



# Experiment 5

## The estimation of GOT and GPT level in serum

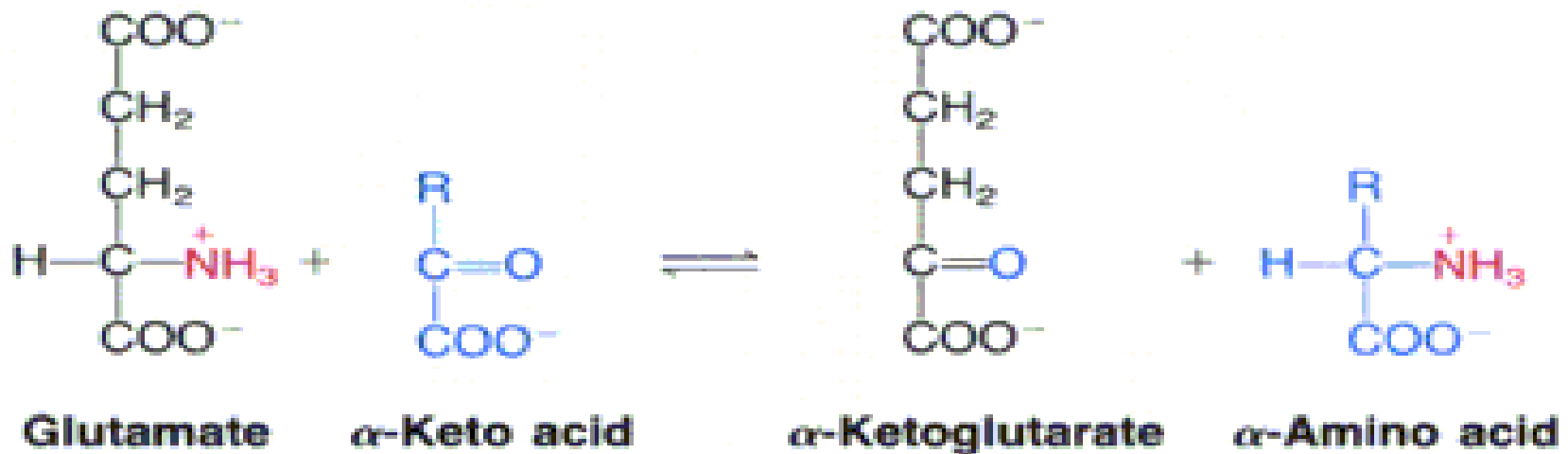
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# Objective:

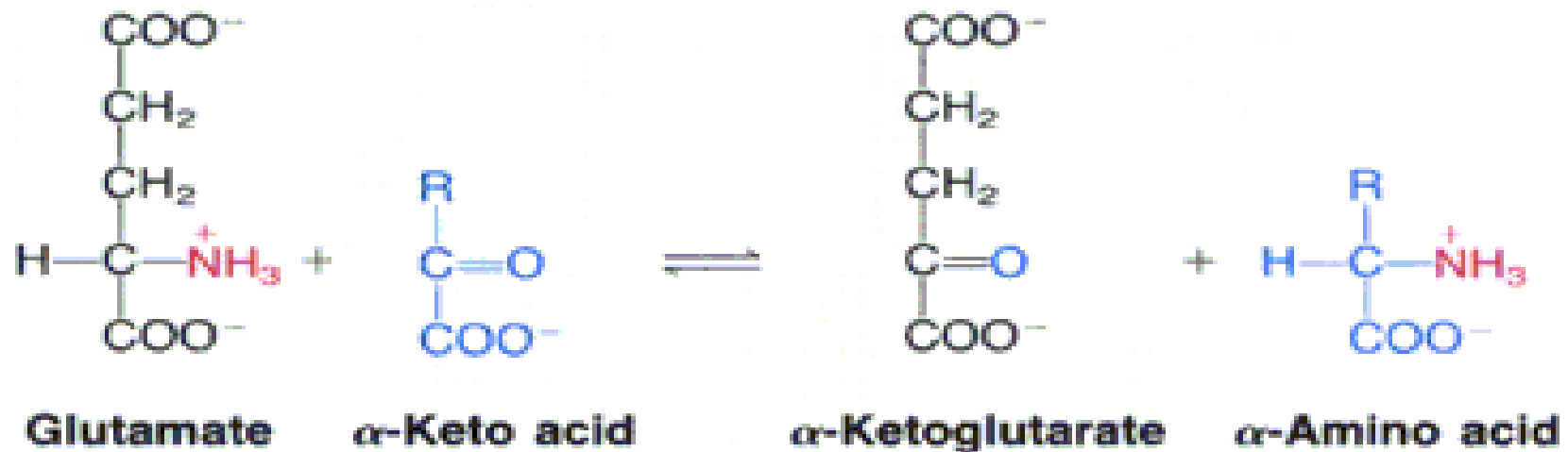
- To estimate the GOT and GPT activities of a sample of serum using a chemical method.

# Transaminase:

- **Transaminase:** is a name for a category of enzymes involved in exchange of an oxygen from a -keto acid and an amine from an amino acid.



# Transaminase



- This carbon skeleton then becomes an amino acid
- The original amino acid then becomes an  $\alpha$ -keto acid



# Transaminase

Transaminases are present in almost all tissues both in the cytoplasm and in the mitochondria.

# Transamination

- **Transamination:** is the process by which an amino group, usually from glutamate, is transferred to an  $\alpha$ -keto acid, with formation of the corresponding amino acid plus  $\alpha$ -ketoglutarate.
- **Transamination** reactions are catalyzed by **transaminases (aminotransferases)**

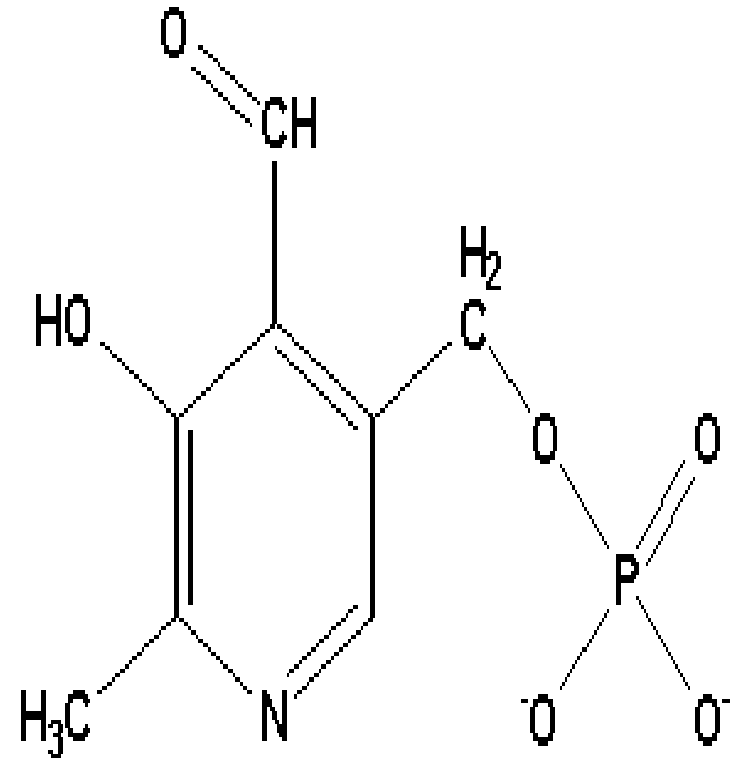


# Transamination

- **Transamination** reactions are generally reversible and they play an important role in the metabolism of amino acids in the body.

# Transamination

- **Aminotransferases** utilize a coenzyme, pyridoxal phosphate, that is derived from vitamin B6. The functional part of the cofactor is an aldehyde functional group, -CHO, attached to a pyridine ring

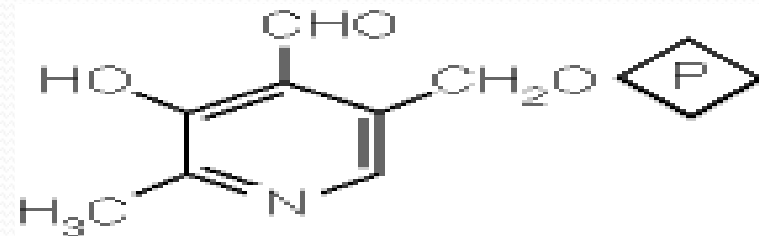


**Pyridoxal Phosphate (PyP; Vitamin B6)**

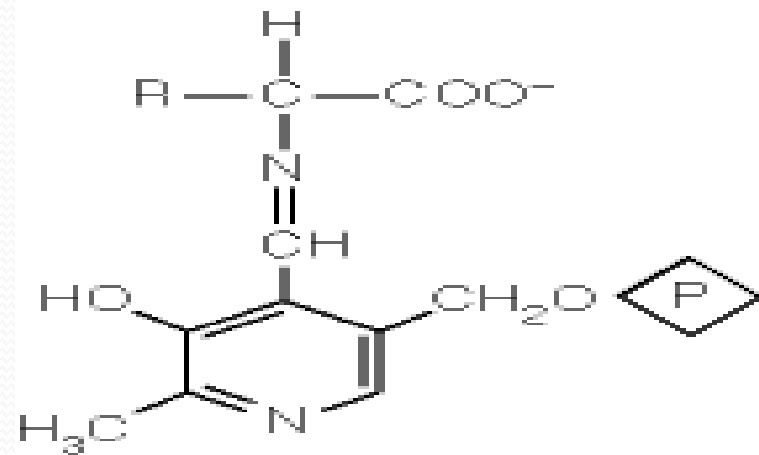


# Transamination

- Catalysis begins with condensation of the aldehyde group with the amino group of an amino acid, to give a Schiff base, or aldimine, intermediate, followed by formation of a resonance-stabilized carbanion with a quinonoid structure



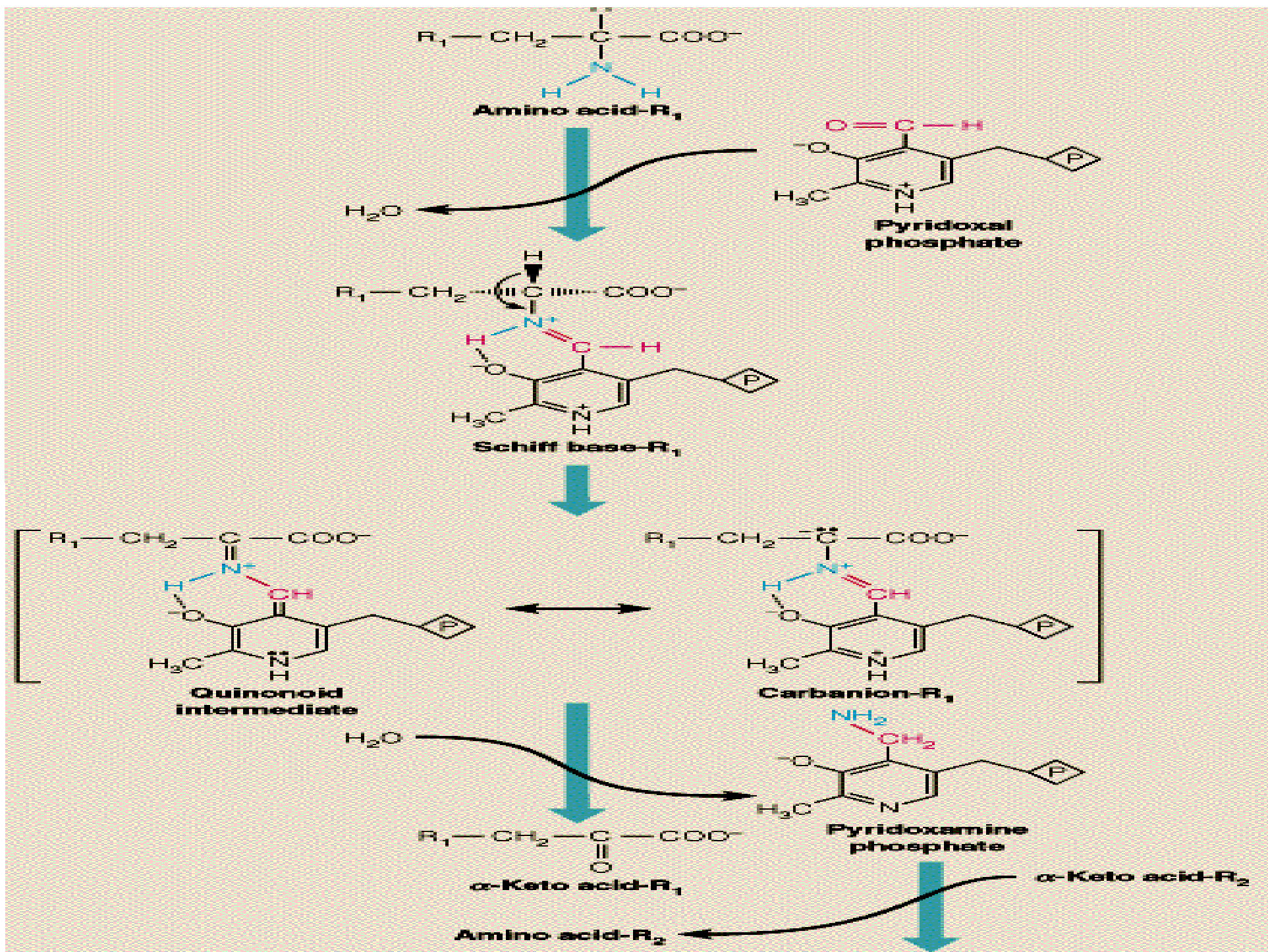
**Pyridoxal phosphate**



**Schiff base between amino acid and pyridoxal phosphate**

# Transamination

- Next, the base is reduced by NADPH in a stereospecific manner to produce the L-isomer of glutamate. The reaction is catalyzed by *glutamine synthase*.
- *After the amino group has been added to glutamate, it is available for subsequent transfer to other amino acids through the process of transamination*



# GOT and GPT

- The most important transaminases identified are  
*glutamate-oxaloacetate transaminase (GOT)*  
*glutamate- pyruvate transaminase (GPT).*
- One of the thousands kinds of liver enzymes, and a kind of transferase
- Large amount of transaminase is released into blood mostly on liver cell damages.
- Thus, detection of serum level tells the existence of liver cell damage.

# GOT and GPT

- The reaction catalysed by GOT is shown below:



- The reaction catalysed by GPT is shown below:



# GOT and GPT

- The activity of these two enzymes are quiet low in serum, whereas its relatively high in such tissues as liver, skeletal muscle and kidney.
- Measurement of SGOT and SGPT is useful in the clinical diagnosis of certain diseases.
- These enzymes will be increased in cases where cells containing large quantity of the enzymes are ruptured, thereby releasing their content into the blood stream.

# GOT and GPT

- Increased levels of SGOT and SGPT are found in cases of:
  - Myocardial infarction ( accompanied with increase in CPK)
  - Viral hepatitis
  - Toxic liver necrosis
  - Cirrhosis
  - Malignant infiltration of the liver



# Normal levels

- The levels of these enzymes should not exceed 9-15 U/L.
- It can be affected by:
  - Gender
  - Age
  - Body weight



# Principle of test

- For routine laboratory work 2,4 dinitro phenyl hydrazine is used to convert both oxaloacetate and pyruvate to the corresponding 2,4-dinitrophenylhydrazine derivatives which can be measured spectrophotomerically at 546 nm.

# Materials

- Substrate for SGOT.
- Substrate for SGPT.
- 1mM 2,4 dinitrophenyhydrazine.
- 0.4 M NaOH.
- Stop clock.
- Test tubes.
- Water bath at 37 °C.
- Pipettes ( 0.2, 1 and 10 ml ).
- Spectrophotometer and cuvettes.

# Method

Tube no.	Tube 1 (Blank 1) SGPT	Tube 2 (Sample 1) SGPT
Substrate for SGPT	1 ml	1 ml

Incubate at 37 °C , Leave tubes in water bath, then add

Distilled water	0.2 ml	-----
Serum	-----	0.2 ml

Start the stop watch immediately and incubate for *exactly* **30 min**

Add 2,4- dinitrophenyl hydrazine	1 ml	1 ml
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Mix the contents and allow to stand for **20 min** at room temperature

0.4 M NaOH	1 ml	1 ml
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Mix the contents and allow to stand for **5 min** at room temperature

# Result

- The data shown in the tables is used to convert absorbance at 546 nm into enzymatic activity in U/L of serum.
- Draw graphs using the data in the tables with absorbance on the (y – axis) and enzymatic activity in U/L on the (x – axis).
- From this graph , estimate the activity in U/L of SGPT ( table 2).

# Result cont'ed

