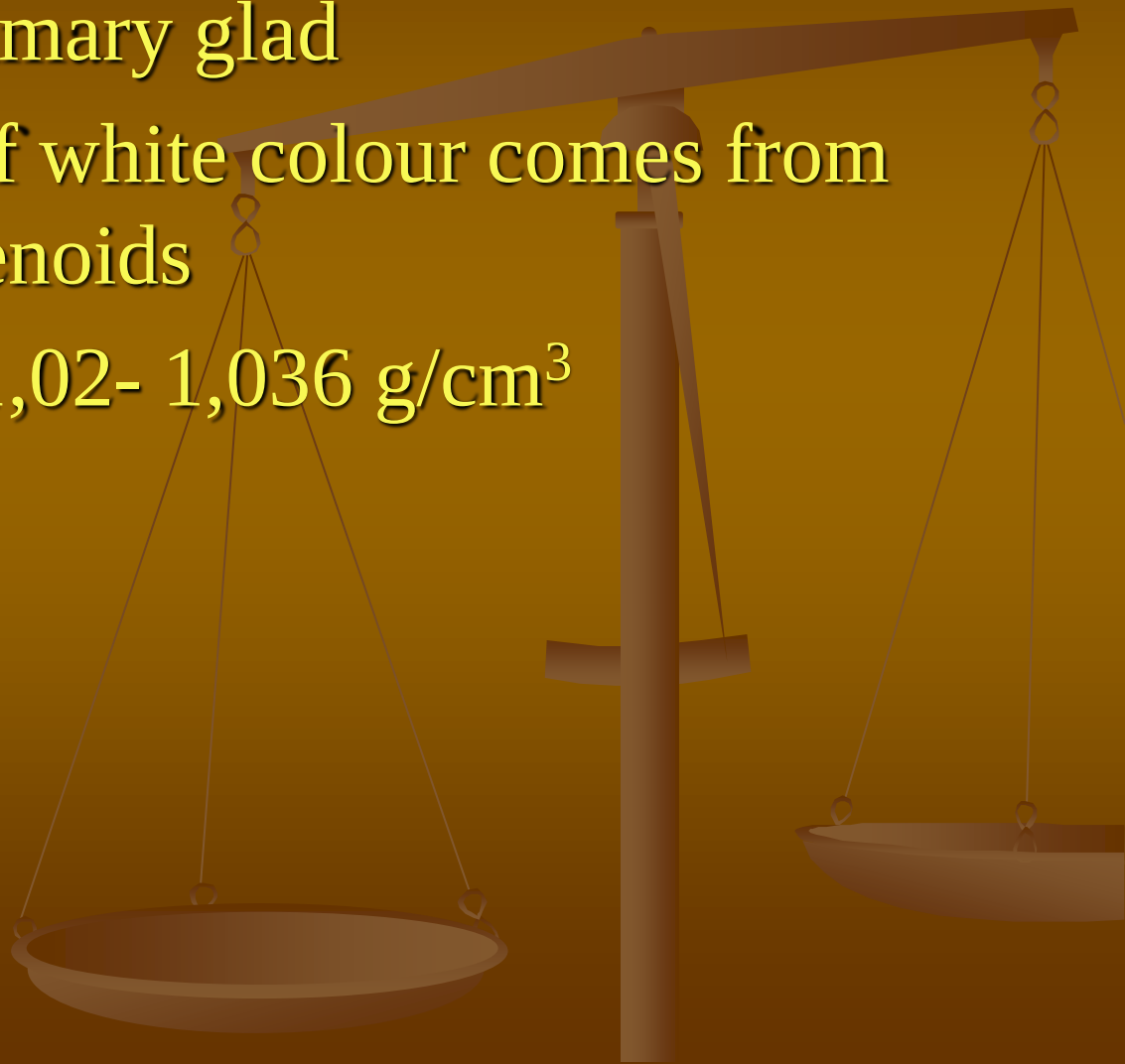
# The aim:

Identification of selected components of milk and bile as well as the examination of properties of these body fluids



# Physical properties:

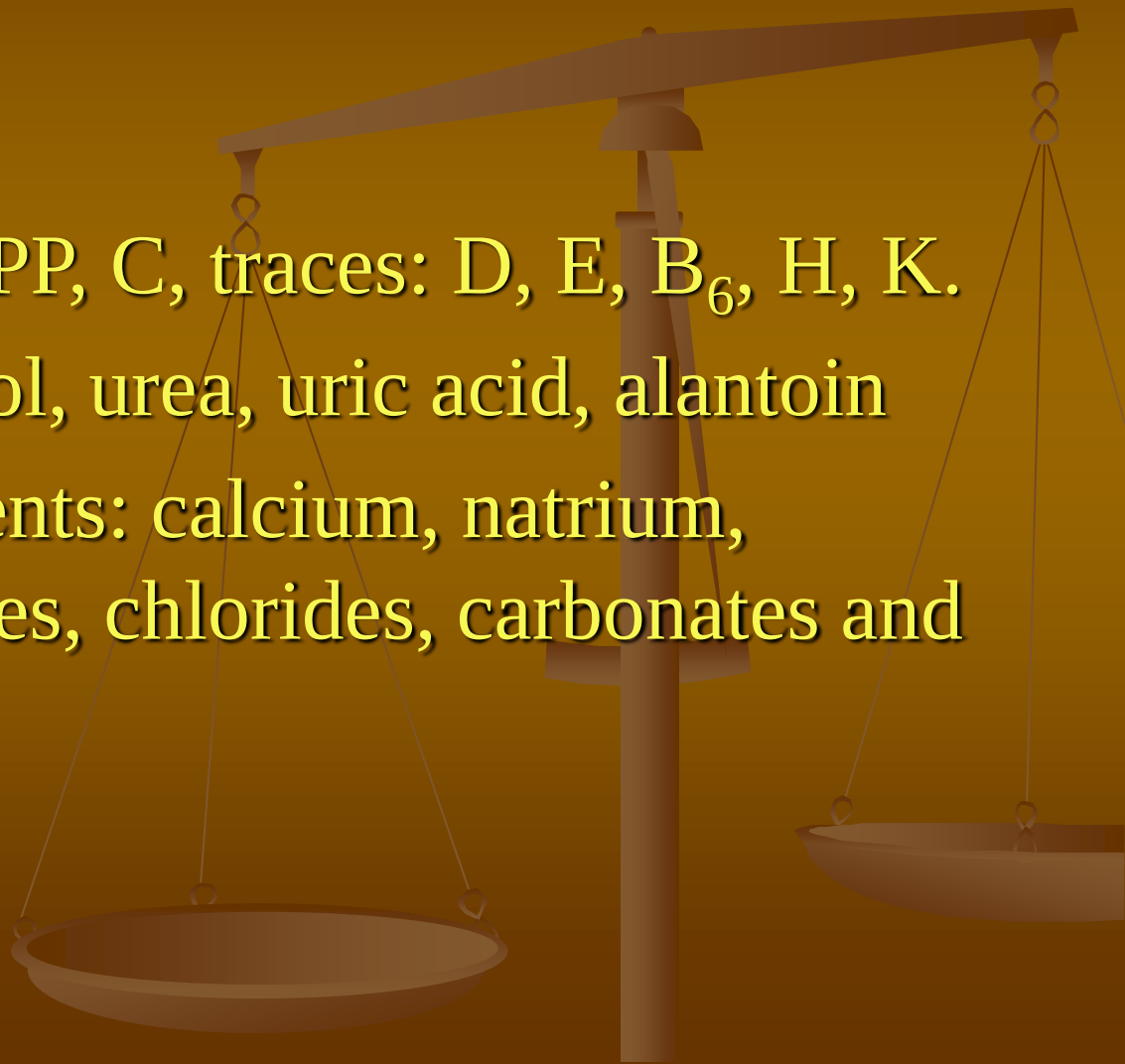
- Exsudate of mammary glad
- Yellow shadow of white colour comes from flavins and carotenoids
- Specific gravity 1,02- 1,036 g/cm$^3$



# Components of milk:

- Proteins: caseins (phosphoproteins), lactoalbumins, lactoglobulins (lack in humans)
- Enzymes: alkaline and acid phosphatase, lipase, alfa-amylase, peroxidase and xanthine oxidase
- Lipids are present as spheroids of triacylglycerols surrounded by proteins
- Around 150 different fatty acids, 60% are saturated

- Sugars: lactose, glucosamine, N-acetyloglucosamine, N-acetylolactosamine,
- Citrates
- Hormons
- Vitamins: A, $B_2$, PP, C, traces: D, E, $B_6$, H, K.
- Traces: cholesterol, urea, uric acid, alantoin
- Mineral components: calcium, natrium, kalium, phosphates, chlorides, carbonates and sulphates.



# Colostrum:

- Milk of early lactation (appears few days before and after parturition)
- The content of proteins is 4-5 times higher as in milk. Especially high content express albumins and gamma globulins
- The presence of gamma globulins decides about passive resistance during first hours of newborn life
- In comparison to milk - higher contents of calcium, iron, phosphorum and chloride but lower of natrium, kalium and water
- Specific gravity 1,046- 1,080 $g/cm^3$

# Immunoglobulins of colostrum

- In regular milk the content of immunoglobulins accounts around 0,06%. They are in colostrum in significantly higher amounts. Their content increase in cases of mastitis.
- Are produced by plasmatic cells mammary gland.
- Three groups of immunoglobulins are known:
  - Type G (IgG) – 90% of total globulins of bovine milk (IgA dominate in humans), molecular weight 150-170 kDa.
  - Type M (IgM) – molecular weight 900 kDa.
  - Type A (IgA) – molecular weight 300-500 kDa.

# IgG

**IgG** are most probably the most important antibodies for secondary answer. Are characterised by the presence of heavy chain γ. There are 4 subclasses:

**IgG1**, binds proteins of Staphylococci and Streptococci. Appears as first in immunological answer and the most strongly activates the complement

**IgG2**, binds similar proteins as above;

**IgG3**, binds proteins of Streptococcus, activates the complement but in weaker way as IgG1;

**IgG4**, binds both Staphylococci and Streptococci. Appears later as IgG1, does not activate the complement.

The meaning of these antibodies is related to high affinity to antigen and the activation of the complement. This property expresses also IgM

# IgM

**IgM** – produced by plasmocytes. Contain heavy chain.

IgM - pentamer molecules known as „snow lobule” which are modified to „crab-shape” after binding antigen. They activate the complement but express low affinity to antigen. They are present only in primary answer.

The increase of IgM in humans may mean severe disease eg boreliosis, toxoplasmosis or liver cirrhosis.

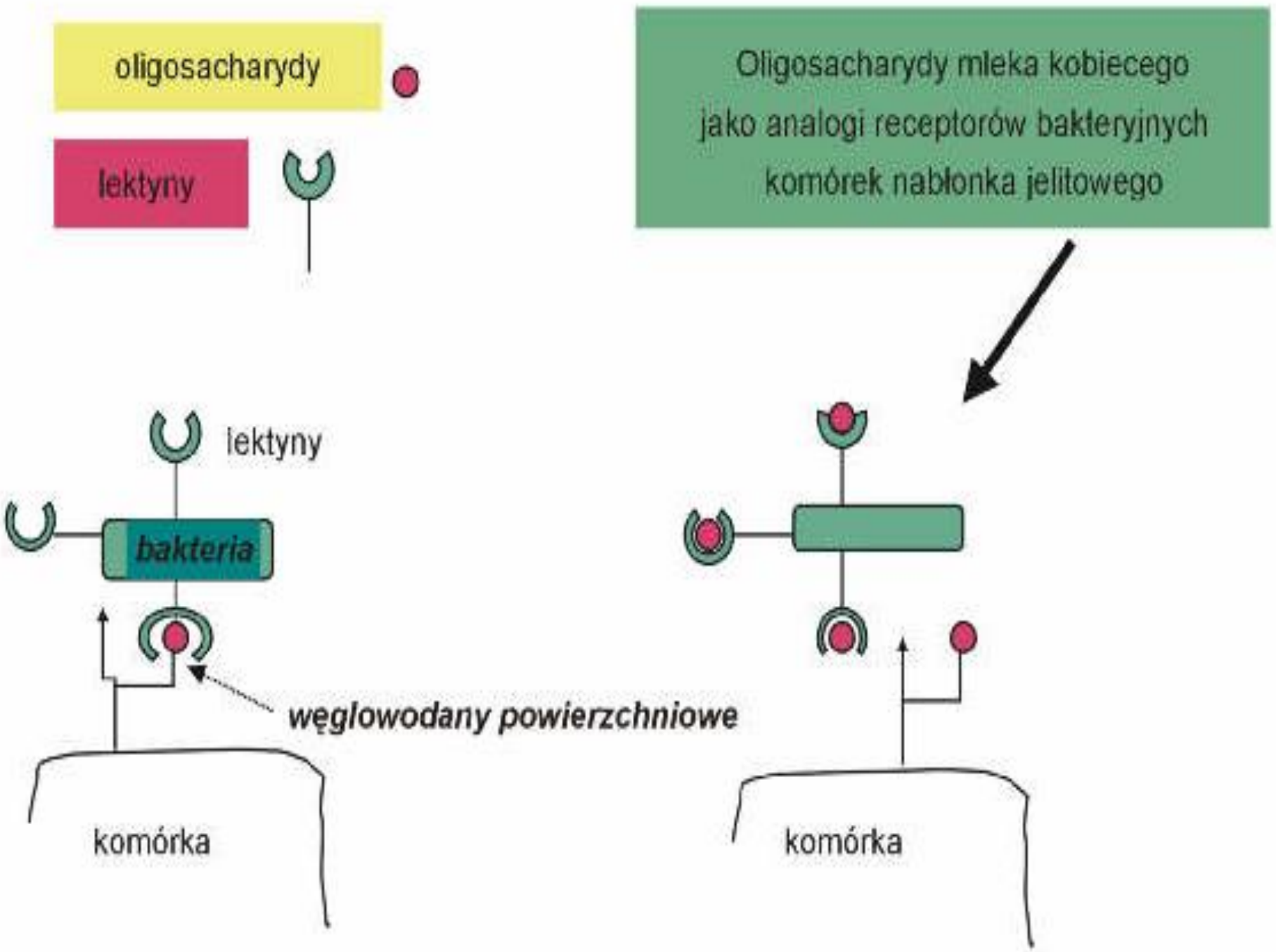
# IgA

**IgA** – contain heavy chain α and participate in the defence of mucosa.

IgA – produced in high amounts, even up to 9,2 g per day. Two subclasses are known:

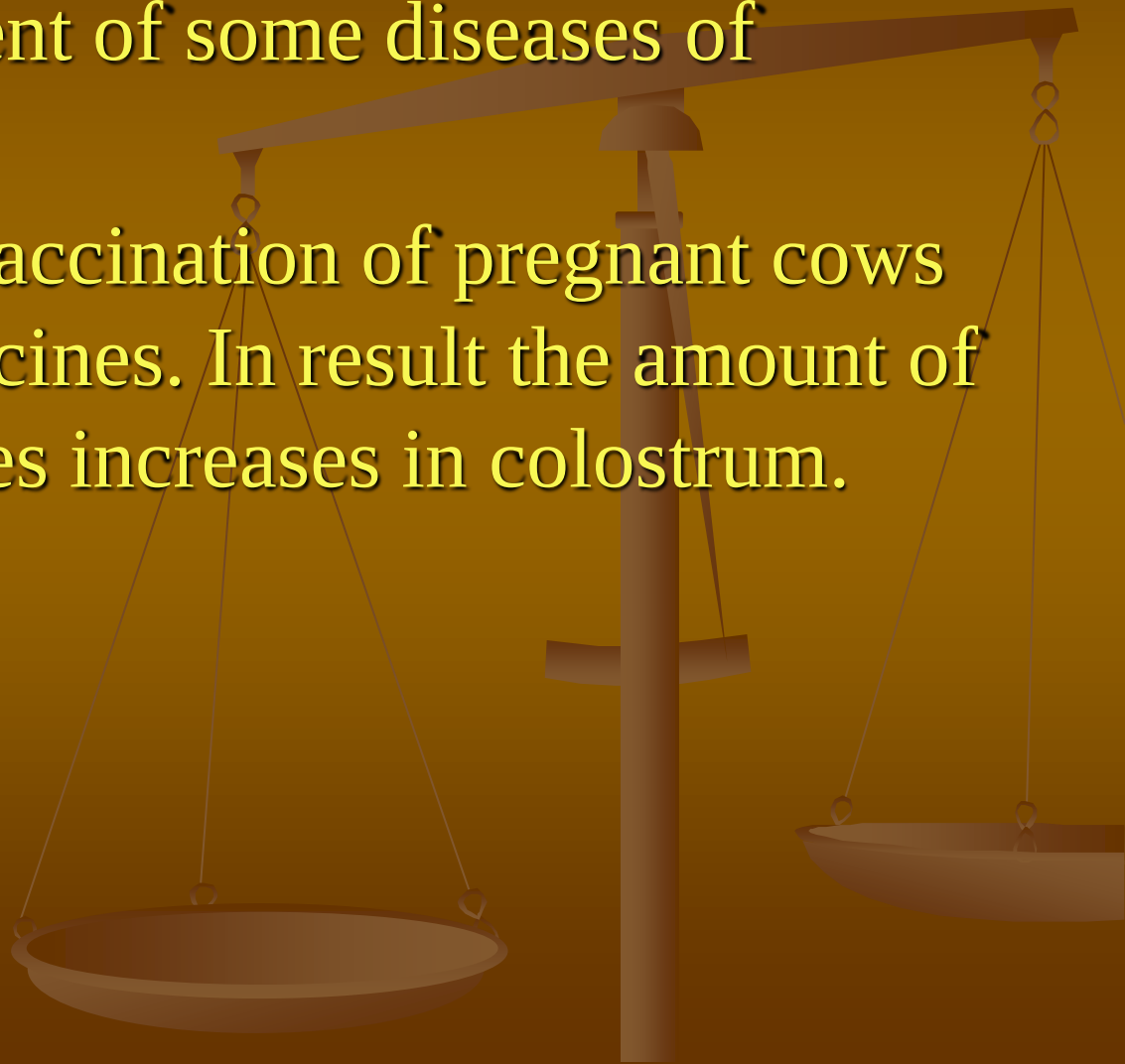
IgA1, mainly present in plasma as monomers.

IgA2, mainly dimers secreted to the surface of mucosa. They are not degraded by digestive enzymes in intestine or by bacterial proteases.


oligosacharydy
lektyny
Oligosacharydy mleka kobiecego
jako analogi receptorów bakteryjnych
komórek nabłonka jelitowego
lektyny
bakteria
węglowodany powierzchniowe
komórka
komórka

# Hyperimmunized colostrum

- Is used in treatment of some diseases of digestive tract
- Is produced via vaccination of pregnant cows with selected vaccines. In result the amount of selected antibodies increases in colostrum.



# Bile:

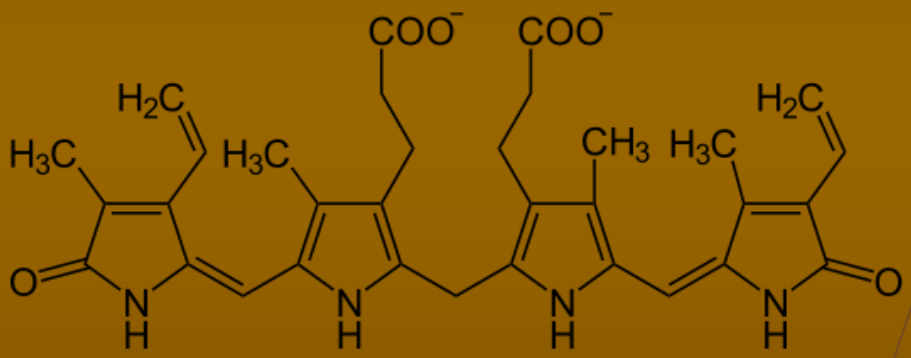
- Exsudate of liver cells which is collected in gall bladder. Cholecystokinin is responsible for the removal of bile from bladder.
- As exsudate it stimulates digestion and absorption of lipids.
- As excretion – bile pigments, some drugs or microelements like iodine can be removed from body with bile

# Properties:

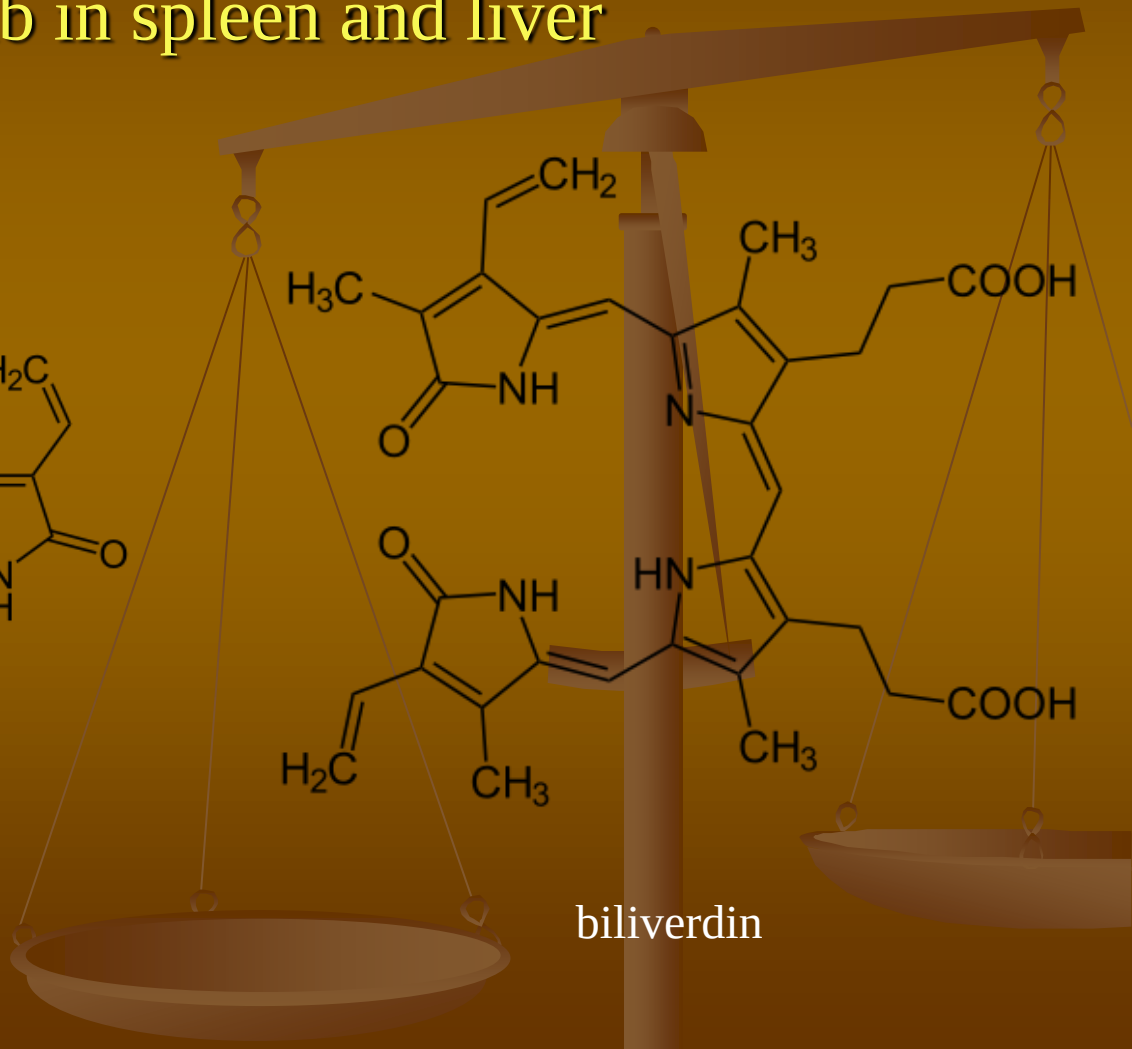
- Bitter taste comes from bile acids
- pH 7,4 - 8,0
- Specific gravity of liver bile - 1,004 g/cm$^3$, bladder bile - 1,020 g/cm$^3$
- Colour of bladder bile – golden-yellow or braun-yellow
- It is sticky due to the presence of mucins

# The components of bile:

Bile pigments: bilirubin and biliverdin – are formated during breakdown of Hb in spleen and liver



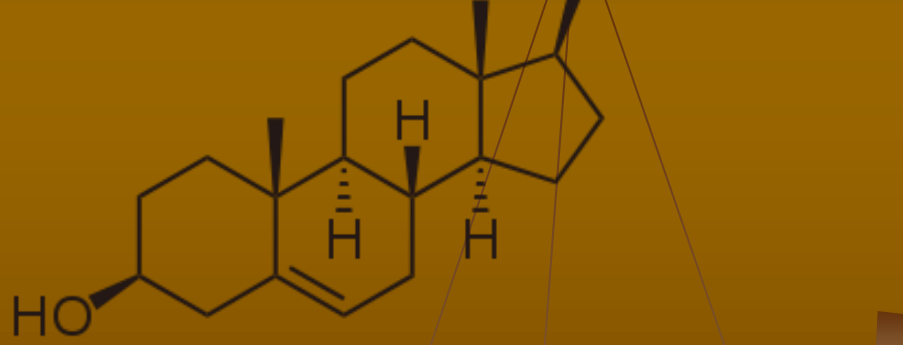

bilirubin




biliverdin

# The components of bile:

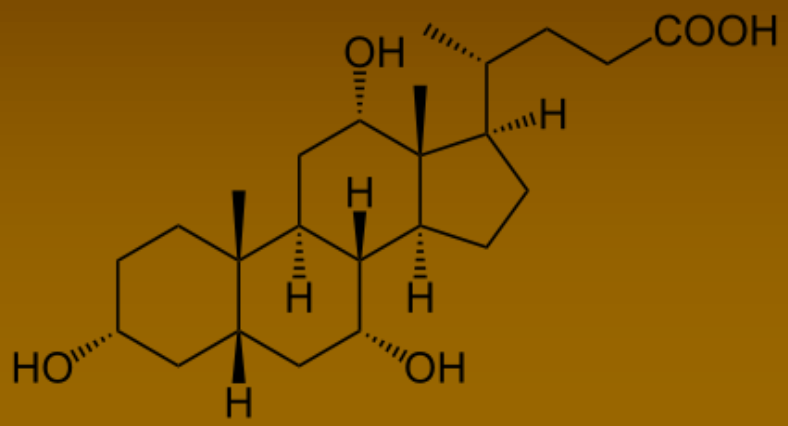
## Cholesterol and lipids



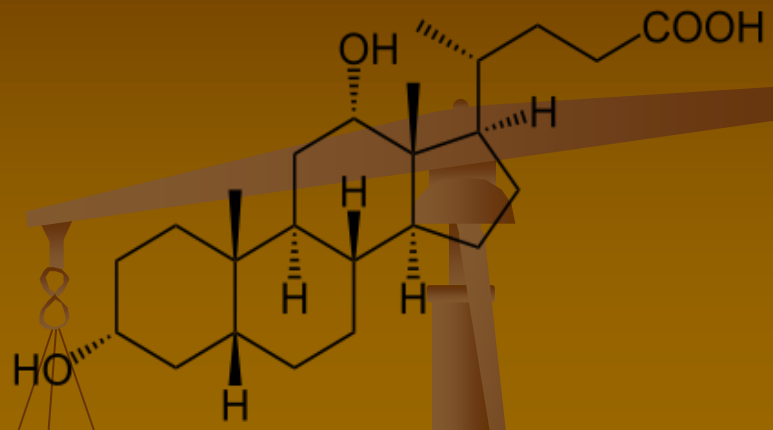

cholesterol

# The components of bile:

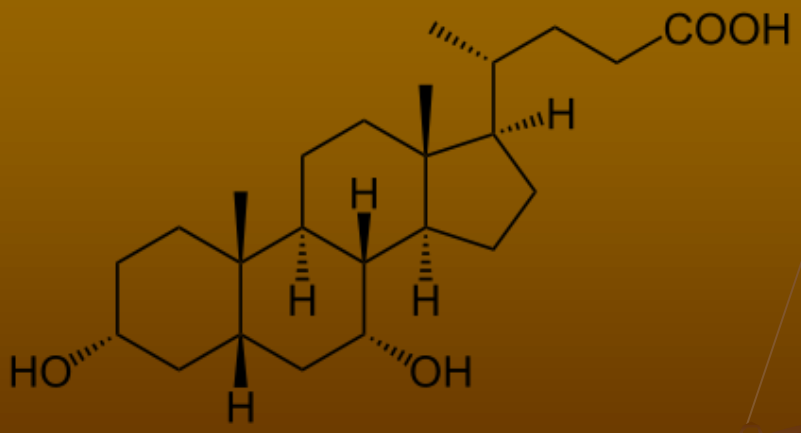
## Salts of bile acids



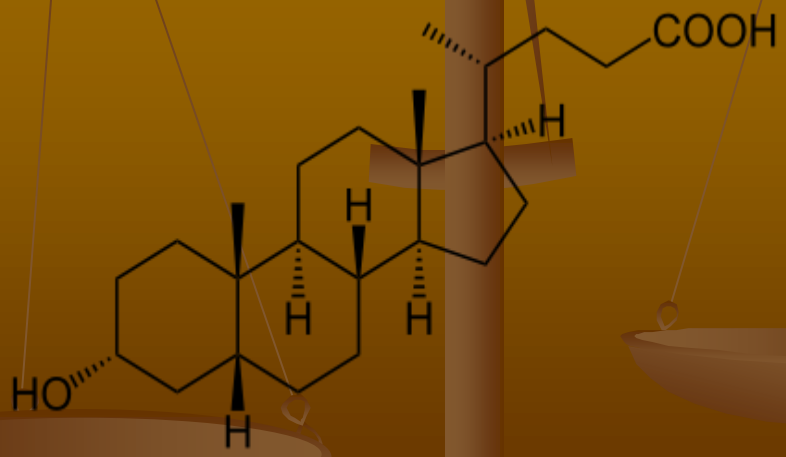

Cholic acid
3,7,12-trihydroxycholate




deoxycholic acid
3,12-dihydroxycholate

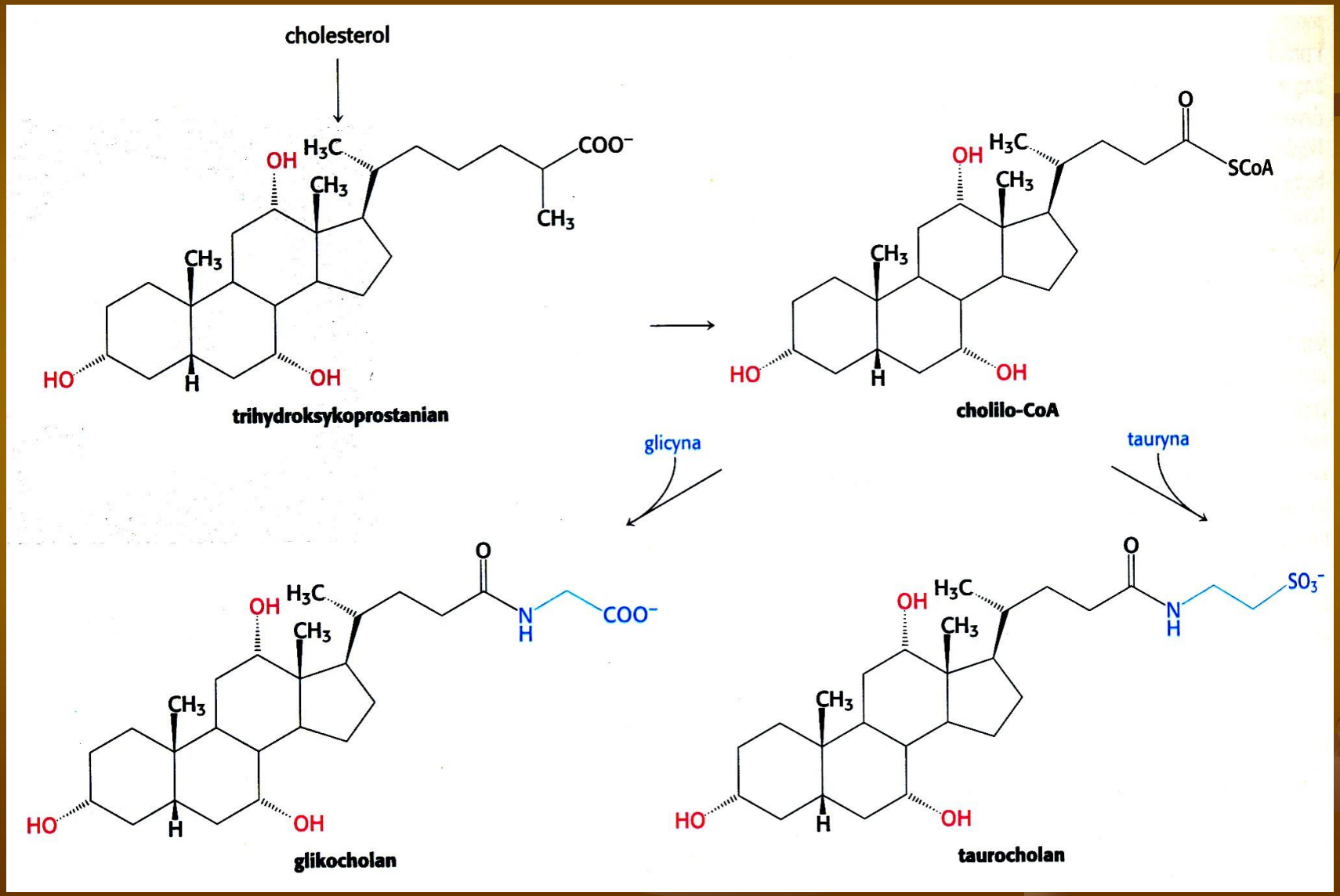



cheneodeoxycholic acid
3,7-dihydroxycholate




litocholic acid
3-hydroxycholate

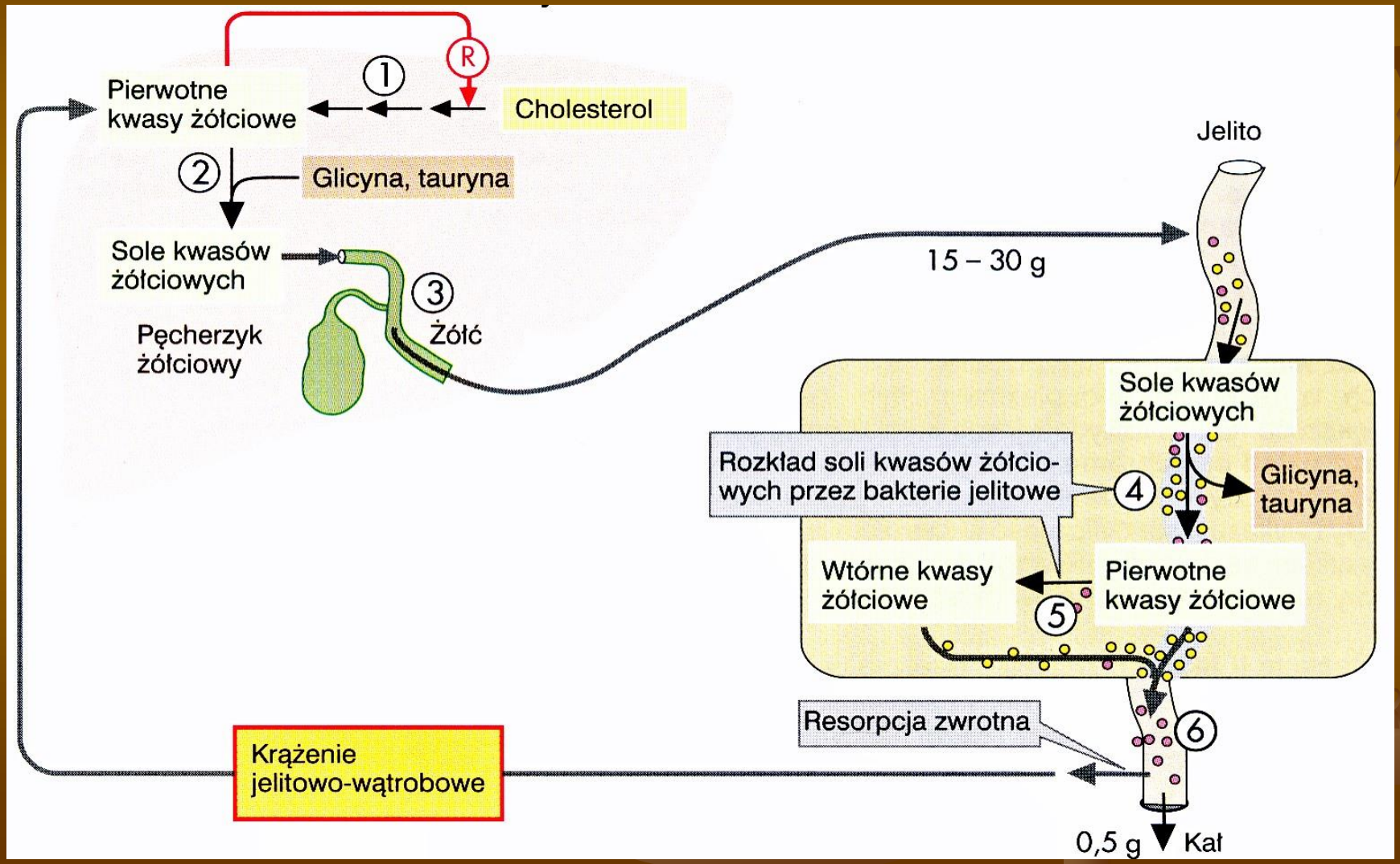
# The synthesis of salts of bile acids



# The functions of bile acids:

- Activate pancreatic lipase in small intestine
- Emulsify lipids
- Absorb fatty acids in small intestine
- Stimulate the absorption of lipid soluble vitamins, especially K
- Keep cholesterol in bile as dissolved
- Stimulate the production of bile, which is secreted as long as bile acids are absorbed from digestive tract

# Liver-intestine circulation of bile acids



## Experiment 1. Chemical composition of milk

### Protocol.

### Obtaining of milk proteins

Take 10cm$^3$ of milk to beaker, add 20 cm$^3$ $H_2O$ and 7 drops of glacial $CH_3COOH$. Caseous sediment of casein and lipids will precipitate. Drain sediment of casein and lipids with 2 filter papers and remove. Add 1,5 cm$^3$ 10% $Na_2CO_3$ to obtained filtrate, pH of sample should be 8 (check with strip indicator). Boil the solution. Precipitated sediment of lactoalbumin and lactoglobulin filtrate with filter paper and remove.

Use obtained filtrate for further determinations:

### 1. Detection of lactose

Take 1 cm$^3$ of filtrate to glass tube and add 1 cm$^3$ of Benedict or Fehling reagent. Boil for few minutes. As positive result of presence of lactose red sediment of $Cu_20$ will be formed.

### 2. Detection of $Cl^-$ ions

Take 1 cm$^3$ of filtrate to glass tube, add 4 drops of concentrated $HNO_3$, and 0,1 mol/dm$^3$ $AgNO_3$. As positive result white, caseous precipitate of AgCl will be formed.

### 3. Detection of $Ca^{+2}$ ions

Take 1 cm$^3$ of filtrate to glass tube and add 0.5 cm$^3$ ammonium oxalate (szczawian amonu - $(NH_4)_2C_2O_4$). As positive result white cloudiness of calcium oxalate ($CaC_2O_4$) will be formed.

### 4. Detection of $PO_4^{-3}$ ions

Take 1 cm$^3$ of filtrate to glass tube, add 1 cm$^3$ of concentrated $HNO_3$ and 0.5 cm$^3$ ammonium molybdate solution (molibdenian amonu). Heat it carefully over the burner to boil. As positive result yellow precipitate of ammonium phosphoro-molybdate will be formed.

**Experiment 2. Detection of fat in milk**

**Protocol.**

**1.** Detection of fat in milk

Take 3 $cm^3$ of „Phenoloftalein" milk and add 2 $cm^3$ of pancreatic lipase. After mixing the solutions incubate in 40° C. Observe when milk will decolor, explain the course of experiment.

## Experiment 3. Detection of bile acids

### Protocol.

**1. Reaction of Hay with "sulfur flower" (kwiat siarkowy)**

Take 2 glass tubes and add 2 $cm^3$ of water to each. Add 1 drop of bile to one tube. Add "sulfur flower" to both tubes and compare the results. "Sulfur flower" will fall down in tube with bile – explain this reaction.

**2. Emulsifying properties of bile**

Take 2 glass tubes and add 5 $cm^3$ of water and few drops of oil to both. Add one drop of bile to one tube. Both tubes mix vigorously and observe the behaviour of emulsion in both tubes.

**3. Reaction of Pettenkofer – detection of bile acids**

Take 1 $cm^3$ of bile, add few cristals of sacharose and gently 1 $cm^3$ of concentrated $H_2SO_4$. Red ring will be formed on the border between solutions.

**4. Detection of bilirubin – reaction of Gmelin**

Take 1 $cm^3$ of bile and add gently concentrated $HNO_3$. Coloured rings of products of bilirubin oxidation will be formed on the border of solutions.