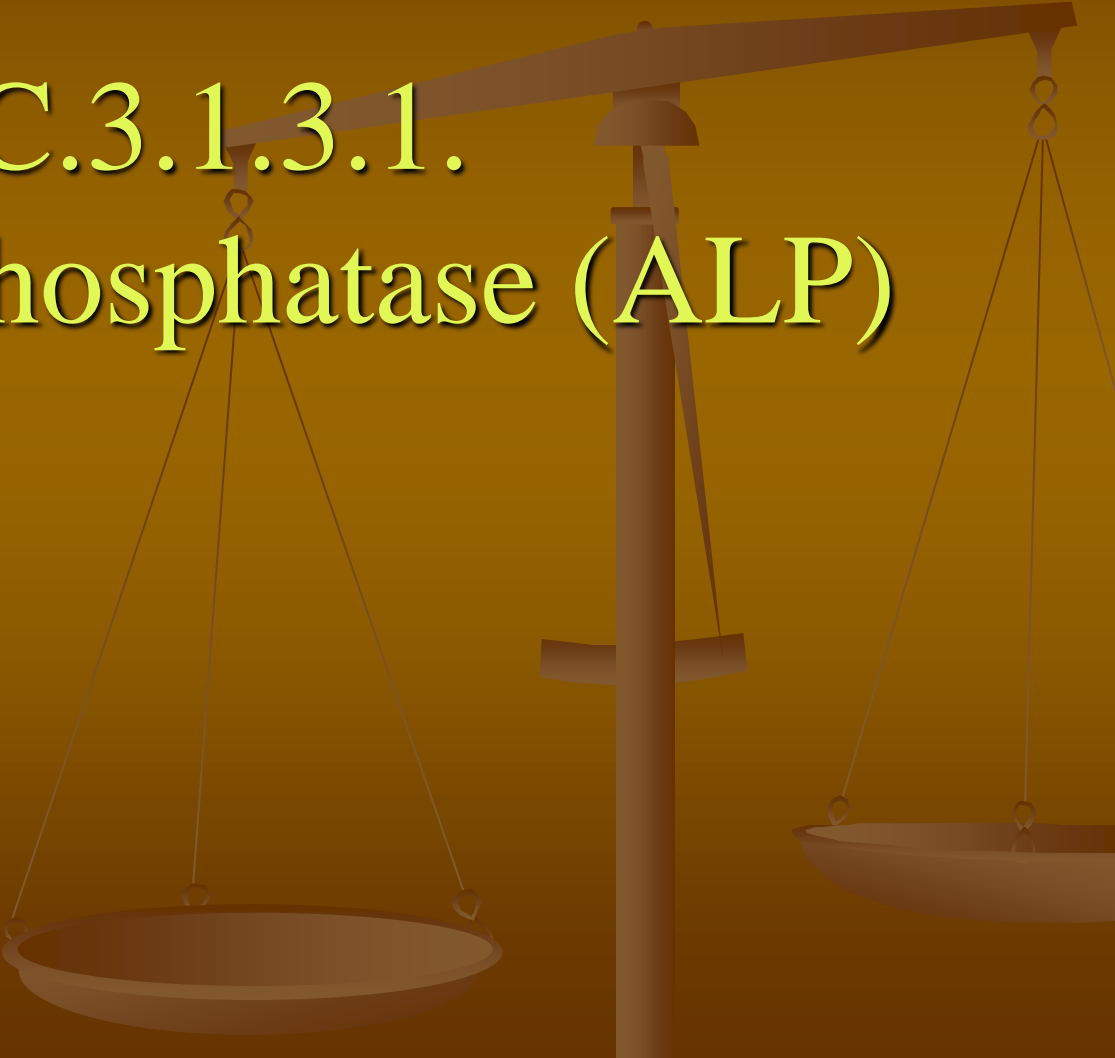


A faint, stylized illustration of a balance scale is visible in the background. The scale is tilted, with the right pan being lower than the left pan. The text is overlaid on this background.

Hydrolases

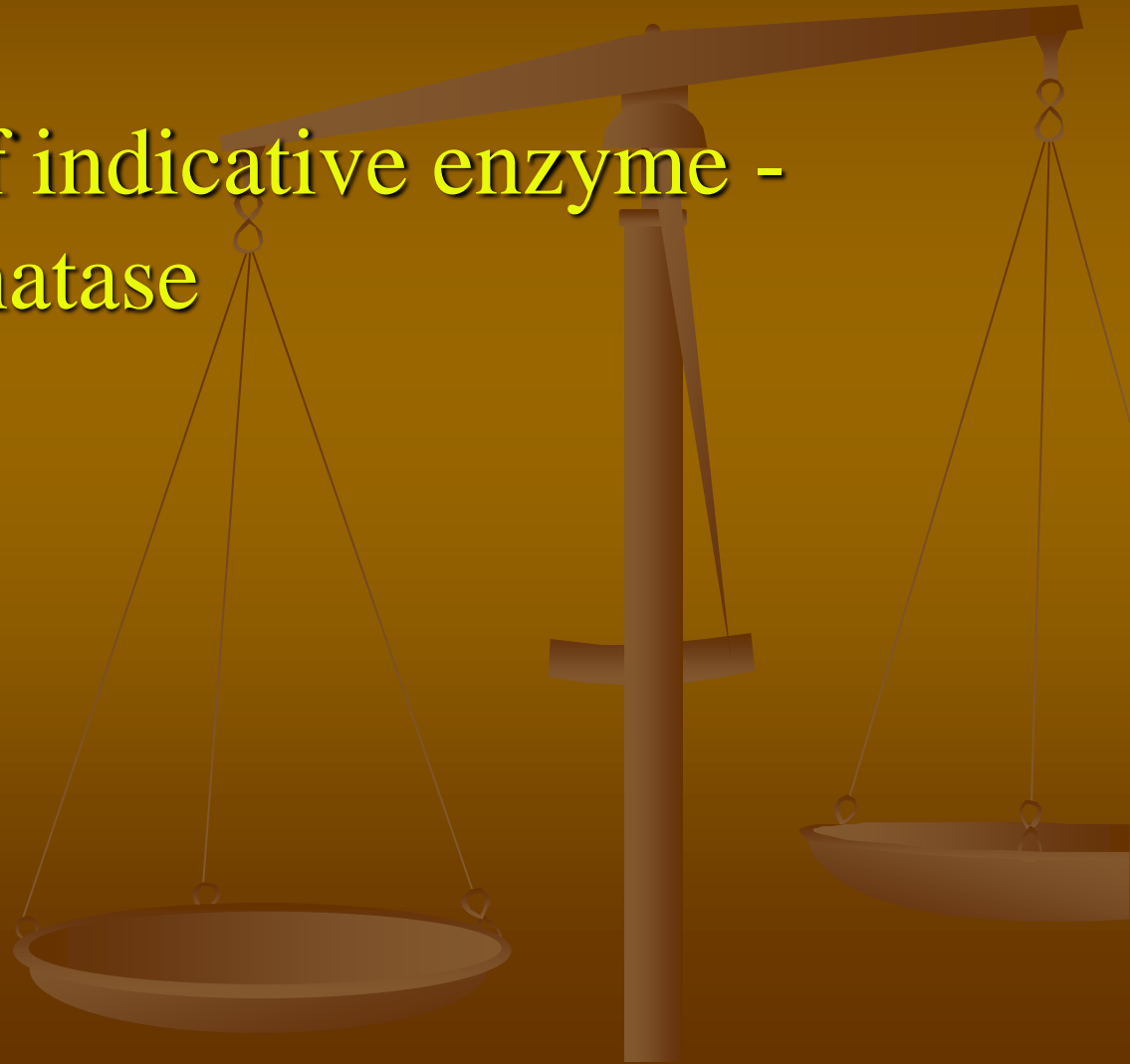
Alkaline and Acid Phosphatase



E.C.3.1.3.1.  
Alkaline Phosphatase (ALP)

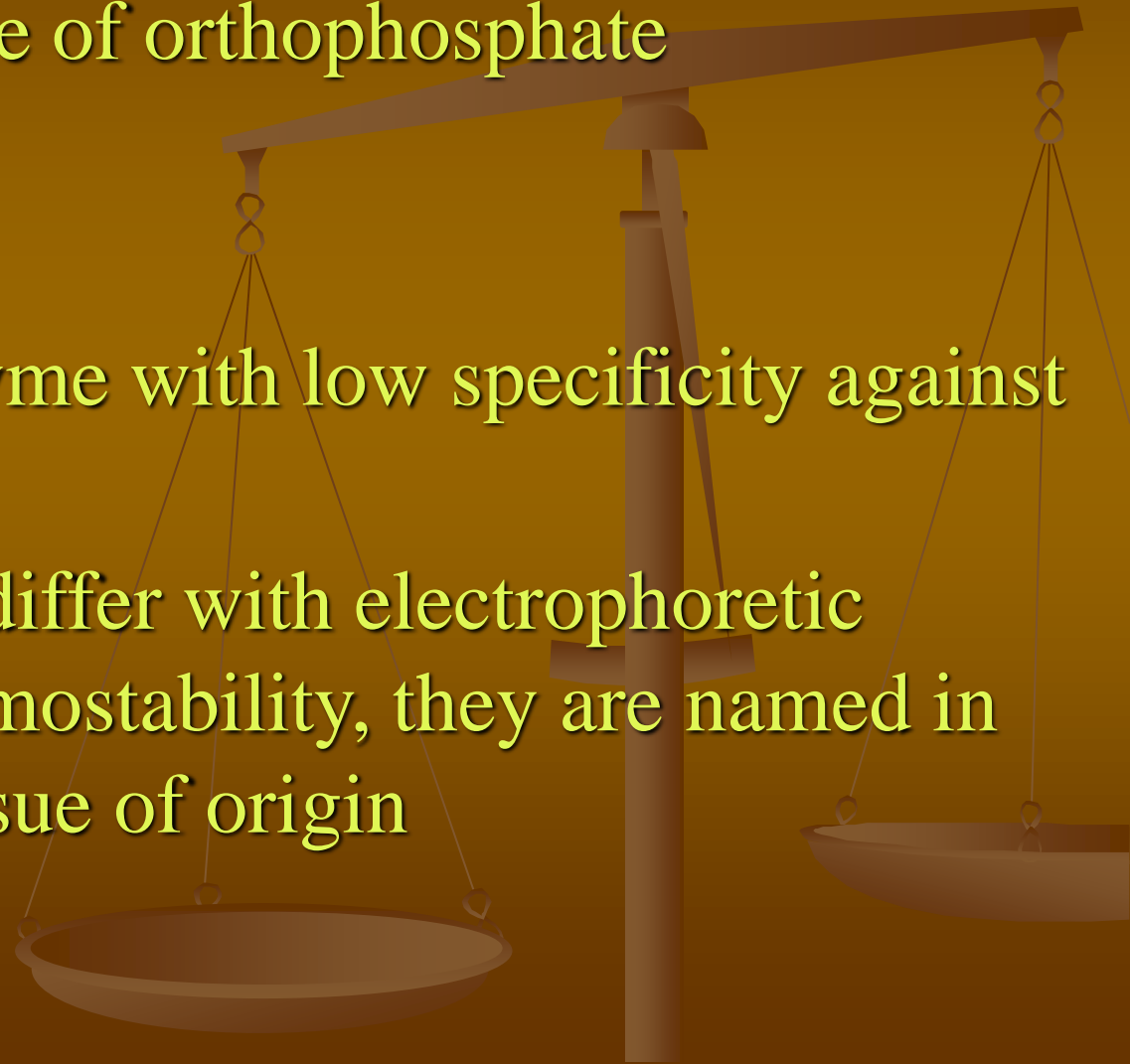
The aim:

The estimation of indicative enzyme -  
alkaline phosphatase



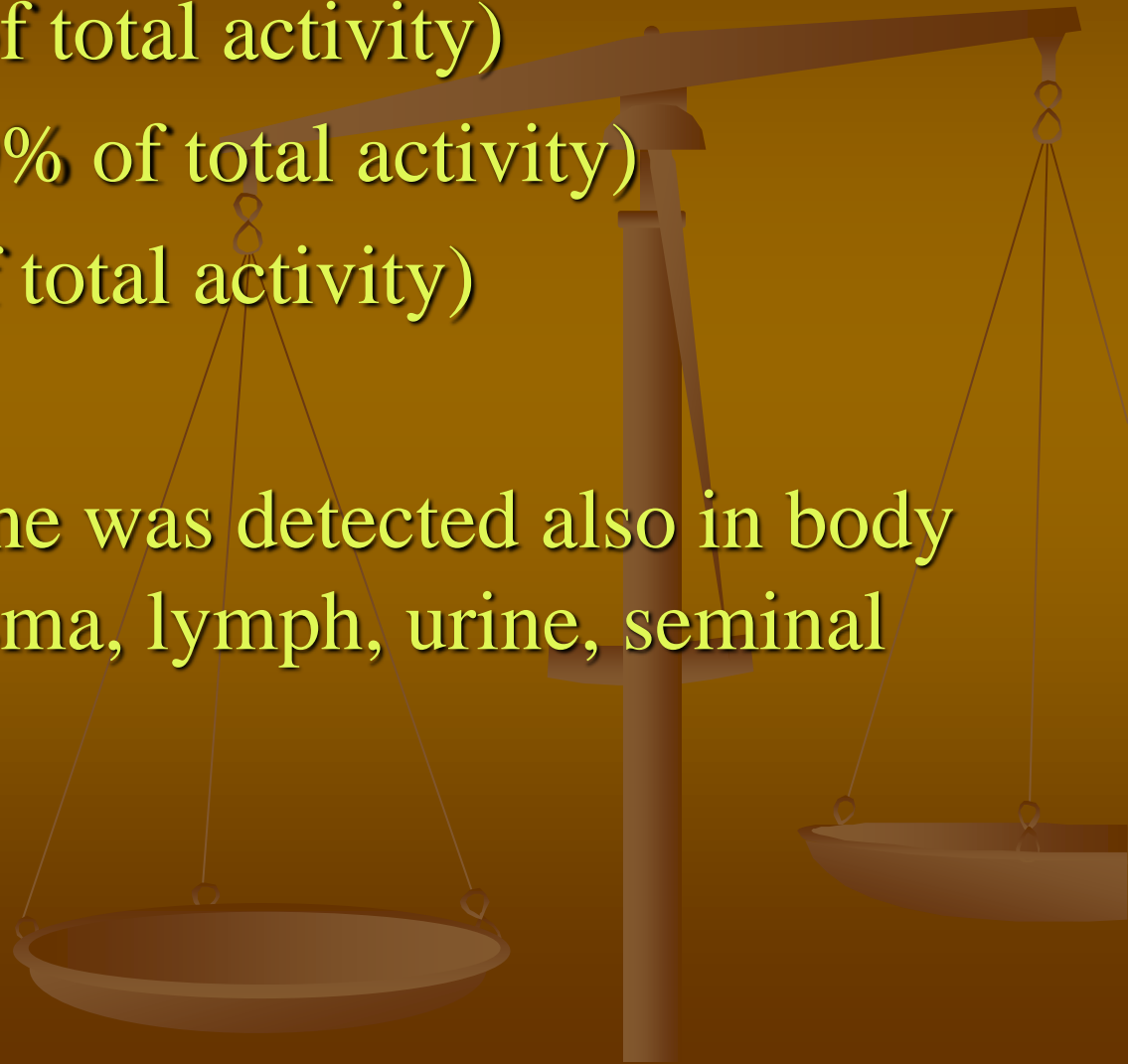
# Properties:

- Phosphohydrolase of orthophosphate monoesters
- pH 8,5-10,5
- Heterogenic enzyme with low specificity against substrate
- Izoenzymes can differ with electrophoretic motility and thermostability, they are named in accordance to tissue of origin

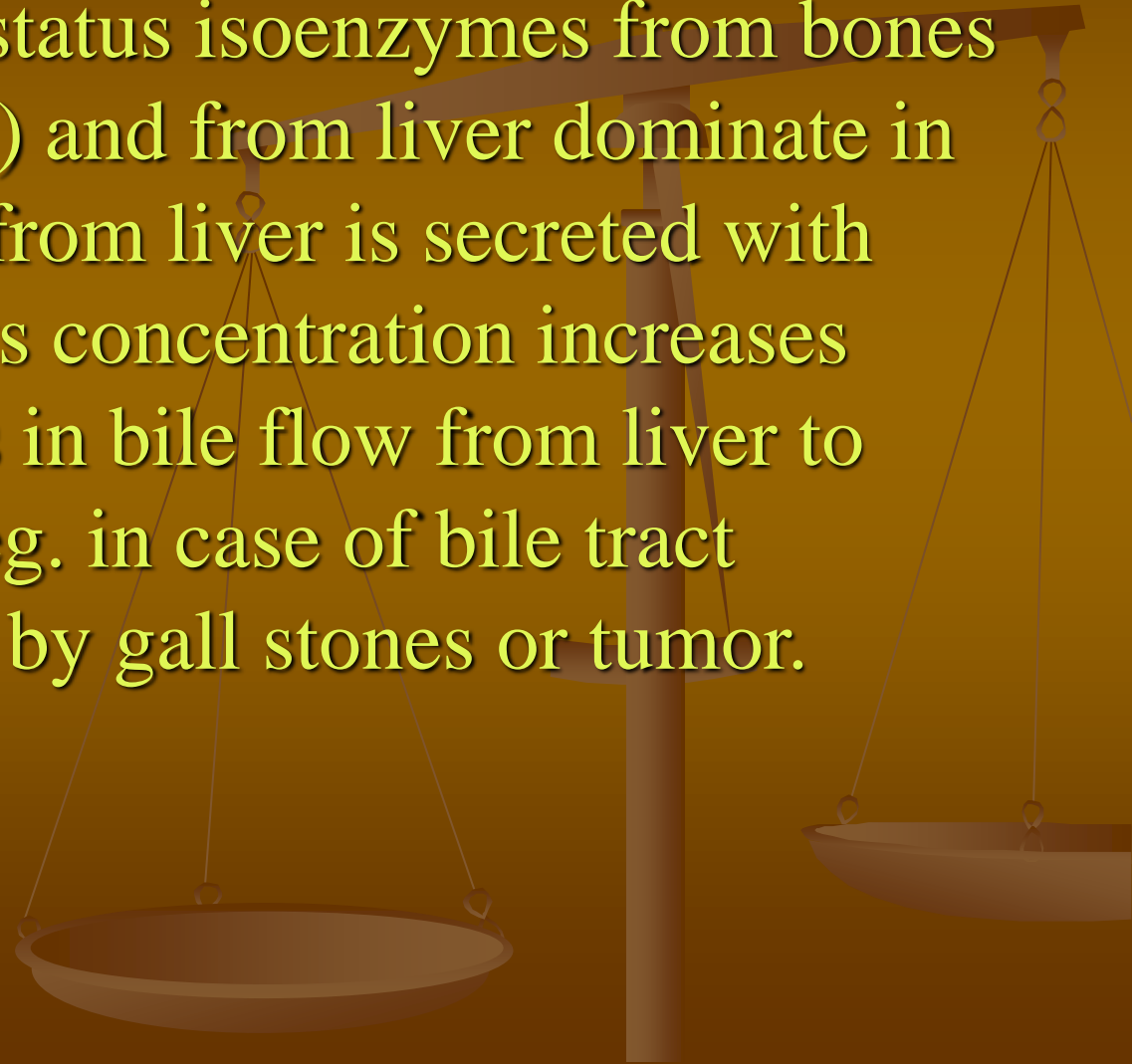


# Localization:

- Bones (50-60% of total activity)
- Intestine wall (30% of total activity)
- Liver (10-20% of total activity)
- Placenta
- Activity of enzyme was detected also in body fluids (blood plasma, lymph, urine, seminal plasma)

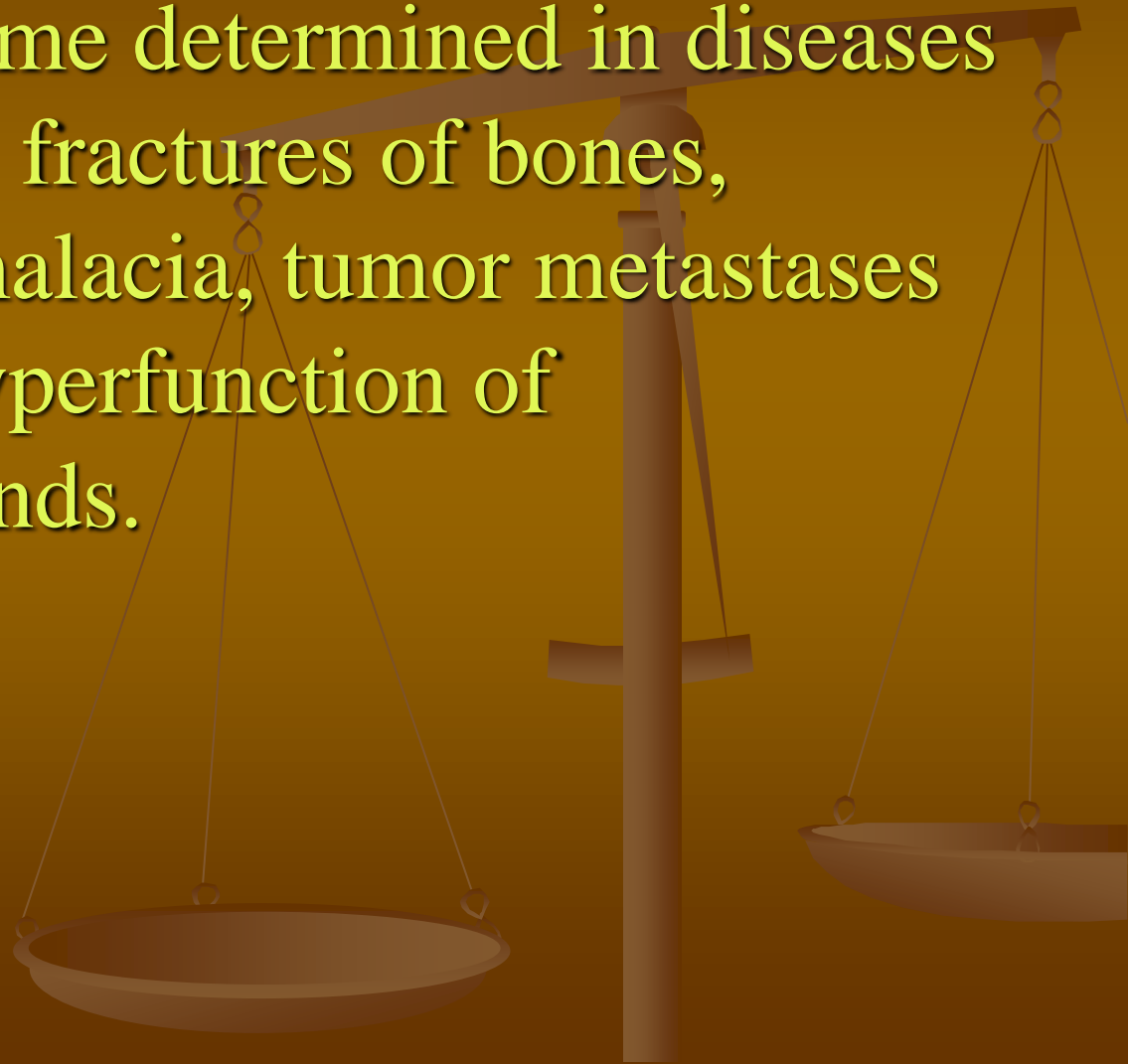


- In physiological status isoenzymes from bones (from osteoblasts) and from liver dominate in plasma. Isoform from liver is secreted with bile that is why its concentration increases when difficulties in bile flow from liver to intestine appear eg. in case of bile tract occlusion caused by gall stones or tumor.



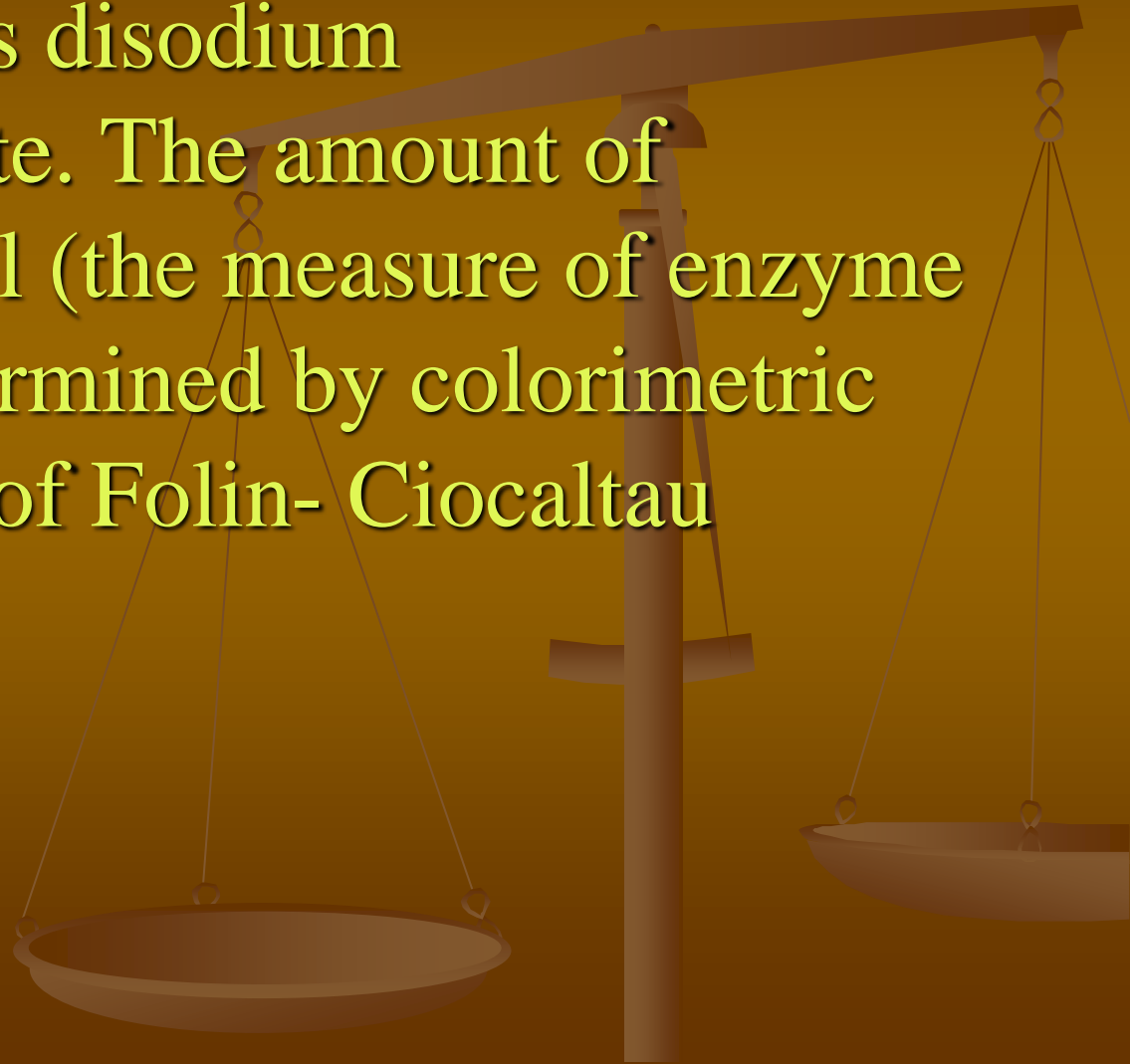
# Meaning:

- Indicative enzyme determined in diseases with bile stasis, fractures of bones, rachitis, osteomalacia, tumor metastases to bones and hyperfunction of parathyroid glands.



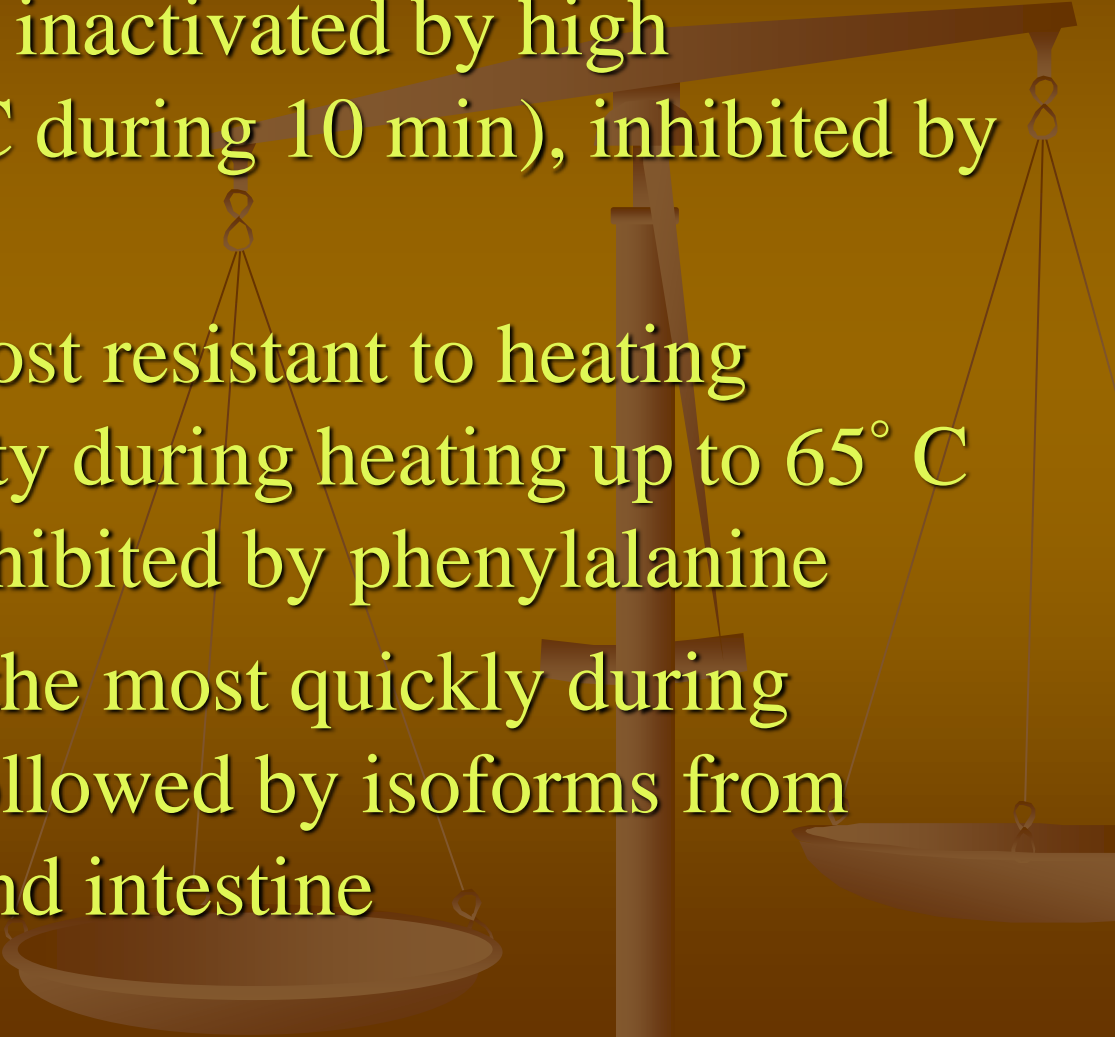
# The determination of activity:

- ALP hydrolyses disodium phenolphosphate. The amount of liberated phenol (the measure of enzyme activity) is determined by colorimetric method by use of Folin- Ciocaltau reagent.



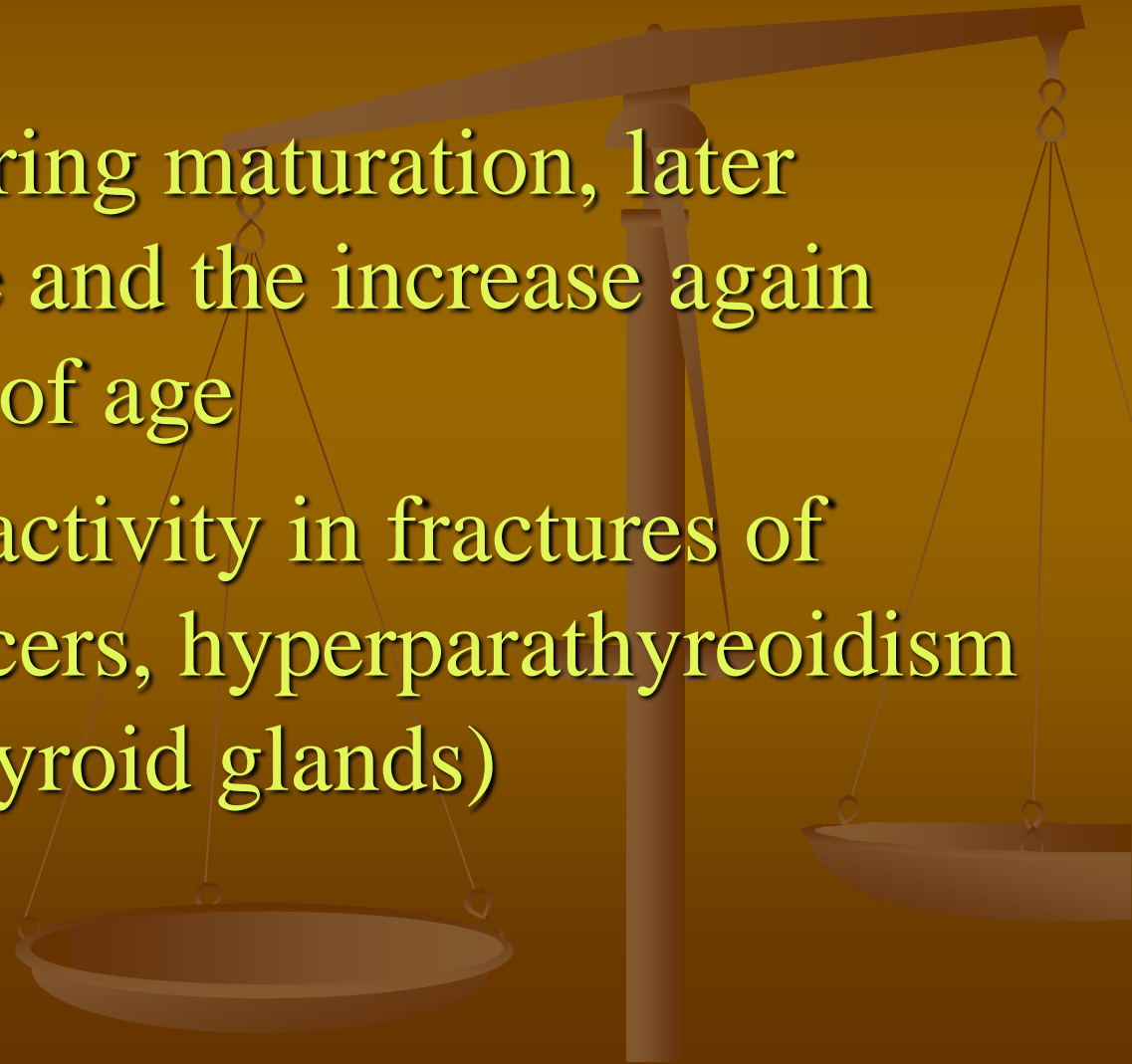


# Isoenzymes of alkaline phosphatase

- Intestinal – easily inactivated by high temperature (56°C during 10 min), inhibited by phenylalanine
  - Placental – the most resistant to heating (retains the activity during heating up to 65° C during 30 min, inhibited by phenylalanine
  - Liver – migrates the most quickly during electrophoresis followed by isoforms from bones, placenta and intestine
- 

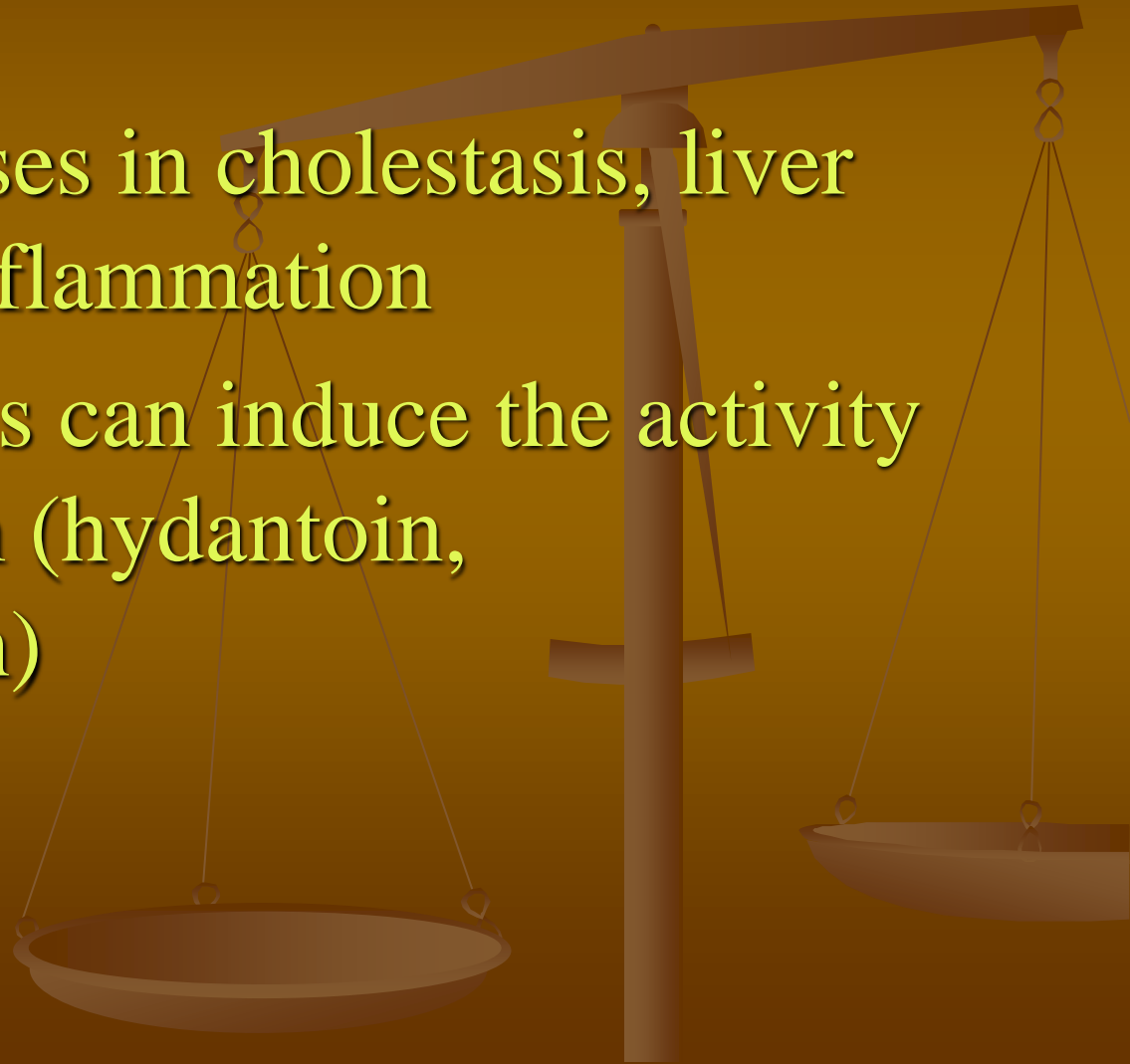
# Isoform from bones:

- High activity during maturation, later gradual decrease and the increase again around 50 years of age
- The increase of activity in fractures of bones, bone cancers, hyperparathyreoidism (tumor of parathyroid glands)

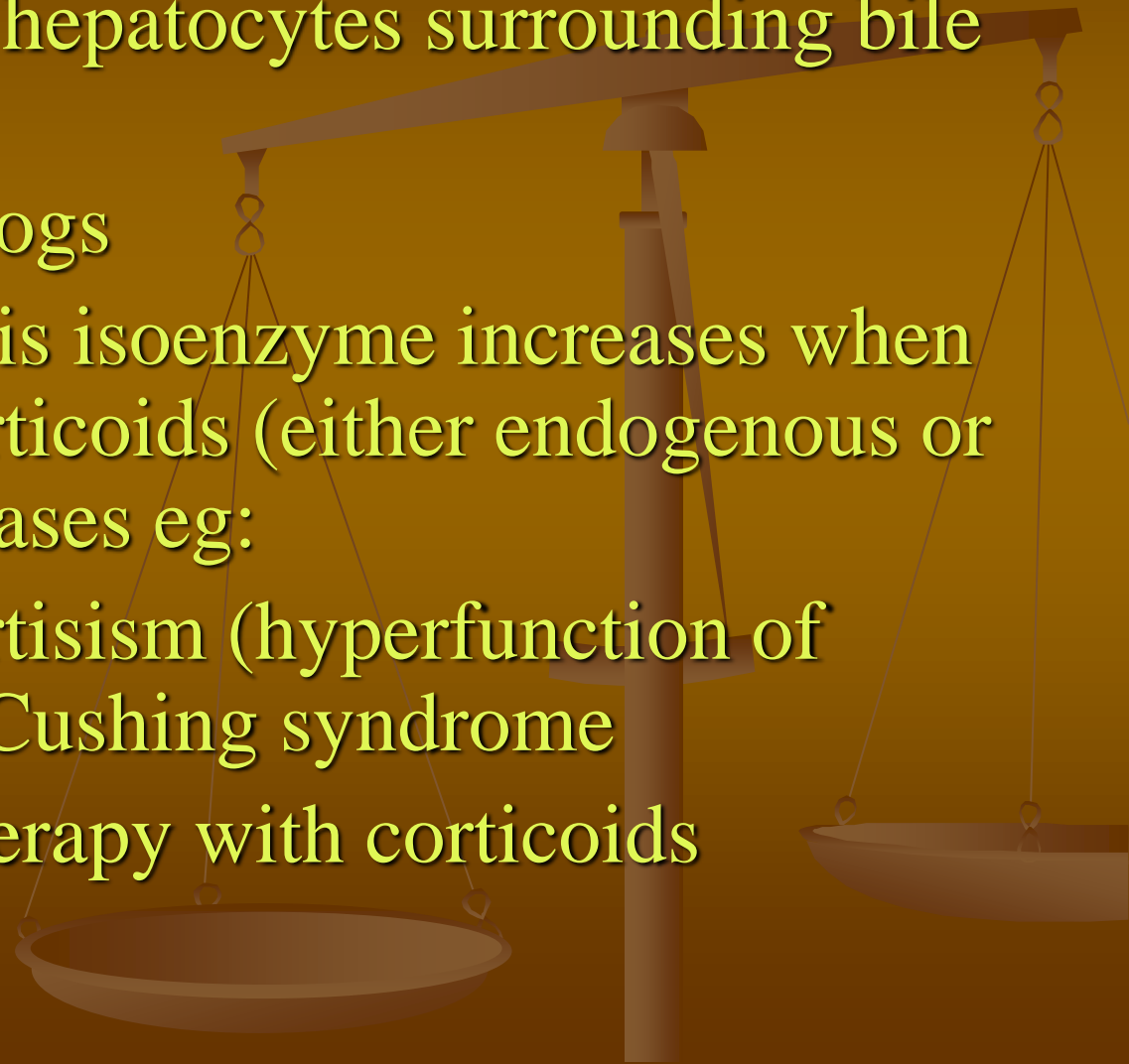


# Isoform from liver:

- Activity increases in cholestasis, liver tumors, liver inflammation
- Some medicines can induce the activity of liver isoform (hydantoin, chlorpromazin)

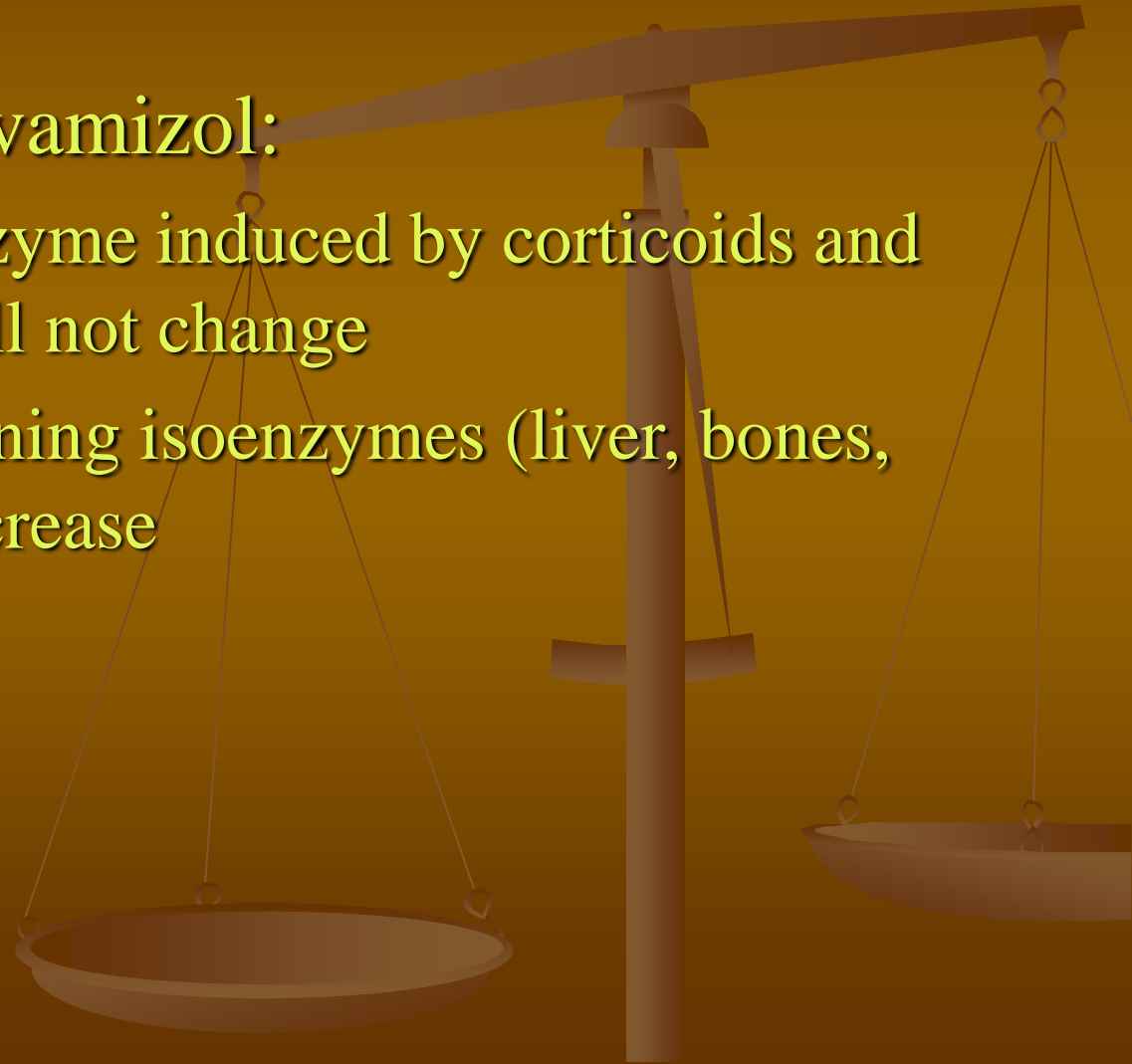


- The modification of liver isoform is isoenzyme induced by corticosteroids (C-ALP)
- It is produced by hepatocytes surrounding bile tracts
- It is detected in dogs
- The activity of this isoenzyme increases when the content of corticoids (either endogenous or exogenous) increases eg:
  - Hiperadrenocortisism (hyperfunction of adrenal cortex – Cushing syndrome)
  - Long lasting therapy with corticoids



# How to differentiate isoenzymes?

- Inhibition with levamisol:
  - Activity of isoenzyme induced by corticoids and from intestine will not change
  - Activity of remaining isoenzymes (liver, bones, placenta) will decrease

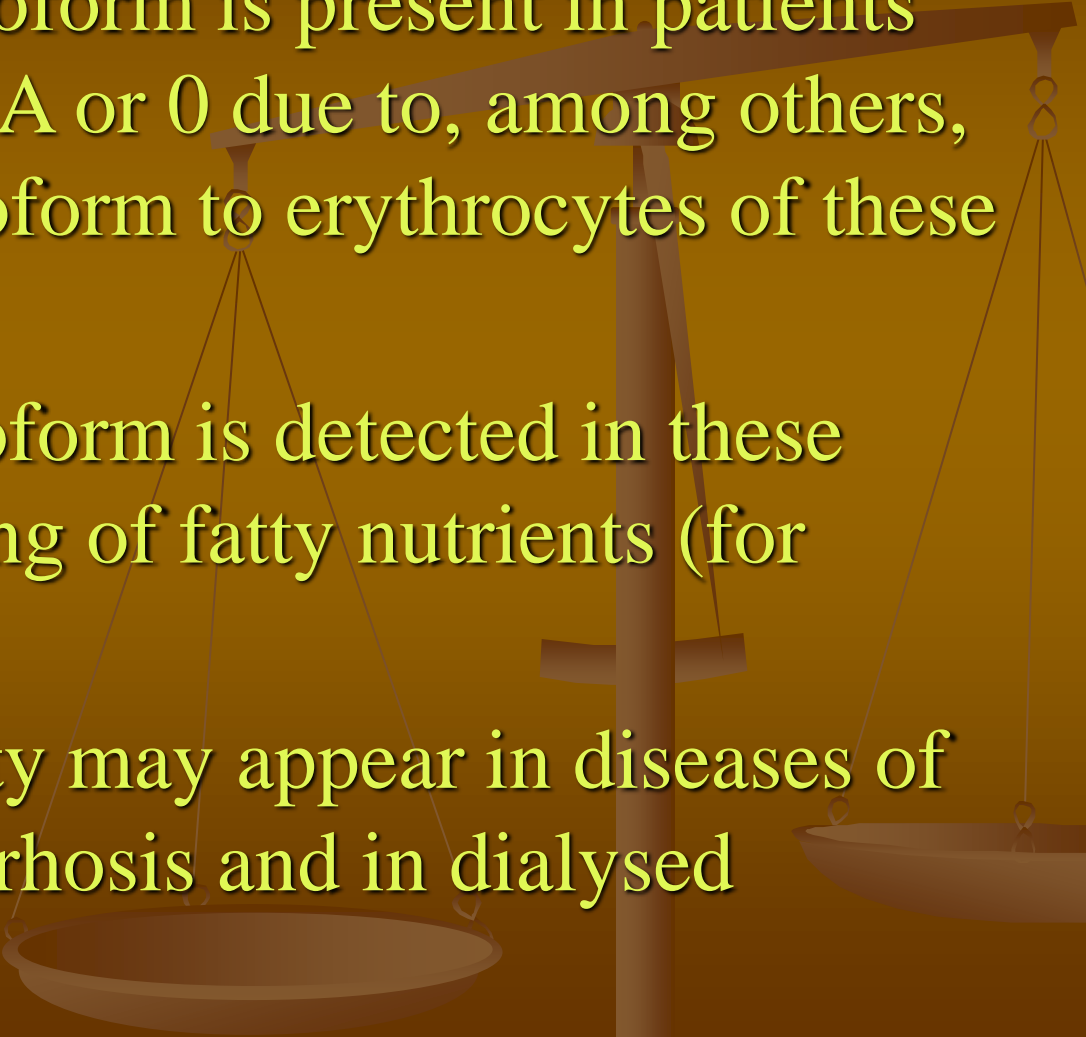


# Placental isoform:

- Activity increases slightly during physiological pregnancy while significantly during pathologies

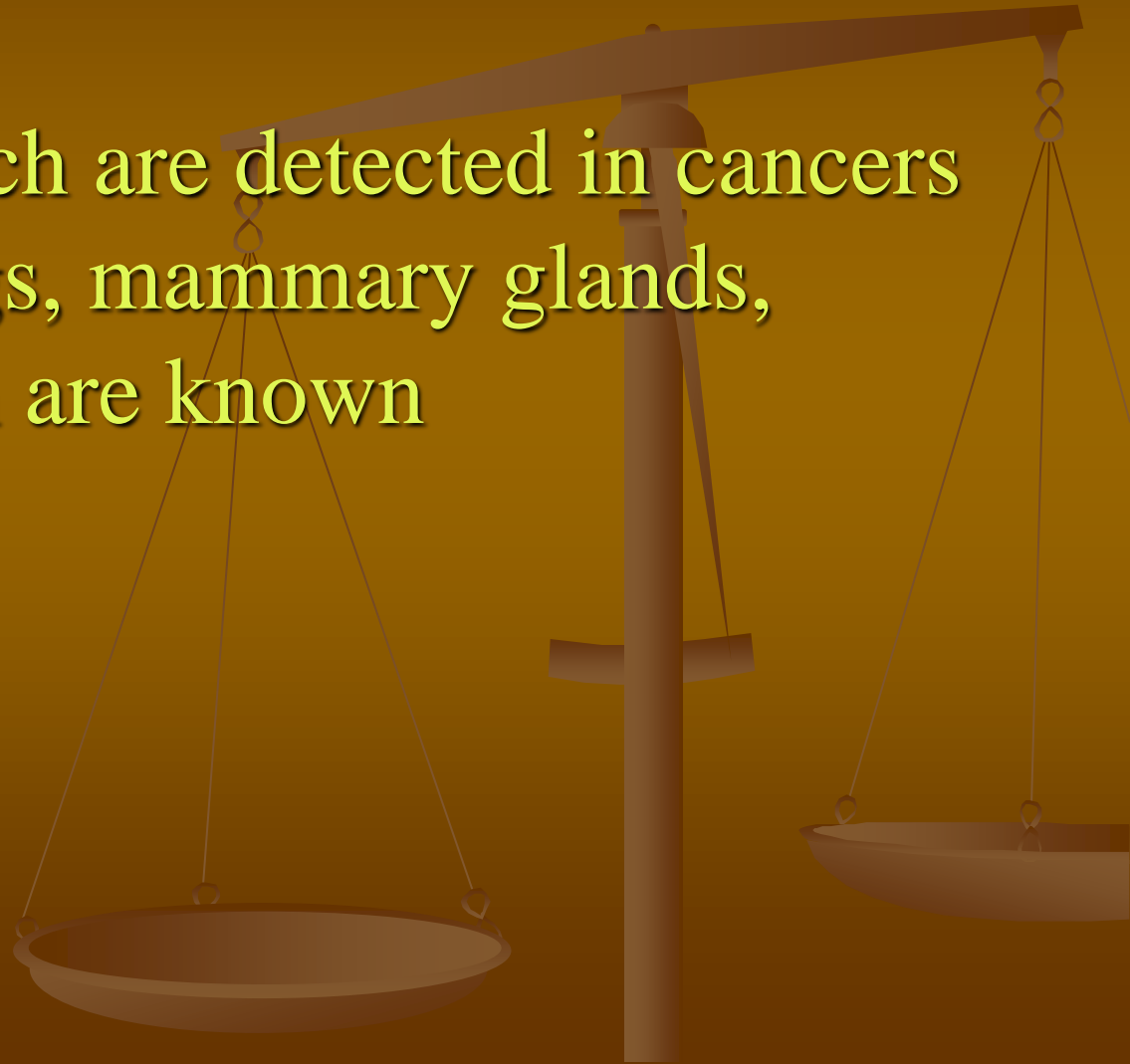


# Intestine isoform:

- Activity of this isoform is present in patients with blood group A or 0 due to, among others, binding of this isoform to erythrocytes of these blood groups.
  - More intestine isoform is detected in these patients after eating of fatty nutrients (for 25%)
  - Increase of activity may appear in diseases of intestine, liver cirrhosis and in dialysed patients.
- 

# Cancer-placental isoform :

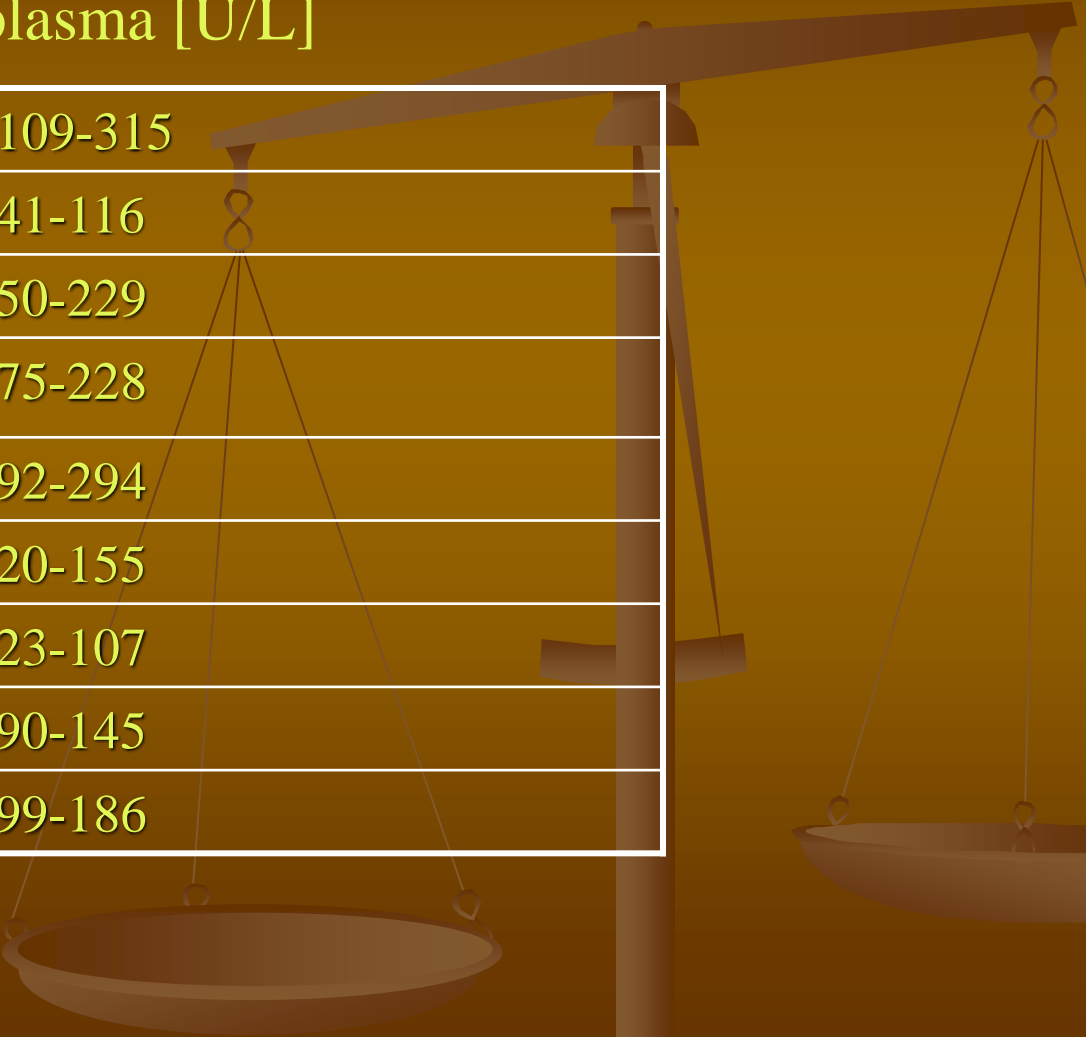
- 4 fractions which are detected in cancers of ovaries, lungs, mammary glands, pancreas, colon are known





# Reference values for alkaline phosphatase

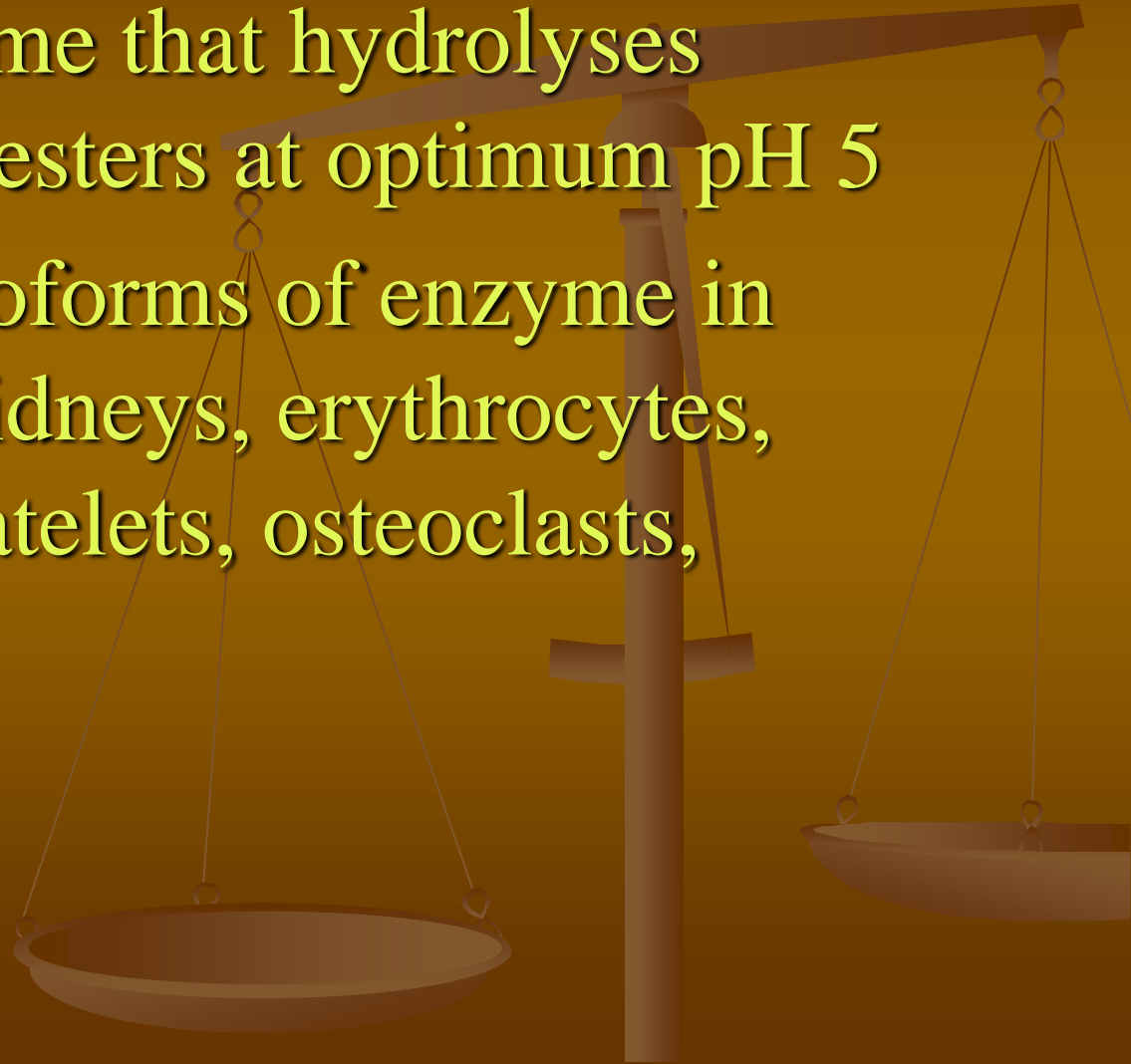
Activity of ALP in blood plasma [U/L]



|                |         |
|----------------|---------|
| Horses         | 109-315 |
| Cattle         | 41-116  |
| Sheep          | 50-229  |
| Goats          | 75-228  |
| Pigs           | 92-294  |
| Dogs           | 20-155  |
| Cats           | 23-107  |
| Rabbits        | 90-145  |
| Golden hamster | 99-186  |

# Acid phosphatase:

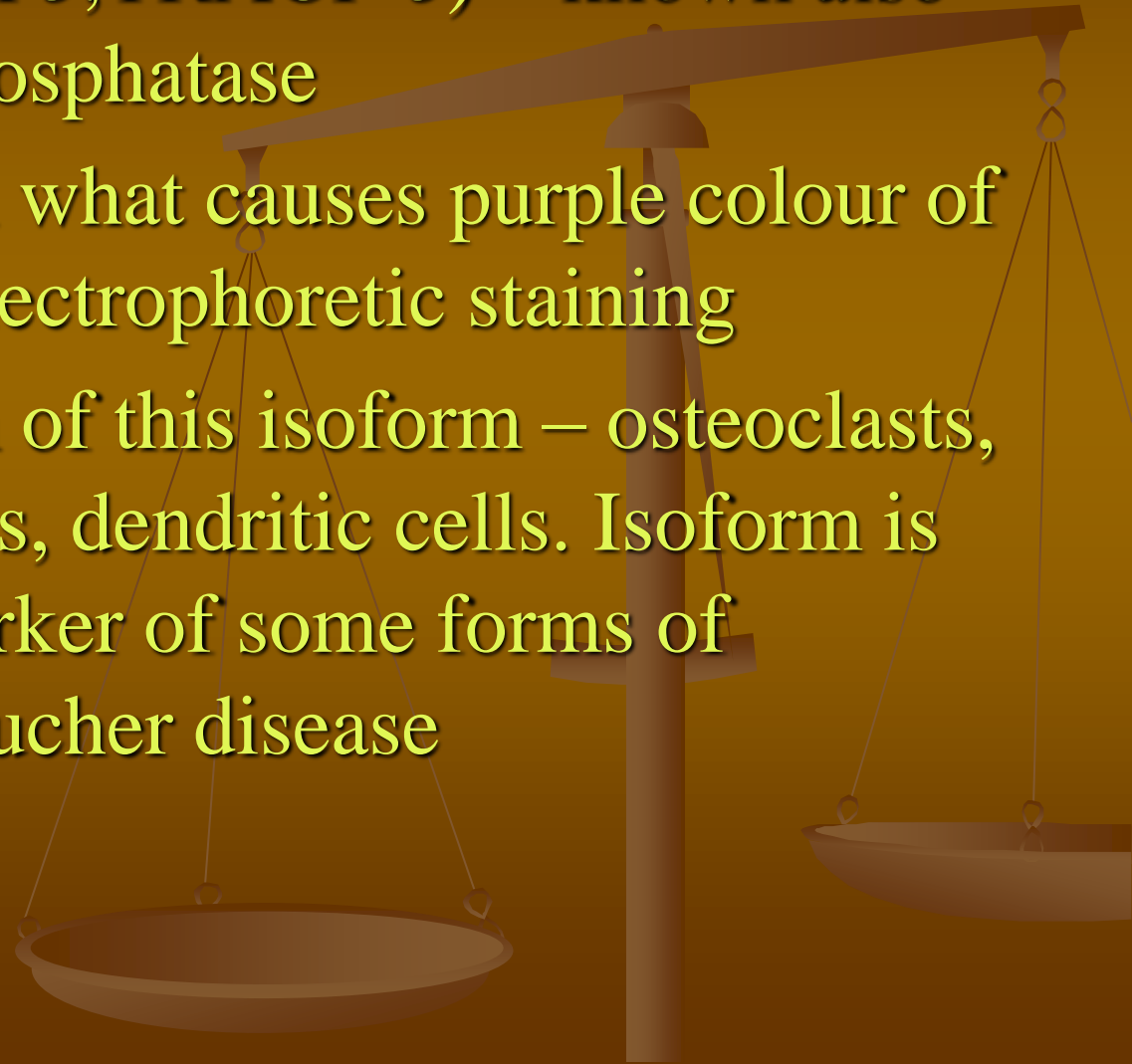
- Lysosomal enzyme that hydrolyses monophosphate esters at optimum pH 5
- There are few isoforms of enzyme in prostata, liver, kidneys, erythrocytes, spleen, blood platelets, osteoclasts,



# Isoenzymes of acid phosphatase

- Clinical meaning of prostata isoenzyme:
  1. Isoenzyme from prostata [1] (PAP, ACP3, ACPP) , covers 30% of total activity of acid phosphatase
  2. Is inhibited by tartrate
  3. Activity increases in the course of prostata cancer, myocardial infarction, thrombotic inflammation of veins
  4. Good marker for monitoring successful cancer therapy and renewal of cancer

- Isoenzyme [2] – tartrate – resistant acid phosphatase (ACP5,TRACP-5) – known also as purple acid phosphatase
- Contains iron ion what causes purple colour of fraction during electrophoretic staining
- Main localisation of this isoform – osteoclasts, lung macrophages, dendritic cells. Isoform is cytochemical marker of some forms of leukemia and Gaucher disease



# Reference values of acid phosphatase

Activity of ACP in plasma [U/L]

|      |        |
|------|--------|
| Dogs | 30-120 |
| Cats | 20-63  |

Half-life period of acid phosphatase is different in different animal species.

The change of activity in cats is detected after 6 hours while in dogs after 3 days.

# When the determination of acid phosphatase should be done?

- Suspicion of liver and bile tract diseases (inflammation of liver, differentiation of jaundice, damage to liver by drugs, obturation of bile tracts due to gall stones or cancer).
- Suspicion of diseases of bone system (pain of bones, pathological fractures in the course of cancers).
- Disturbances of calcium and vitamin D<sub>3</sub> balance (osteomalacia, hyperfunction of parathyroid glands, in case of symptoms like: deformities in bone system, radiographic and scyntigraphic alterations in bones).
- Suspicion of cancers that produce acid phosphatase.

# Indicative enzymes

Tabela 9.2. Klasyfikacja enzymów (1) używanych rutynowo w diagnostyce

| Klasa           | Nazwa rekomendowana                             | Nazwa zwyczajowa | Nazwa standardowa | Nazwa systematyczna   |
|-----------------|---|------------------|-------------------|---|
| oksydoreduktazy | dehydrogenaza mleczanowa                        | LDH              | LD                | oksydoreduktaza L-mleczan: NAD                              |
|                 | dehydrogenaza glukozy-6 fosforanowa             | G-6-PDH          | G6PD, GPD         | oksydoreduktaza D-glukozy-6-fosforan: NADP                  |
| transferazy     | aminotransferaza asparaginianowa                | GOT, AspAT       | AST               | L-asparagino: $\alpha$ -ketoglutaran aminotransferaza       |
|                 | aminotransferaza alaninowa                      | GPT, ALAT, SGPT  | ALT               | transferaza L-alanino-2-oxoglutaran                         |
|                 | kinaza kreatynowa                               | CPK              | CK                | fosfotransferaza ATP-kreatyna                               |
|                 | $\gamma$ -glutamylotranspeptydaza (transferaza) | GGTP             | GGT               | glutamylotransferaza glutamina: D-glutamyl                  |
|                 | acylotransferaza lecytyna: cholesterol          | LCAT             | LCAT              | lecytyna:cholesterol acyltransferaza                        |
| hydrolazy       | fosfataza alkaliczna                            | ALP              | ALP               | fosfohydrolaza monoestrów ortofosforanowych (pH alkaliczne) |
|                 | fosfataza kwaśna                                | ACP              | ACP               | fosfohydrolaza monoestrów ortofosforanowych (kwaśne pH)     |
|                 | $\alpha$ -amylaza                               | AMY              | AMS               | 4-glukanohydrolaza $\alpha$ -1,4 glukagonu                  |
|                 | lipaza triglicerydów                            | LPS, LP          | LPS               | acylohydrolaza triglicerydów                                |
|                 | cholinoesteraza                                 | CHE              | CHE               | acylohydrolaza acylocholinyl                                |

# Indicative, secretive and excretive enzymes

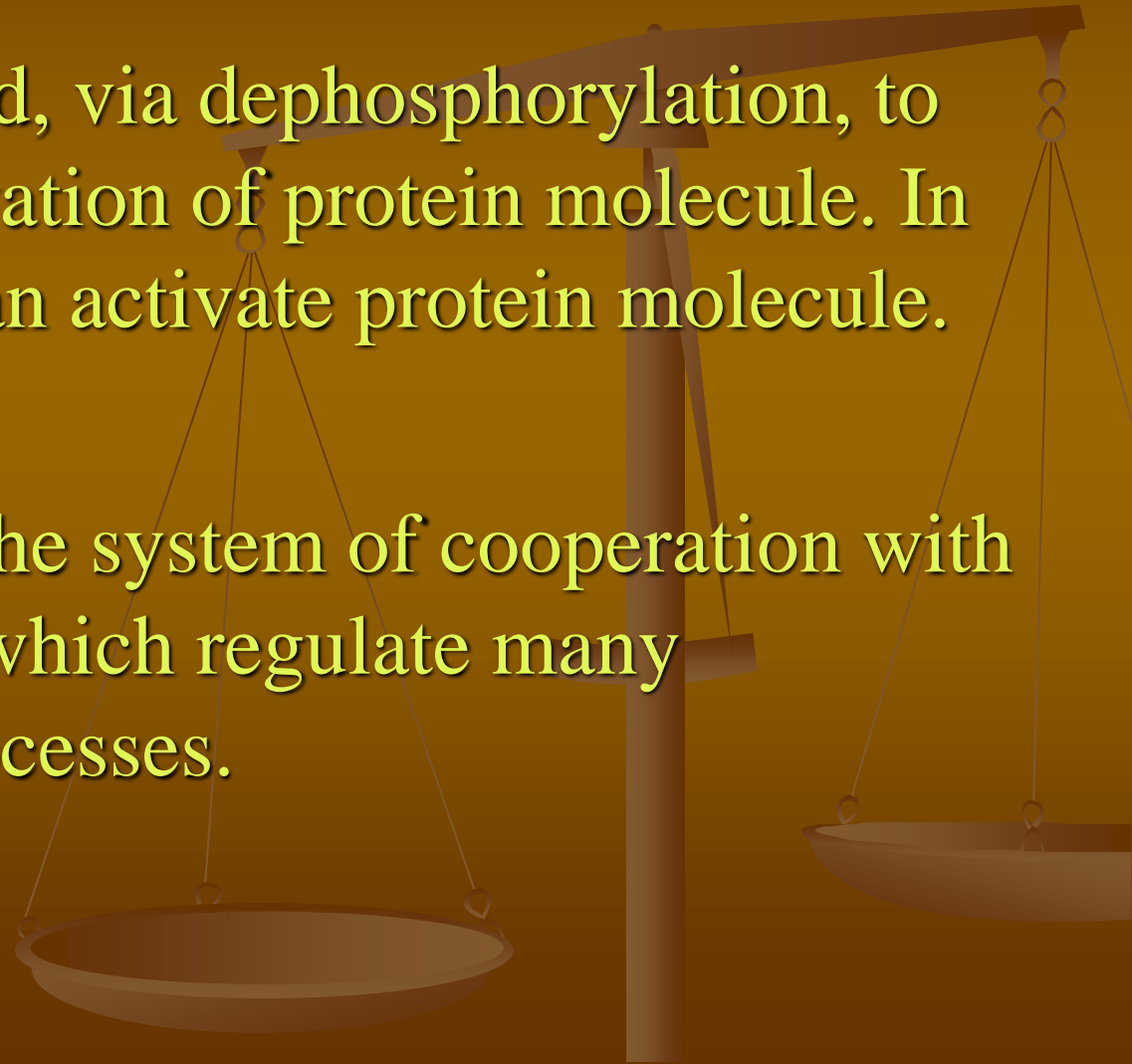
Tabela 9.3. Klasyfikacja (2) wg sposobu uwalniania enzymów używanych rutynowo w diagnostyce laboratoryjnej

| Nazwa                                 | Skrót      | Grupa                   |
|---------------------------------------|------------|-------------------------|
| dehydrogenaza mleczanowa              | LDH        | wskaźnikowy             |
| dehydrogenaza glutaminowa             | GLD        | wskaźnikowy             |
| aminotransferaza asparaginianowa      | AST        | wskaźnikowy             |
| aminotransferaza alaninowa            | ALT        | wskaźnikowy             |
| kinaza kreatynowa                     | CK         | wskaźnikowy             |
| $\gamma$ -glutamylotranspeptydaza     | GGTP (GGT) | wskaźnikowy/ekskrecyjny |
| fosfataza alkaliczna                  | ALP        | wskaźnikowy/ekskrecyjny |
| fosfataza kwaśna                      | ACP        | wskaźnikowy/ekskrecyjny |
| $\alpha$ -amylaza                     | AMS        | ekskrecyjny             |
| lipaza                                | LPS, LP    | ekskrecyjny             |
| cholinoesteraza                       | CHE        | sekrecyjny              |
| acylotransferaza lecytyna:cholesterol | LCAT       | sekrecyjny              |



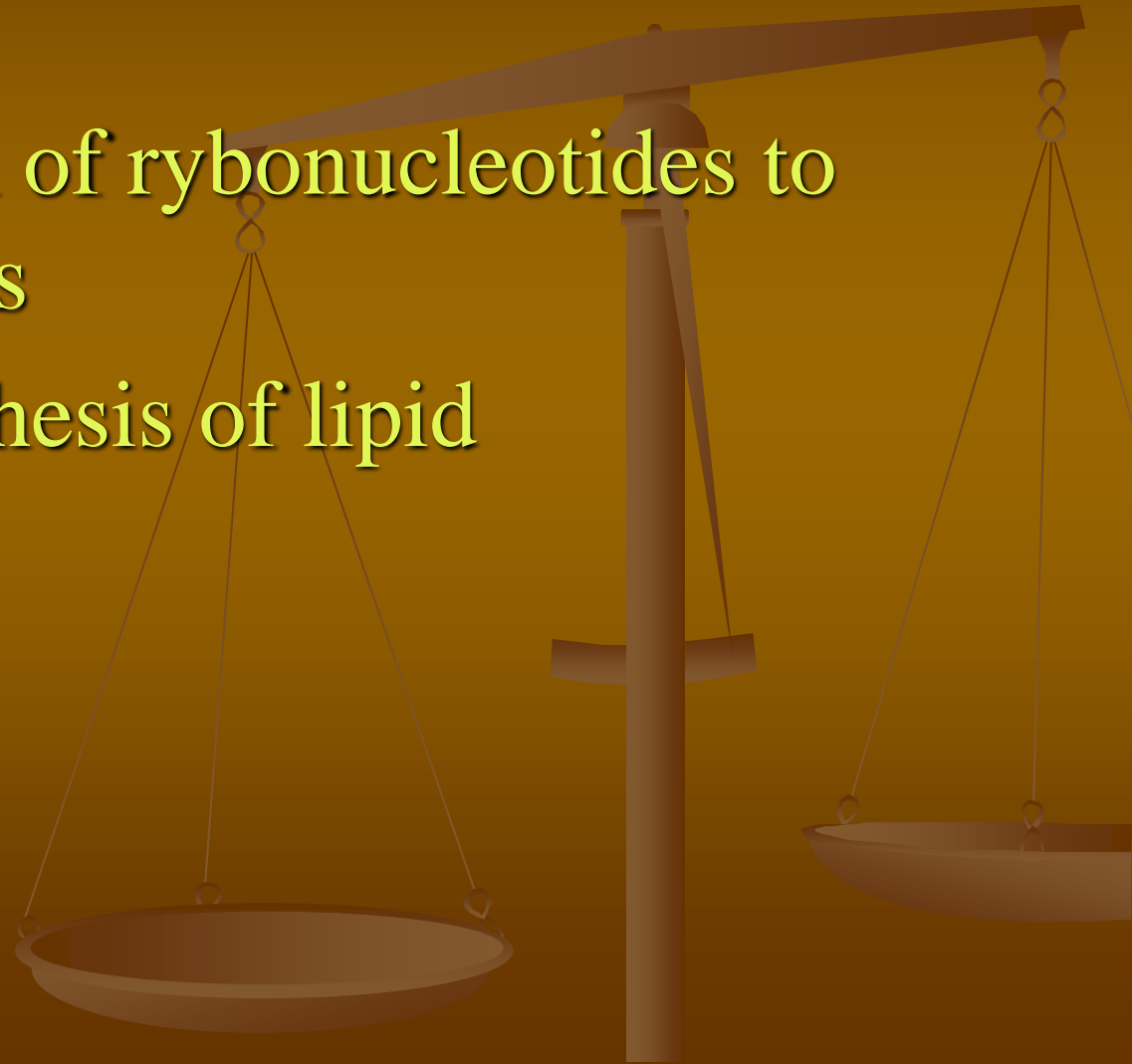
# Protein phosphatases

- Enzymes that lead, via dephosphorylation, to biological inactivation of protein molecule. In rare cases they can activate protein molecule.
- Enzymes create the system of cooperation with phosphokinases which regulate many physiological processes.



# Phosphatases

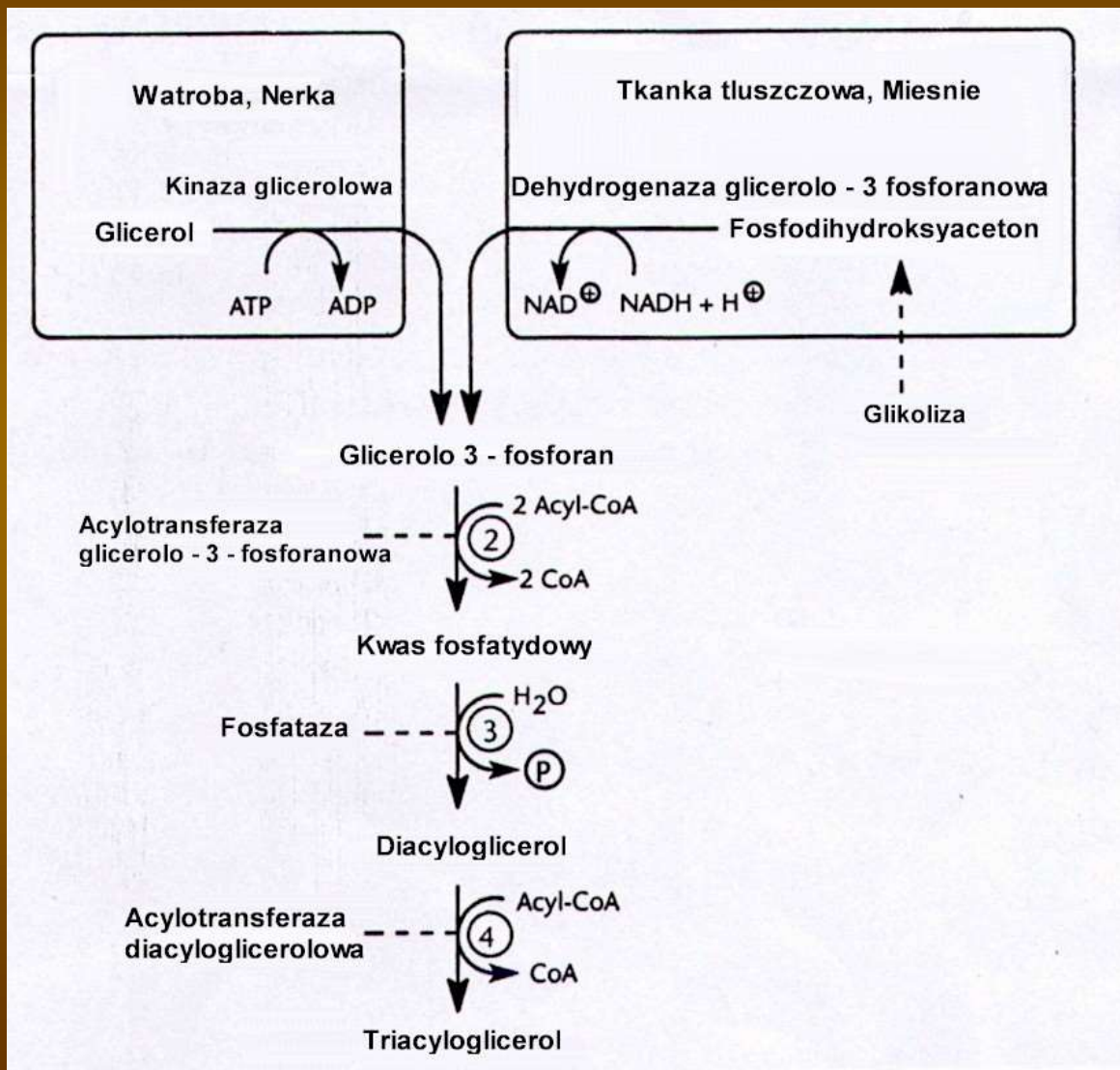
- The conversion of ribonucleotides to ribonucleosides
- During biosynthesis of lipid



# Biosynthesis of lipids

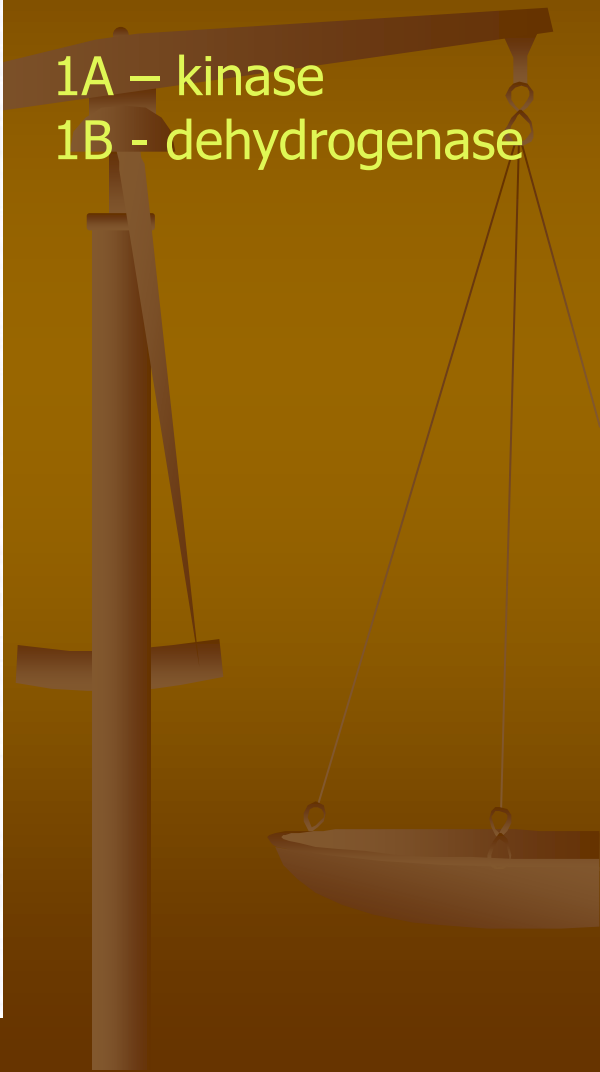
1 A

1 B



1A – kinase

1B - dehydrogenase



## Exercise 1

Phosphatase hydrolyzes disodium phenylphosphate. The amount of liberated phenol (the measure of enzyme activity) is determined colorimetrically with Folin-Ciocalteu reagent.

Unit of King-Armstrong is expressed in mg of phenol which is liberated from disodium phenylphosphate by enzyme in 100 ml blood plasma or plasma of semen in temperature 37°C during 15 minutes and pH=10 for alkaline phosphatase.

Protocol:

| Test tube                          | 0                 | 1                   | 2                   |
|------------------------------------|-------------------|---------------------|---------------------|
| Substrate for Alkaline phosphatase | 2 cm <sup>3</sup> | 2 cm <sup>3</sup>   | 2 cm <sup>3</sup>   |
| Blood Plasma                       | -                 | 0.2 cm <sup>3</sup> | 0.2 cm <sup>3</sup> |

Incubation for 30 minutes in 37°C.

|               |                     |                     |                     |
|---------------|---------------------|---------------------|---------------------|
| Blood Plasma  | 0.2 cm <sup>3</sup> | -                   | -                   |
| Folin reagent | 1.8 cm <sup>3</sup> | 1.8 cm <sup>3</sup> | 1.8 cm <sup>3</sup> |

The centrifugation in a laboratory centrifuge for 15 min.

|                                     |                   |                   |                   |
|-------------------------------------|-------------------|-------------------|-------------------|
| Supernatantant                      | 2 cm <sup>3</sup> | 1 cm <sup>3</sup> | 2 cm <sup>3</sup> |
| 15% Na <sub>2</sub> CO <sub>3</sub> | 2 cm <sup>3</sup> | 2 cm <sup>3</sup> | 2 cm <sup>3</sup> |

Incubation for 10 minutes in 37°C.

Read the absorbance of samples 0,1,2 against distilled water at a wavelength of 600 nm.

**Calculations:**

Calculation of enzyme activity:

$$\Delta E = E_B - E_K$$

$$\Delta E \times 1,6 \times 100 \times 7,09 = 1134,4 \times \Delta E = \text{Iu/l}$$