Analysis of plasma enzymes

Tamás Kőszegi

Department of Laboratory Medicine

Biochemical properties of enzymes

Enzymes are proteins!

Activity: **post-translational modification**

prosthetic groups

activation mechanisms (proteolysis, carboxylation, etc)

inactivation mechanisms

(proteolysis, anti-protease, etc)

Plasma enzymes by their origin

Origin: cell constituent (transaminases, glycolytic enzymes, creatine kinase, etc.)

product (coagulation factors, pseudo-cholinesterase, amylase, etc.)

Activity change due to tissue damage

cell constituent - increase

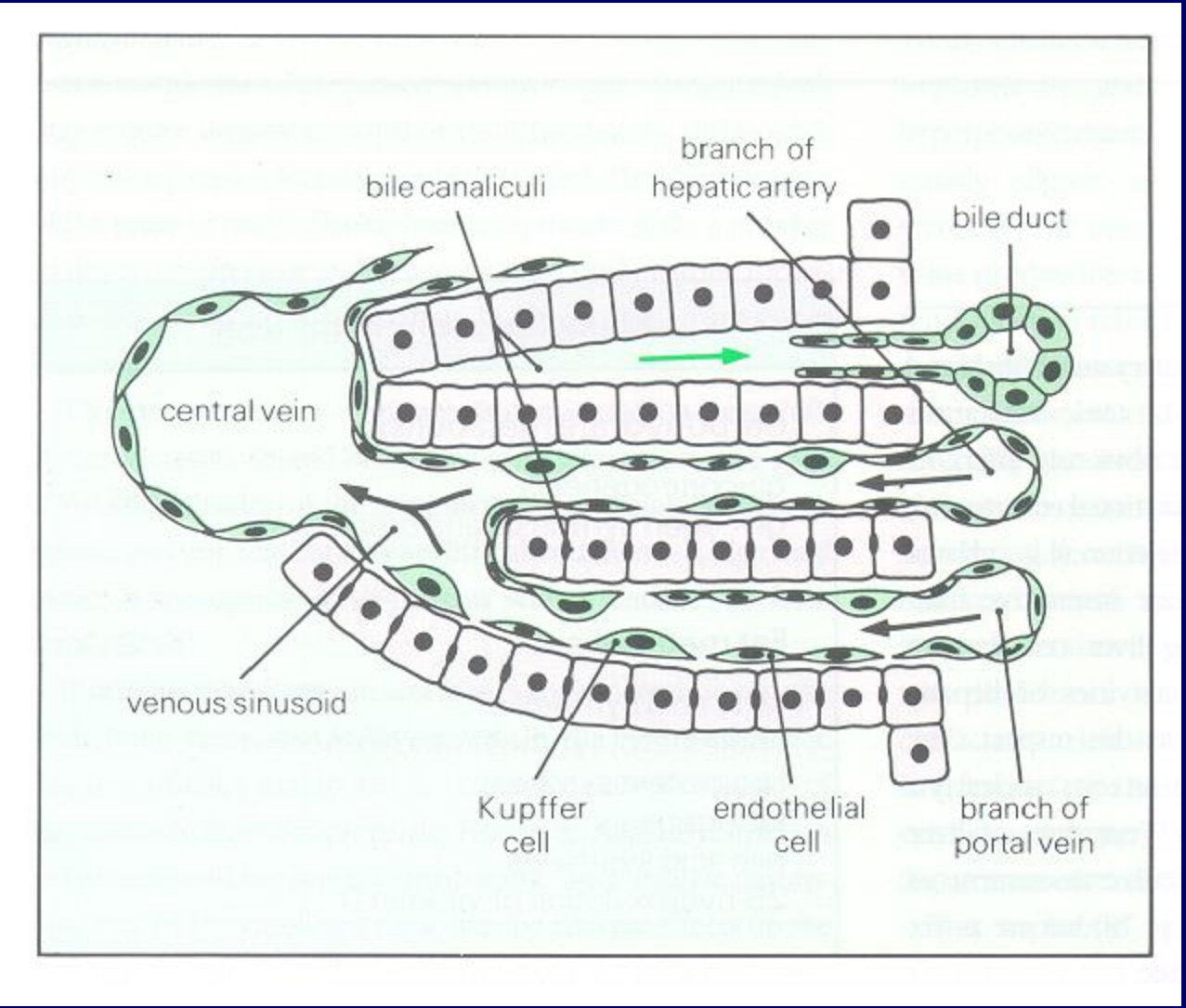
product - decrease

Why we find enzymes in the plasma of healthy individuals?

- **Exert their function in the plasma (products)**

- **Due to normal cell turnover (apoptosis, renewal) they are released into the plasma (product + cell constituent)**

Ultrastructure of liver and pathways for enzymes to get into the circulation



Factors influencing the reference range of a parameter

- Speed of synthesis - secretion
- Blood supply
- Cell turnover - age!
- Presence of isoenzymes
- Pregnancy
- Sex
- ?????

Classification of enzymes

Oxidoreductases (EC Class 1)
Transfer electrons (RedOx reactions)
Transferases (EC Class 2)
Transfer functional groups between molecules
Hydrolases (EC Class 3)
Break bonds by adding H2O
Lyases (EC Class 4)
Elimination reactions to form double bonds
Isomerases (EC Class 5)
Intramolecular rearrangements
Ligases (EC Class 6)
Join molecules with new bonds

Different ways to detect enzymes

Enzymes are proteins!

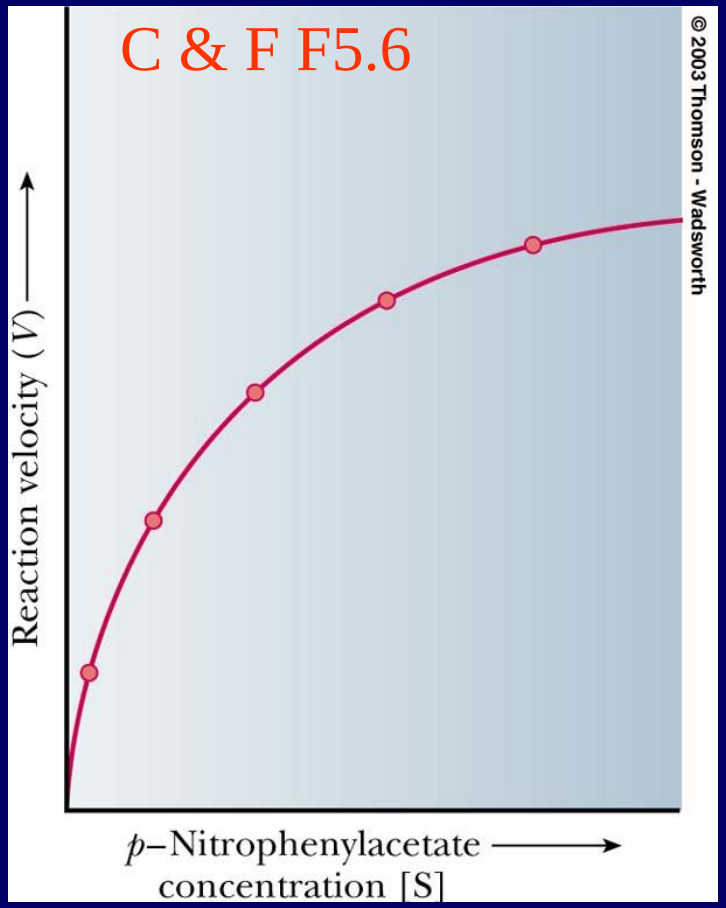
Protein specific antibody (mass/volume)

does not say anything on activity!

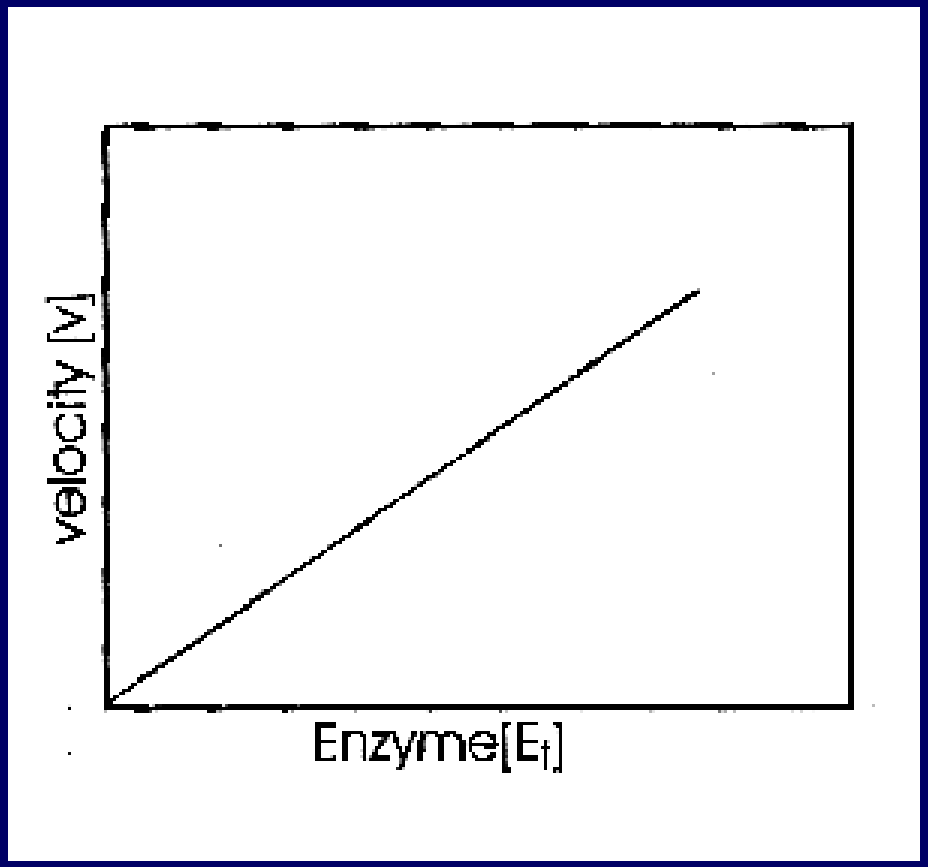
Activity – functional probe (substrate, indicator reaction, IU/l or kat/l)

does not say anything on concentration!

A “simple” enzymatic reaction



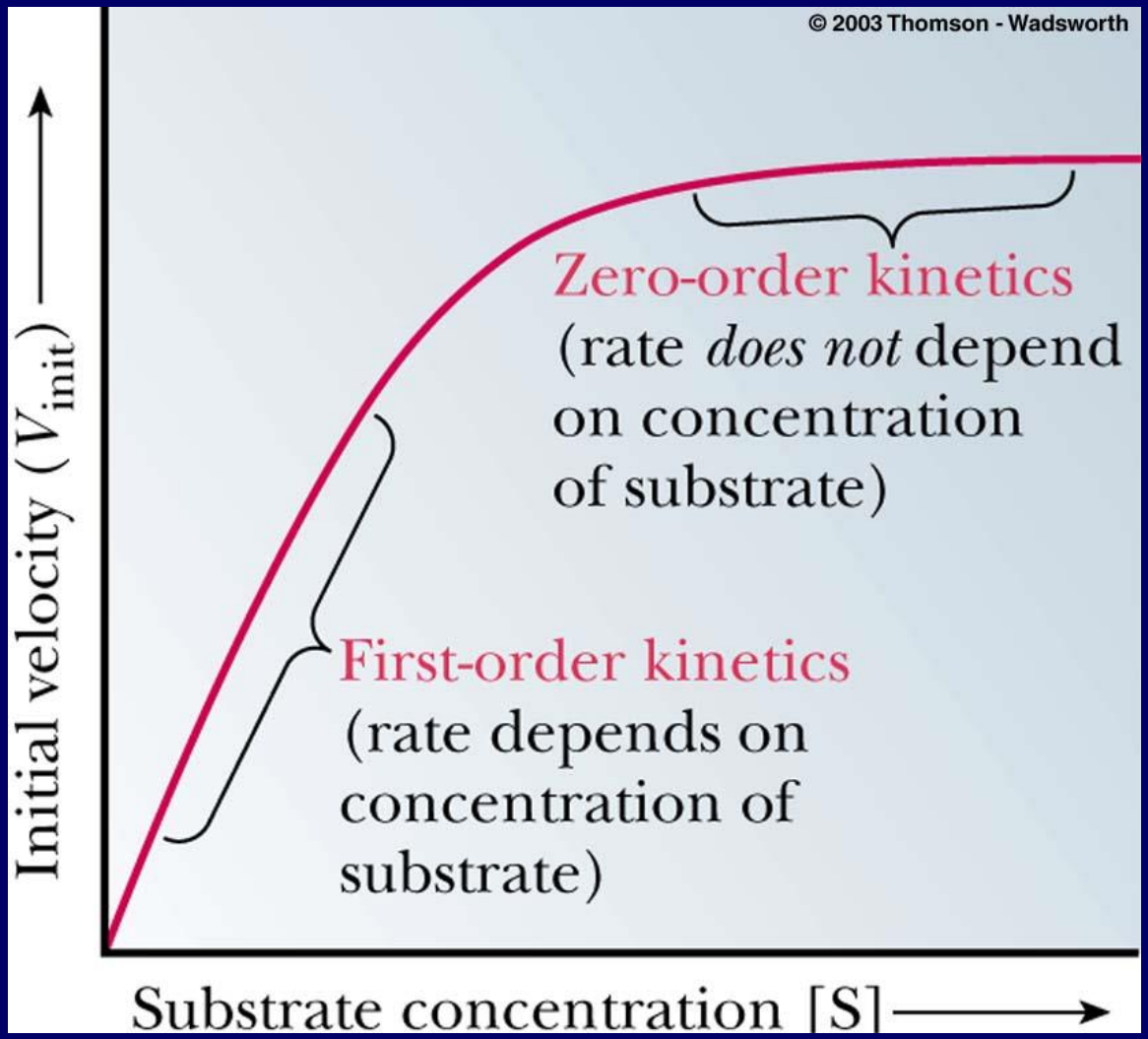
A plot of velocity, v, versus substrate [S] is a rectangular hyperbola (may be sigmoidal for a complex enzyme)



A plot of velocity, v, versus enzyme concentration, [E] is linear – *we say the reaction is 1st order in E*

v versus [S] for an enzyme reaction

C & F 5.8



[E] is held constant. Shows how nature of kinetics changes as [S] increases

The Michaelis-Menten Equation

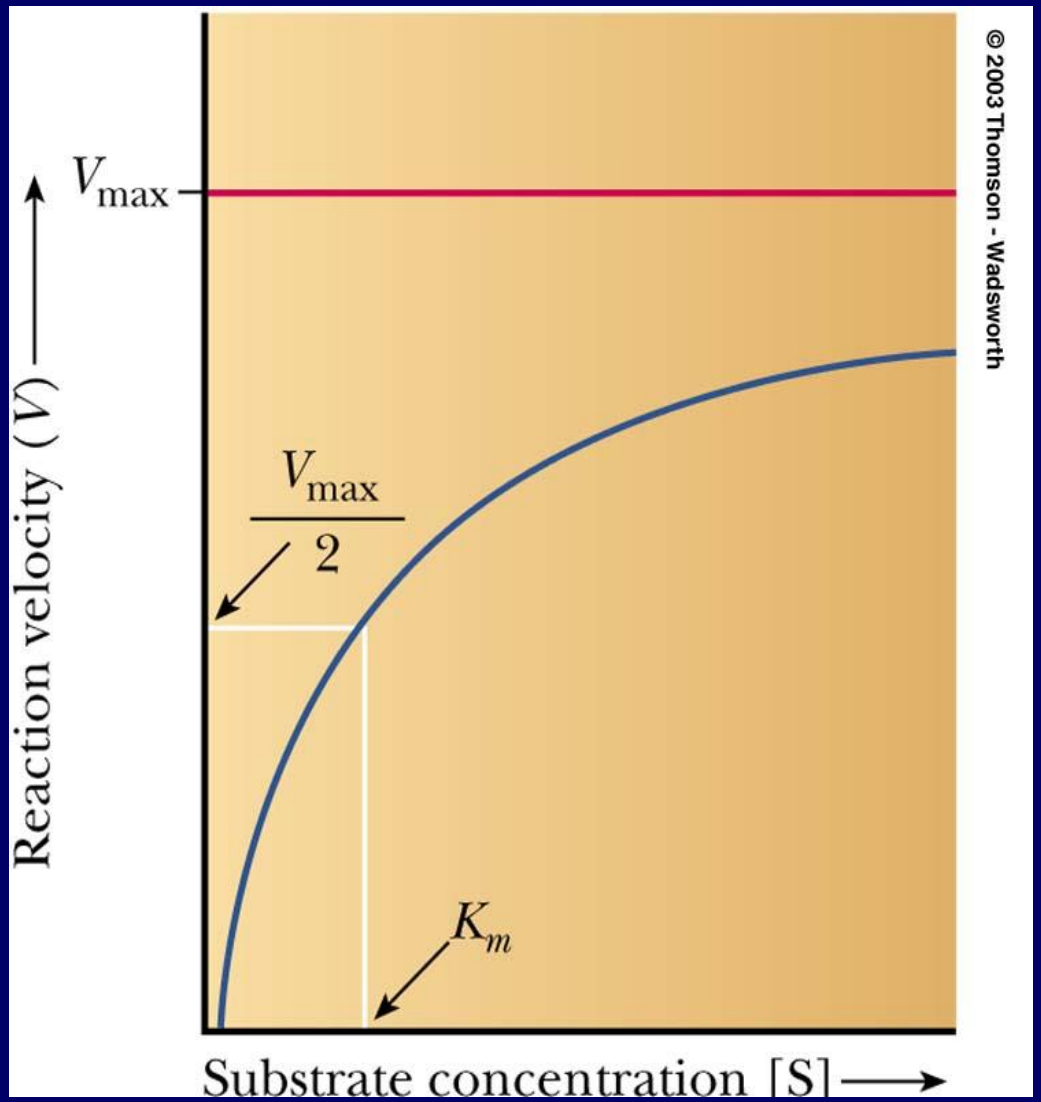
$$v = \frac{V_{max}[S]}{K_m + [S]}$$

K_m is the Michaelis constant.
V_{max} is the maximum velocity
[S] is the substrate concentration
Fits the hyperbolic shape of curve

Trivial understanding of K_m

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

- Look at what happens when $v = V_{max}/2$:
  - $V_{max}/2 = V_{max}[S]/(K_m + [S]$
  - or $K_m + [S] = 2[S]$
  - $K_m = [S]$
- In other words we may think of K_m as the substrate concentration at half maximal velocity



C & F 5.9

The dual nature of the Michaelis-Menten equation

Combination of 0-order and 1st-order kinetics

- When [S] is low, the equation for rate is 1st order in S – linear relation between [S] and v
  - $v = (V_{max}/K_m)[S]$ = constant x [S]
- When [S] is high, the equation for rate is 0-order in S: v is independent of [S]
  - $v = V_{max}$ = constant

General requirements for measuring enzymatic activity

Activity measurement: optimized, kinetic method!

$S >> K_m$ (approx. 20x)

pH, cofactors, temperature

Indicator reaction, coupled reaction,

kinetic photometric detection, linearity check

0-order reaction!

Why do we measure plasma enzyme activities?

- To detect tissue damage
- To assess extent of tissue damage
- To localize the damaged tissue
- To show effects of inhibitors, drugs, and toxins
- To assess the function of different organs
- To monitor therapeutic efficiency

Probing of product-type enzymes

- **Coagulation factors: complicated cascade, functional/activation probe/monitoring**
- **Pseudo-cholinesterase: reduced synthesis inhibition (intoxication)**
- **Digestive enzymes (lipase, amylase, trypsin)**

The pancreas paradox!

The pancreas paradox!
Acute injury (inflammation)

- **Indicates tissue damage? - yes**
- **Indicates extent of tissue damage? - no**
- **Reflects origin of tissue damage? – y/n**

analysis of isoenzymes!

- **Monitoring therapy? – y/n**
- **Additional tests? - necessary**
- **Urine enzyme tests? – y/n, macroamylase**

Probing of intracellular constituent-type enzymes

- Indicates tissue damage? - yes
- Indicates extent of tissue damage? – y/n
- Reflects origin of tissue damage? – partially
  analysis of isoenzymes!
- Monitoring therapy? – yes
- Additional tests? - necessary
- Timing of sample collection? – important
- Assessment of organ function? – partially

Factors influencing the reference range of a parameter (reminder)

- Speed of synthesis - secretion
- Blood supply
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- Pregnancy
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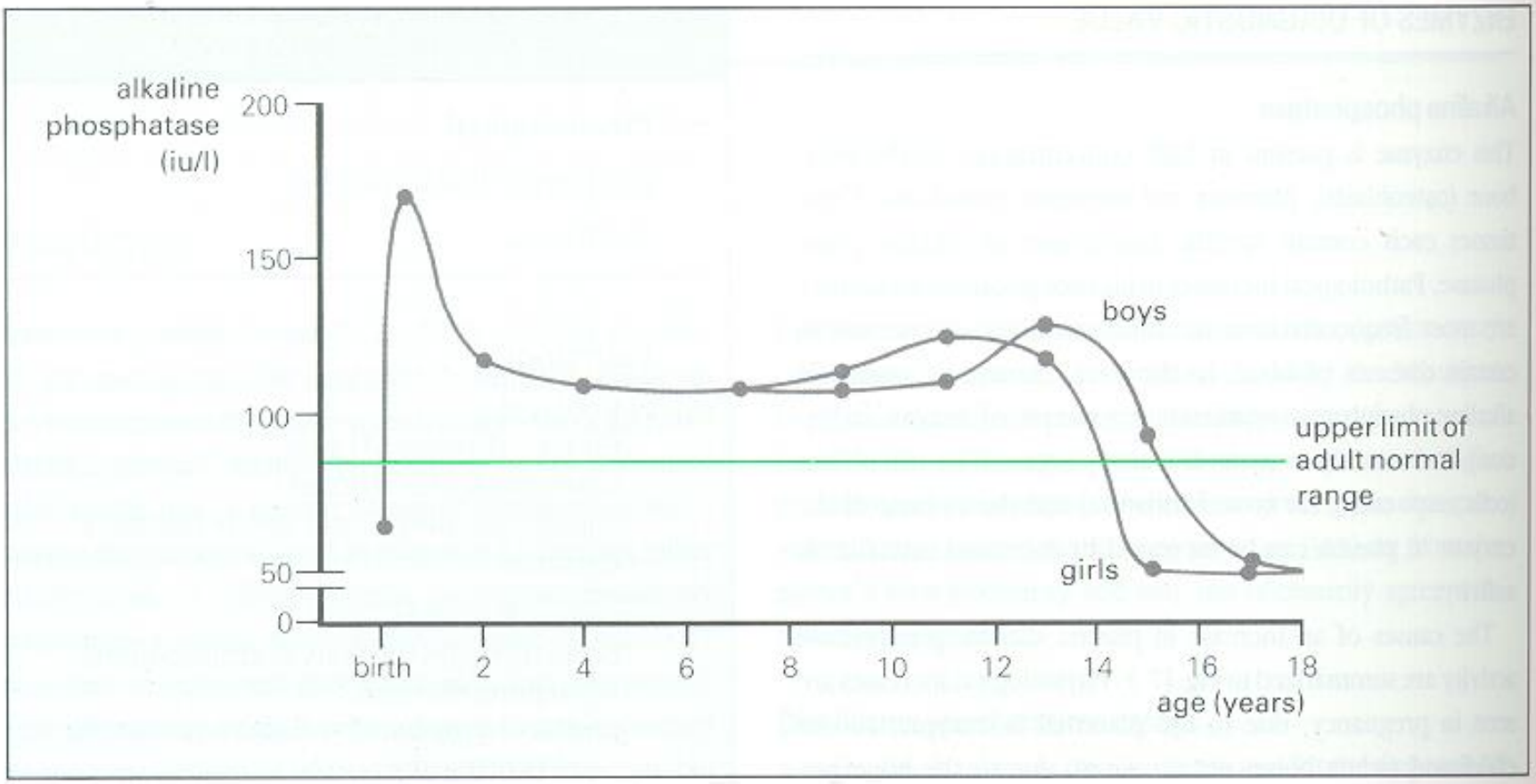
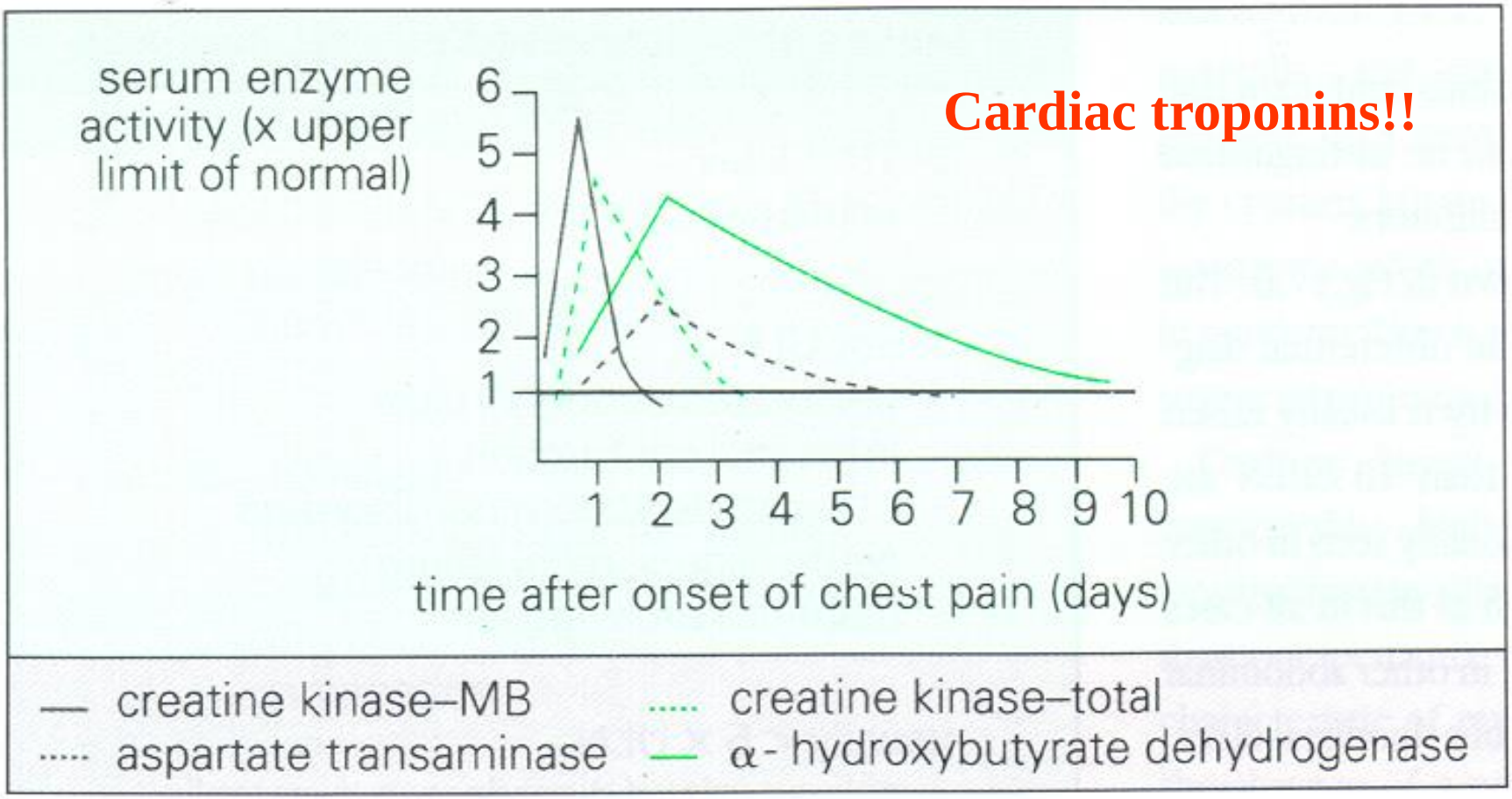
Reference range vs. age in healthy individuals: alkaline phosphatase



Factors influencing speed of plasma appearance of intracellular enzymes under pathological conditions

- Cell turnover (e.g. malignant tumor)
- Energetical state of the cells (lack of oxygen, metabolic block, etc.)
- Tissue vascularization
- Compartmentalization of enzymes inside the cells
- ?????

Monitoring of tissue damage: heart infarct



Analytical procedures for isoenzyme testing

- **Electrophoretic separation - detection**

- **Utilization of substrate preference**
- **Specific inhibition: by antibodies (CK-MB)**
  by heat, lectins (AP)
- **Quantitative protein determination:**
  immuno assays (AP, PSA, NSE)

Alkaline phosphatase isoenzymes