

**ALANINE
AMINOTRANSFERASE**

**ASPARTATE
AMINOTRANSFERASE**

□ Objective of the Exp.

- To estimate the level of *Alanine Aminotransferase* in a serum sample using a chemical method.

Types of Enzymes Present in Plasma

□ *There are 2 Types of Enzymes Present in Plasma*

Certain enzymes, proenzymes, and their substrates

are

present all the time in the circulation of normal individuals

and

perform physiologic function in blood.

These enzymes are known as

Functional Plasma Enzymes.

Types of Enzymes Present in Plasma

□ *There are 2 Types of Enzymes Present in Plasma*

- **Plasma also contains numerous other enzymes**

**(normally present in low concentrations in blood)
which perform no known physiologic function
in blood.**

- *These which are known as:*

Nonfunctional Plasma Enzymes.

Levels of Nonfunctional Plasma Enzymes in Plasma

Tissue damage or necrosis resulting from injury or disease is generally accompanied by increase in the levels of several of the nonfunctional plasma enzymes (see Table 1).

Information from enzymes measurements in serum

- ⇒ Presence of disease
- ⇒ Organs involved
- ⇒ Aetiology /nature of disease: differential diagnosis
- ⇒ Extent of disease-more damaged cells-more leaked enzymes in blood
- ⇒ Time course of disease

Levels of Nonfunctional Plasma Enzymes in Plasma

Tissue damage or necrosis resulting from injury or disease is generally accompanied by increase in the levels of several of the nonfunctional plasma enzymes (see Table 1).

There are many nonfunctional plasma enzymes.

These enzymes include

Alanine Aminotransferase (ALT)

and

Aspartate Aminotransferase (AST).

Levels of Nonfunctional Plasma Enzymes in Plasma


⇒ **Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes are the most abundantly present in the liver** and is elevated in blood as a result of leakage from damaged cells.

Levels of Nonfunctional Plasma Enzymes in Plasma

⇒ In viral hepatitis the enzyme levels are increased 20-50 times above the upper limit of the normal range

Table 1. Principal non-functional serum enzymes used in clinical diagnosis.

Nonfunctional Plasma Enzymes	Major Diagnostic Use (The Disease that Causes the Elevation in the Nonfunctional Plasma Enzymes)
<p>Aminotransferases</p> <ul style="list-style-type: none"><li data-bbox="241 1029 1211 1166">• Aspartate aminotransferase (AST, or SGOT)<li data-bbox="241 1289 1211 1426">• Alanine aminotransferase (ALT, or SGPT)	<p>Myocardial Infarction</p> <p>Viral Hepatitis</p>



**What is the
Function of
ALT and AST?**

The Function of ALT and AST in Tissues

Transamination Reactions

□ **Aminotransferases** (formerly called transaminases); such as **ALT** and **AST**, are a family of enzymes that catalyses specific reactions known as **Transamination Reactions** involved in **Protein Metabolism**.

- **ALT** and **AST** transfer amino groups from amino acids to carbon skeletons.

The Function of ALT and AST in Tissuescont.

Transamination Reactions

- Aminotransferases: ALT and AST are Enzymes Catalyses Specific Reactions Known as Transamination Reactions Involved in Protein Metabolism
- **The transferring of amino groups to α -Ketoglutarate by aminotransferases.**
 α -Ketoglutarate plays a unique role in amino acid metabolism by accepting the amino groups from amino acids, and thus becoming *glutamate*.

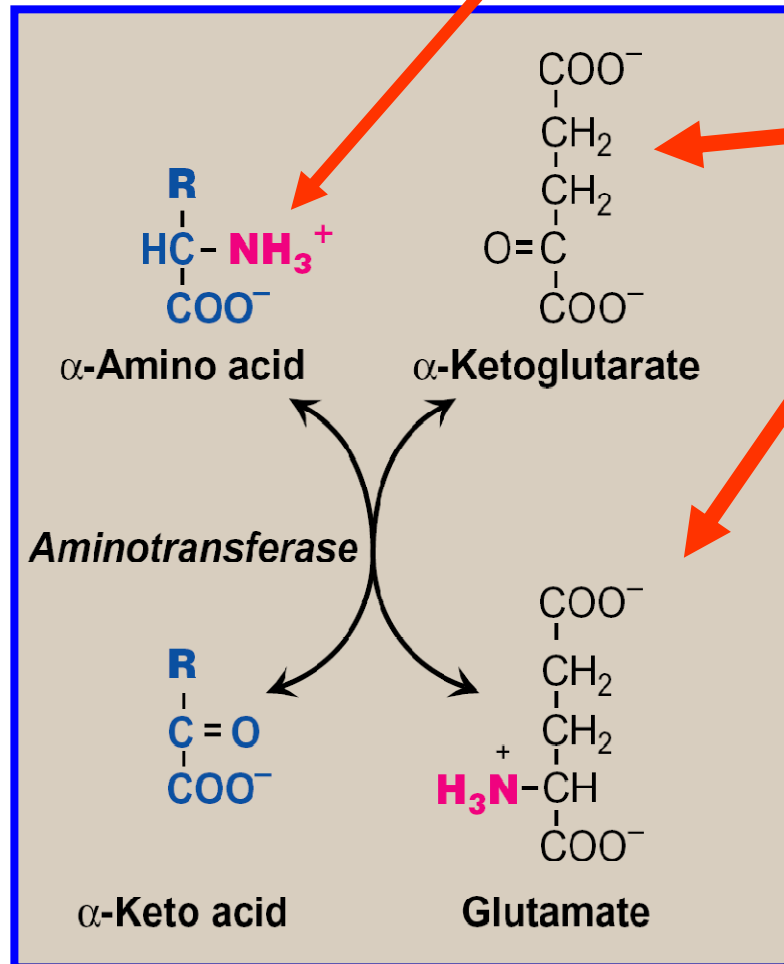
The Function of ALT and AST in Tissuescont.

Transamination Reactions

- **Aminotransferases: ALT and AST are Enzymes Catalyses Specific Reactions Known as Transamination Reactions Involved in Protein Metabolism**
- **The transfer of an amino group to α -ketoglutarate converting it to glutamate by aminotransferases .The first step in the catabolism of most amino acids is the transfer of their α -amino group to α -ketoglutarate (Figure 1). The products are an α -keto acid (derived from the original amino acid) and glutamate (derived from α -ketoglutarate) .**

1- Transamination

Funneling of amino groups to glutamate



α -ketoglutarate accepts the amino group from amino acids to become **glutamate**
By: aminotransferases (transaminases)

Glutamate:

- Oxidat. Deam \rightarrow ammonia \rightarrow urea cycle
Or
- gives amino group to oxalacetate to produce aspartate--- urea cycle
Or
- gives amino group to carbon skeleton to produce new amino acid

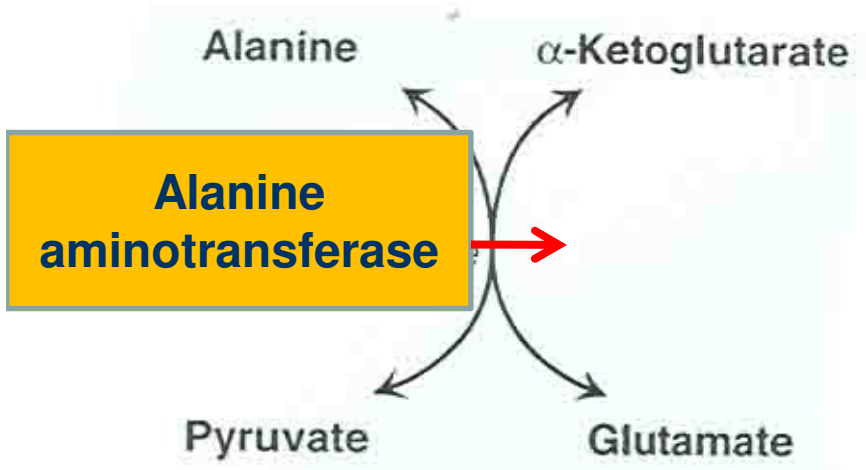
All amino acids (with the **exception** of lysine & threonine) participate in transamination

Aminotransferases

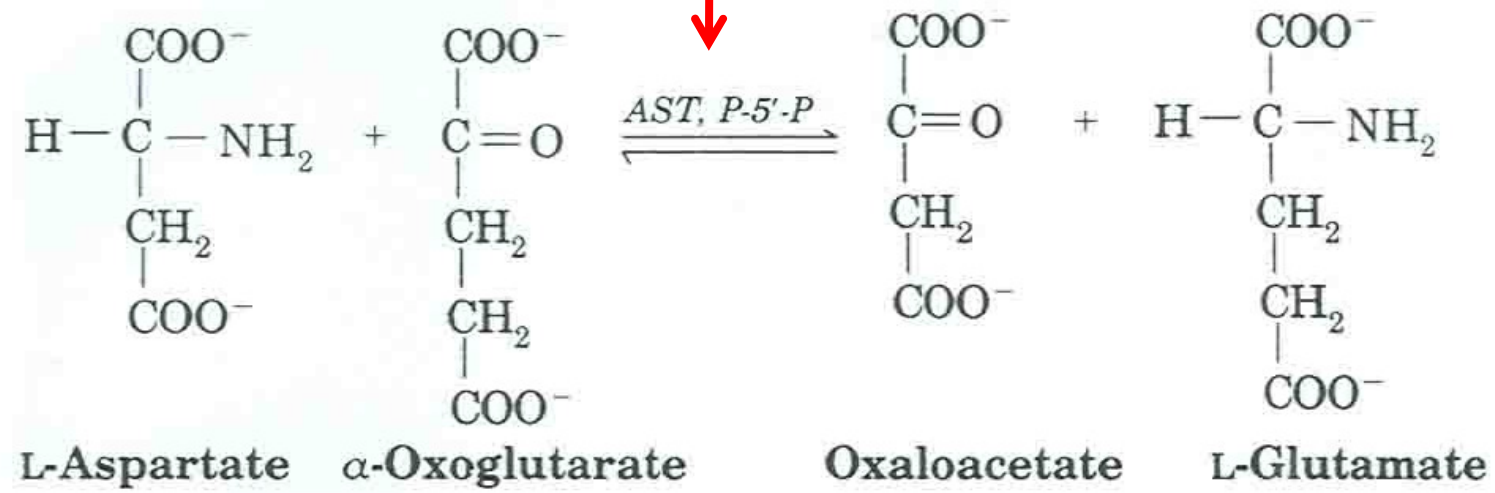
1. Substrate Specificity of Aminotransferases:

- Each **aminotransferase** is specific for one or at most a few amino group donors.
- **Aminotransferases** are named like the following, the word “aminotransferase” comes after the specific amino group donor name (i.e. the amino acid name), because the acceptor of the amino group is almost always α -Ketoglutarate. The two most important aminotransferases are named as : alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

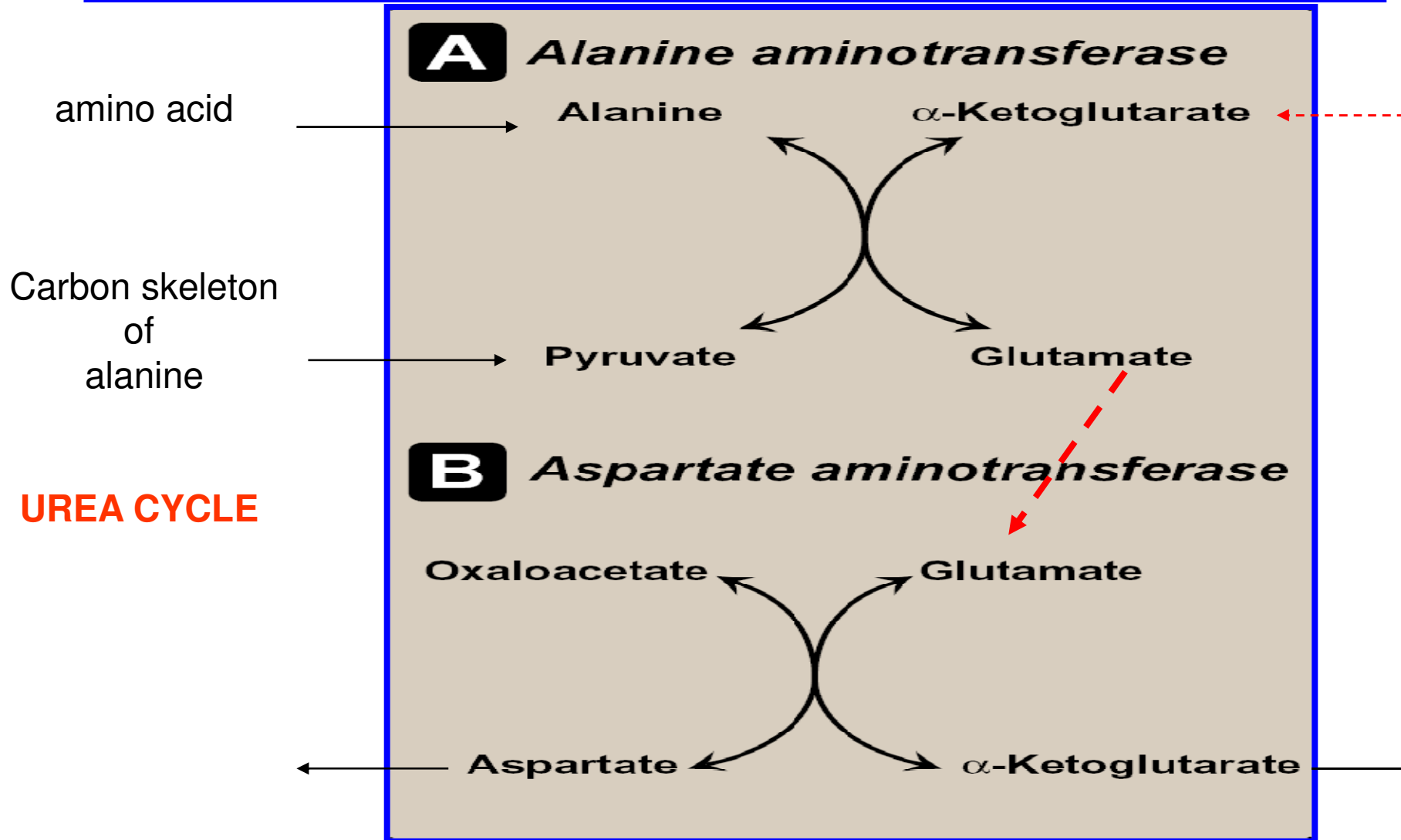
The Two Most Important Transamination Reactions are those which are Catalyzed by ALT and AST



Aspartate aminotransferase



ALanine AminoTTransferase (ALT) & ASpartate AminoTTransferase (AST)



ALANINE AMINOTRANSFERASE

□ ALANINE AMINOTRANSFERASE

- ALANINE TRANSAMINASE (ALT) (EC 2.6.1. 2)
- L-Alanine: α -Ketoglutarate Aminotransferase
- Formerly known as Glutamate Pyruvate Transaminase, (GPT)

ALANINE AMINOTRANSFERASE

.....cont.

- **Alanine aminotransferase (ALT)**, also called glutamate-pyruvate transaminase (GPT), catalyzes the transfer of the amino group of alanine to α -ketoglutarate, resulting in the formation of pyruvate and glutamate (see Figure 2).

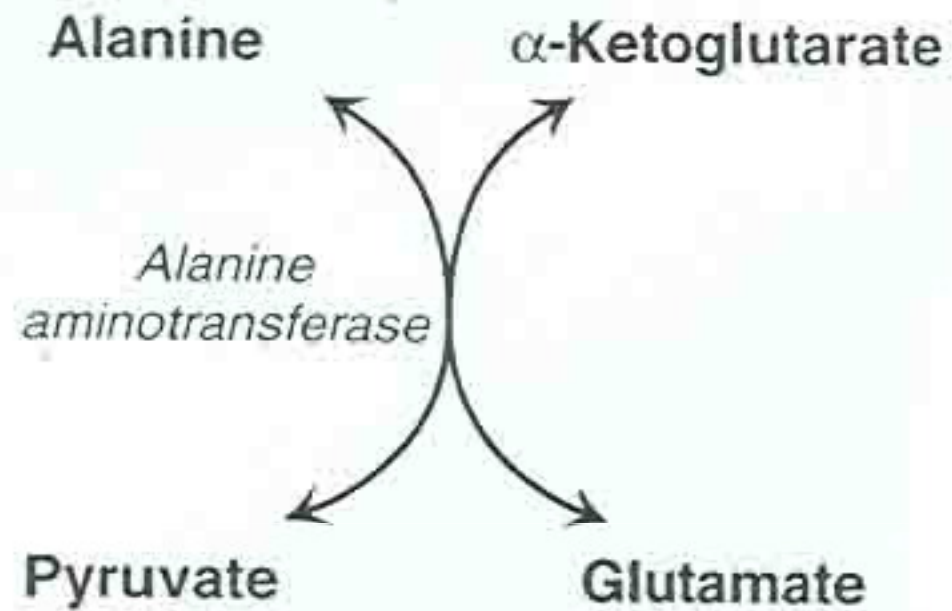


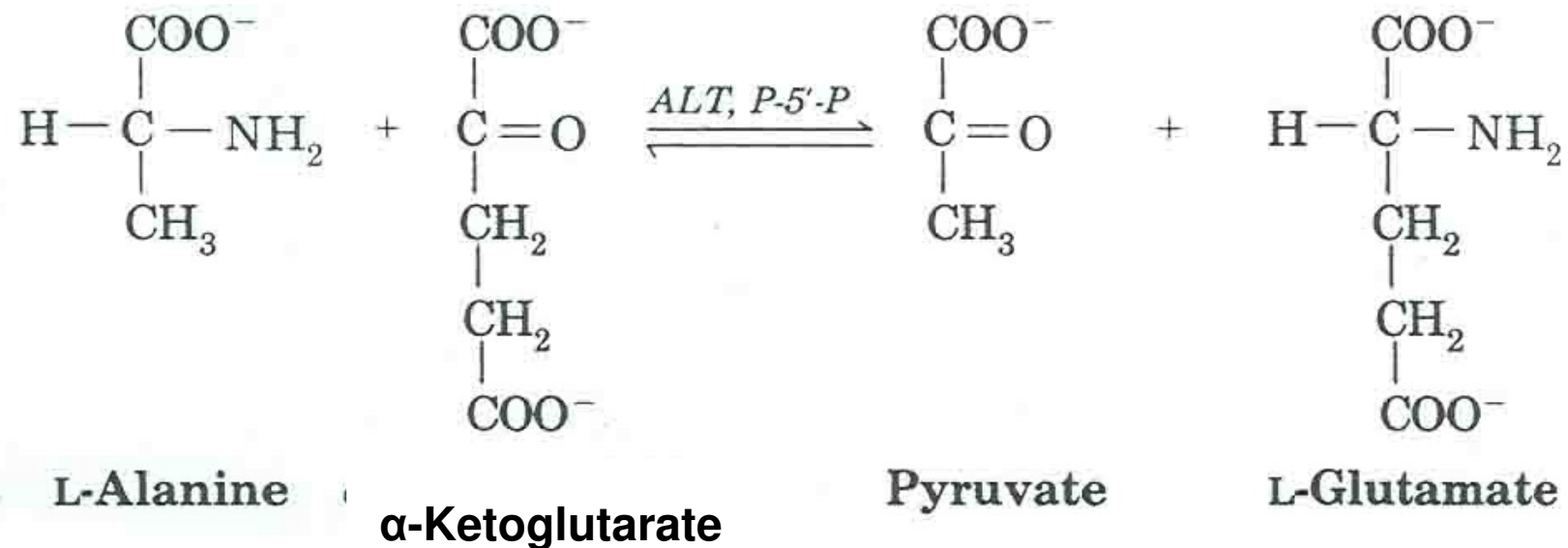
Figure **2**
Reaction catalyzed by *alanine aminotransferase*.

ALANINE AMINOTRANSFERASE

.....cont.

- **Alanine aminotransferase (ALT)** reaction is readily reversible; however, during amino acid catabolism, this enzyme (like most other aminotransferases) functions in the direction of glutamate synthesis. Thus, glutamate acts as a “collector” of nitrogen from alanine.

ALANINE AMINOTRANSFERASE



Where can we Find Alanine Aminotransferase?

- **Alanine Aminotransferase (ALT)**
is
present in many tissues.

*However it is found primarily in the
liver.*

ASPARTATE AMINOTRANSFERASE

□ ASPARTATE AMINOTRANSFERASE

- ASPARTATE TRANSAMINASE (**AST**)

(EC 2.6.1.1)

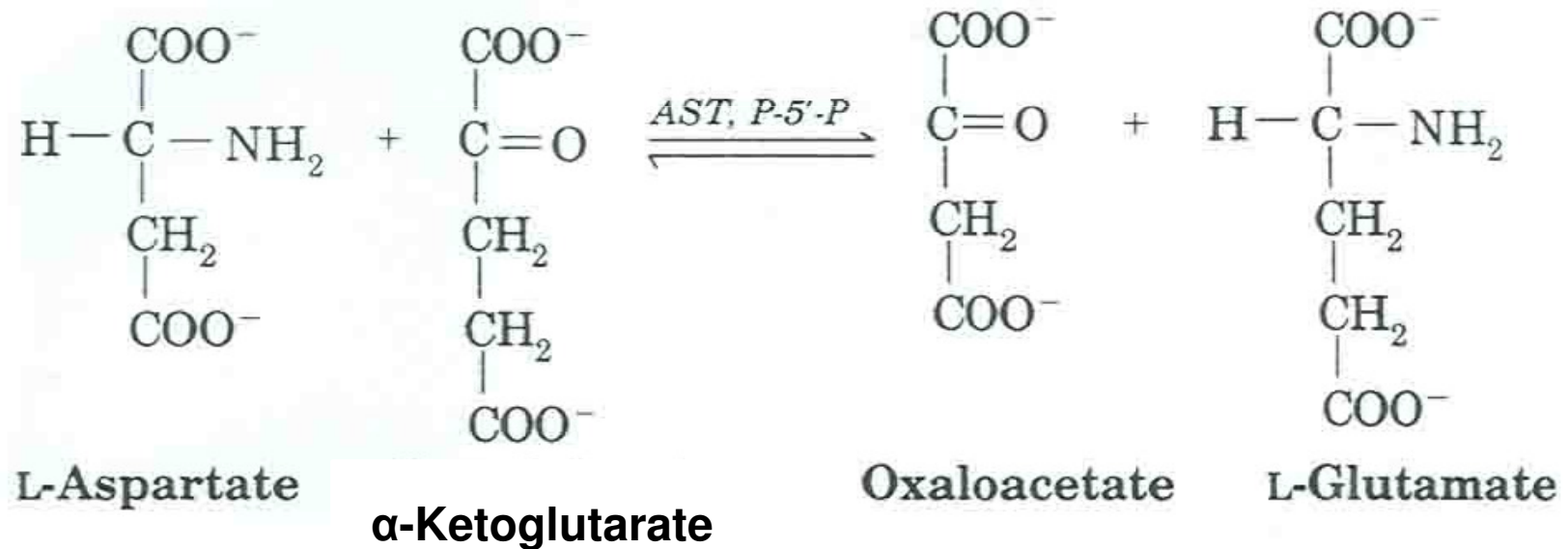
- **L-Aspartate: α -Ketoglutarate Aminotransferase;**

- Formerly known as **Glutamate Oxaloacetate Transaminase, (GOT).**

ASPARTATE AMINOTRANSFERASEcont.

- **Aspartate aminotransferase (AST), also called glutamate: oxaloacetate transaminase (GOT)**, is an exception to the rule that aminotransferases transfer amino groups to α -Ketoglutarate to form glutamate. Since during amino acid catabolism, aspartate aminotransferase transfers amino groups from glutamate to oxaloacetate, to form α -Ketoglutarate and aspartate respectively, aspartate is then used as a source of nitrogen in the urea cycle.

ASPARTATE AMINOTRANSFERASEcont.



ASPARTATE AMINOTRANSFERASEcont.

- **Aspartate Aminotransferase (AST)**

raise in acute liver damage, but since it is also present in different other tissues it is therefore not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage

ASPARTATE AMINOTRANSFERASEcont.

- **Aspartate Aminotransferase (AST)**

is found in the

**Liver, Red Blood Cells, Cardiac Muscle,
Skeletal Muscle**

Any disease in these organs may raise the:

AST level significantly .

- Other less important sources of AST that dose'nt raise the AST level significantly in the blood : kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes in decreasing order of concentration.

THE CO-ENZYME OF AMINOTRANSFERASE

2. Mechanism of Action of Aminotransferases:

All aminotransferases require the coenzyme Pyridoxal phosphate (a derivative of vitamin B₆) which functions as a prosthetic group in the amino transfer reactions. PLP is covalently linked to the ϵ -amino group of a specific lysine residue at the active site of the enzyme. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate Pyridoxamine phosphate.

THE CO-ENZYME OF AMINOTRANSFERASE

- The pyridoxamine form of the coenzyme then reacts with an α -keto acids to form an amino acid and regenerates the original aldehyde form of the coenzyme.
- Figure 3 shows these two reactions of a transamination catalyzed by aspartate aminotransferase.

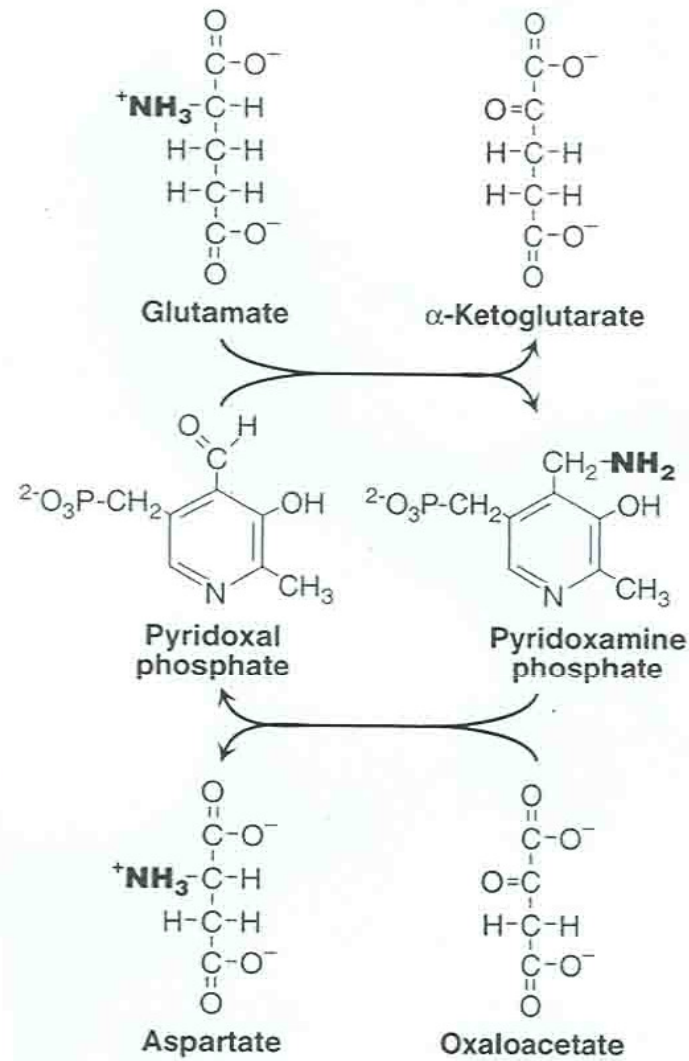



Figure **3**

Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the *aspartate aminotransferase* reaction.



**What Elevates
Aminotransferases
Level in the
Blood**

Aminotransferases Levels in the Blood

- **Aminotransferases**

(both **ALT** and **AST**)

are normally:

Intracellular Enzymes



Their Normal Levels in the Blood
are Low.

Aminotransferases Levels in the Blood cont.

- Thus, the Presence of Elevated Levels of Aminotransferases in the Plasma Indicates?**

Elevation of Plasma Aminotransferases

- Thus, the Presence of Elevated Levels of Aminotransferases in the Plasma Indicates.....?



Damage to Cells Rich in these Enzymes:
(Liver , Heart Muscle and Skeletal Muscle).

Plasma Levels
of
Aminotransferases
are
very Important in the:
Diagnosis of Several
Diseases



**How to Interpret
ALT and AST
Elevations.....?**

Diagnostic Value of Plasma Aminotransferases

- **If high ALT only** : liver disease mainly.
(ALT is almost specific for liver)
- **If high AST only** : either liver disease ,
cardiac or skeletal muscles damage.
(Remember that AST is non spesific)
- **If Both ALT and AST are high** : liver
disease mainly.
(As the liver is almost the only organ that contains both
ALT and AST in large amount in it's cells).

Diagnostic Value of Plasma Aminotransferasescont.

❑ **Liver Disease**: Plasma AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis and prolonged hypotension. Detected AST and ALT elevation in the blood occurs very early in the disease course .

Diagnostic Value of Plasma Aminotransferasescont.

Because
ALT and AST
normally present
in high concentrations in liver
and
in low concentration in blood

Liver blood tests of ALT and AST level values

are used to examine
Liver Function Tests
(LFT)

which are in turn used to detect an injury or an inflammation to the liver.

Diagnostic Value of Plasma Aminotransferasescont.

□ Activities for both enzymes may reach values as high as 100 times the upper reference limit (the higher the level the more severe is the liver disease) , although normally and most frequently 20 to 50 folds elevations are encountered.

Prognostic Value of Plasma Aminotransferases

Liver Diseases:

Frequent enzyme measurements are often useful in determining the course of liver damage. (the more the hepatocyte destructed the higher the peak of ALT level the more serious is the disease and the worst the prognosis (prognosis is the chance of cure))

Prognostic Value of Plasma Aminotransferasescont.

❑ In skeletal or cardiac muscle disease AST is such as in myocardial infarction and muscle disorders.

- **But Liver disease may elevate AST as well.....**
- **How to differentiate between liver disease and cardiac or skeletal muscles disease if AST is elevated?**

How to Differentiate between Liver Disease and Cardiac or Skeletal Muscles Disease if AST is Elevated ?

- It's easylook at ALT if it's high as well it's a liver disease (ie, the source of AST in such a case is the liver)
- If ALT is normal and only AST is high it's either skeletal muscle or cardiac muscle disease and you can differentiate between these two conditions clinically..

**Table 2. DISTRIBUTION OF
DIAGNOSTICALLY IMPORTANT ENZYMES**

Enzyme	Principal Sources	Principal clinical Applications
Alanine Aminotransferase	Liver	Hepatic parenchymal disease
Aspartate Aminotransferase	Liver Skeletal muscle Heart Kidney Erythrocytes	Myocardial infarction, Hepatic parenchymal disease Muscle disease.

Principle of the test:

- In this exp. we will estimate the concentration of ALT in serum, and this estimation will be by estimating the activity of this enzyme.
- The activity of ALT is measured by measuring the concentration of the products of the reaction that is catalyzed by the ALT.
- ALT reaction is reversible, however, in this exp. the products will be pyruvate and glutamate since we will add a substance reacts with pyruvate shifting the reaction toward the direction of pyruvate formation.

Principle of the Test:

- There is a substance known as 2,4 dinitro- phenyl hydrazine reacts with pyruvate (and oxaloacetate) to form the corresponding 2,4- dinitrophenylhydrazine derivative of pyruvate in case the product is pyruvate (and 2,4- dinitrophenylhydrazine derivative of oxaloacetate in case the product is oxaloacetate) which can be measured spectrophotomerically at 546 nm.

Method:

1. Label 2 test tubes 1 and 2 tubes, 1 is the blank for ALT, whereas tube 2 will contain sample of serum for your investigation.
2. Pipette 1 ml of the substrate for ALT into tubes 1 and 2
3. Place all tubes in a water bath at 37° C.
4. Add 0.2 ml of distilled water to the blank tube 1.
5. Add 0.2 ml of serum to tube 2 and immediately start the stop clock.
6. Tubes 1 and 2 should be incubated for exactly 30 minutes.
7. After the incubation period remove the tubes from the water bath and add 1 ml of 2,4 dinitrophenyldrazine to each of them. Mix the contents and allow to stand for 20 minutes at room temperature.
8. Then add 10ml of 0.4 M NaOH to each tube, mix well and allow to stand for a further 5 minutes.
9. Read the absorbance at 546 nm of tube 2 using tube 1 as a blank to zero the spectrophotometer.

Results:

The data shown in the table below is used to convert absorbance at 546 nm into enzymatic activity in U/L of serum. Draw graph using the data in the table 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 ALT (table 3). Record your results in the spaces provided below the table.

Table 3. ALT Activity

Absorbance at 546 nm	ALT activity (U\L)
0.025	2.5
0.050	5.5
0.075	9
0.100	12
0.125	17
0.150	21
0.175	25
0.200	30
0.225	35
0.250	41
0.275	47
0.300	54
0.325	61
0.350	70
0.375	80

Results

- Absorbance at 546 nm of tube " 2" =
- ALT (SGPT) activity of serum sample
(from graph) = U/L.
- **The normal levels of ALT (and AST) are 9-15 U/L and can be affected by age and body weight.**

