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Enzyme Analysis

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Introduction

1. Enzymes are synthesized by the cells of all living organisms. Almost all of enzymes are proteins.
2. They act like catalysts and accelerate the substrate which are metabolic reactions that life depends on.
3. Their catalytic activity depends on the precise conformational structure in the folded polypeptide chains.

Practical Examination 2

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Enzyme Activity Determination

Assay the activity of alanine aminotransferase (ALT) in serum (Mohun's Method)

Enzyme Activity Determination

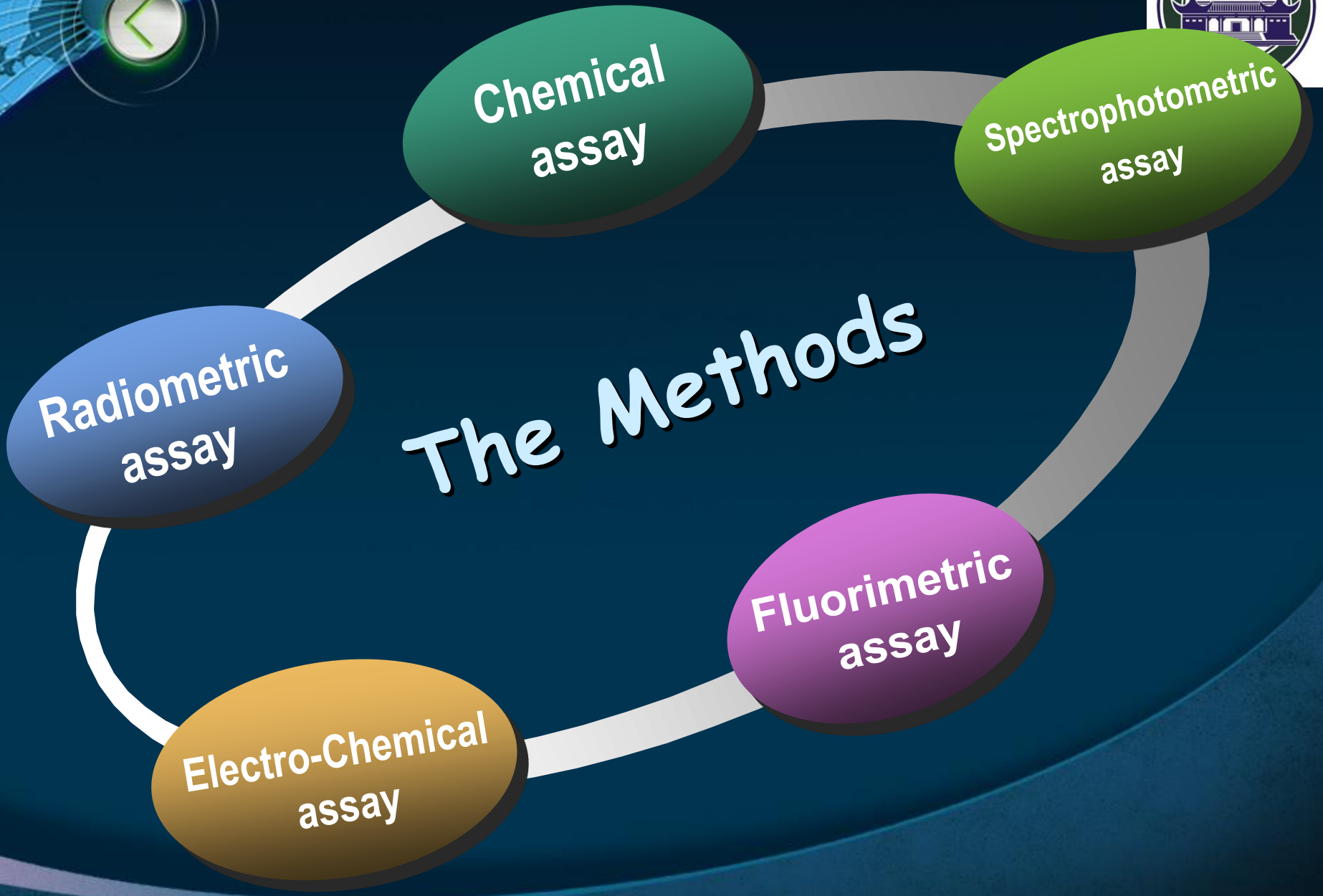
1. Enzyme Activity

The ability of an enzyme to catalyze a specific reaction and a measure of quantity of enzyme present.

2. Determination of Enzyme Activity

The enzyme activity is proportional to the velocity of enzyme-catalyzed reaction, which can be measured as the consumption of substrate or the accumulation of product per unit time under special conditions.

The Methods


Chemical assay
Spectrophotometric assay
Fluorimetric assay
Electro-Chemical assay
Radiometric assay

A unit of enzyme activity **(U)**:

is the amount of enzyme activity which will catalyze the transformation of 1 micromole of the substrate per minute under standard conditions. The unit has a symbol "U".

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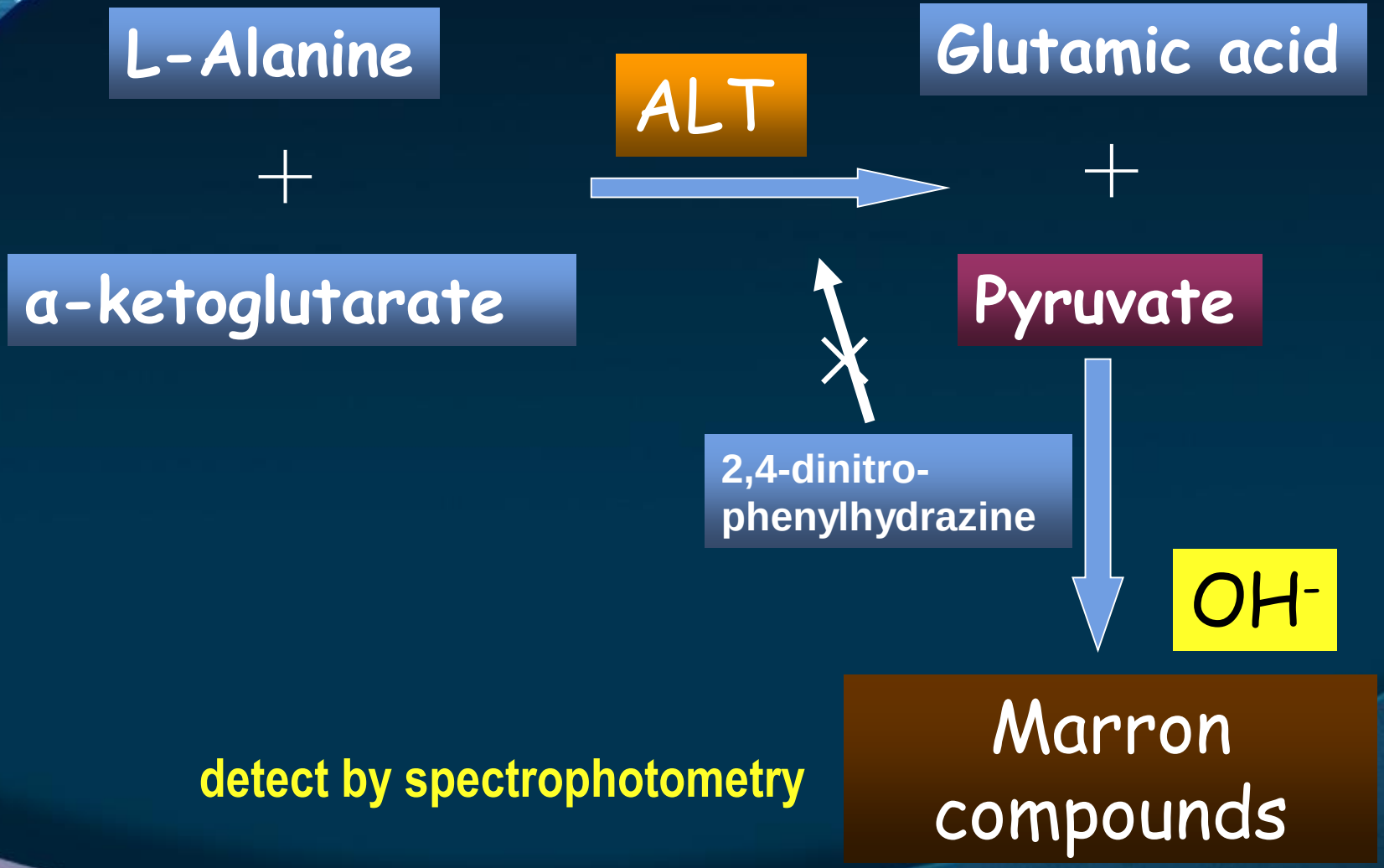
Assay the activity of alanine aminotransferase (ALT) in serum (Mohun's Method)

Introduction

- Activity of serum ALT was determined colorimetrically according to Mohun (1957).
- **Alanine transaminase** or **ALT** is an enzyme that can catalyze the transamination between L-alanine and α-ketoglutarate.
- It is found in serum and in various tissues but is most commonly associated with the liver. The quantitative of serum ALT reflect the damage of liver cell.

Colorimetric determination



2,4-dinitro-phenylhydrazine is added for stopping the reaction and marron compounds are formed which response to α-ketoacid. The absorbance at 520 nm of the product formed from pyruvate is bigger than from α-ketoglutarate. So we can detect the activity of ALT by spectrophotometry.

Procedure

Accurately pipette into 4 tubes respectively

ml	Test (1)	Test Blank (2)	Standard (3)	Standard Blank (4)
Substrate buffer	0.5	0	0.5	0.5

Put the tubes into water bath at 37°C for 2 min

Serum	0.1	0.1	0	0
Pyruvate(200μg/ml)	0	0	0.1	0
Phosphate buffer	0	0	0	0.1

Mix the tubes sufficiently, and put into water bath at 37°C for 30 min

(2)

	(1)	(2)	(3)	(4)
2,4-dinitro-phenylhydrazine	0.5	0.5	0.5	0.5
Substrate buffer	0	0.5	0	0

Mix the tubes, and put into water bath at 37°C for 20 min

0.4 mol/L NaOH	5.0	5.0	5.0	5.0

Mix the tubes, after 10 min at room temperature, read A_{520} within 30 min using diH_2O adjusting A to zero.

Calculation

$$\text{ALT enzyme activity} = \frac{A1-A2}{A3-A4} \times \frac{20}{2.5} \times \frac{1}{0.1}$$

Mohun's Unit

One Unit of ALT activity in serum is defined as the amount of enzyme needed to produce 2.5μg of pyruvate per ml serum after it is incubated with the substrate at 37℃, pH 7.4 for 30 min.

Clinical Significance

The plasma concentration of most enzymes remains constant in a normal individual. It will be altered if there is:

a) change of synthesis of enzymes within the cell;

b) cellular damage;

c) change in the size of enzymes forming tissue ;

d) an alteration in the rate of inactivation and disposal of enzymes;

e) an obstruction to a normal pathway of enzyme excretion.

Clinical Significance

Measurement of serum levels of numerous enzymes is of diagnostic significance. their catalytic activities may serve as qualitative or quantitative indexes of tissue damage.

it may be useful to:

a) assess the severity of the organ damage;

b) differentiate a particular type of disease;

c) follow the trend of the disease;

d) determine post operative risk.

Clinical Significance

正常人各组织GOT及GPT活性 (单位/克湿组织)

组织	GOT	GPT	组织	GOT	GPT
心	156000	7100	胰腺	28000	2000
肝	142000	44000	脾	14000	1200
骨骼肌	99000	4800	肺	10000	700
肾	91000	19000	血清	20	16

Elevated levels of ALT often suggest the existence of other medical problems such as alcoholic or viral hepatitis, congestive heart failure, liver damage, biliary duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems.

The serum transaminase levels are normally low but elevated after extensive tissue destruction.

Liver tissue is rich in Aspartate Transferase (AST) and alanine aminotransferase (ALT), but contains more of ALT than of AST. Therefore measurement of the serum levels (activity) of ALT is to estimate the potential for liver cell damage.