Wolters Kluwer Health | Lippincott Williams & Wilkins

Chapter 13: Enzymes

By Kamisha L. Johnson-Davis

General Properties and Definitions

- Enzymes
  - Specific biologic proteins that catalyze biochemical reactions without altering equilibrium point of reaction or being consumed or changed in composition
  - Found in all body tissues & frequently appear in serum following cellular injury or degradation
  - Proteins, comprising specific amino acid sequence
  - Enzyme may exist in different forms (isoenzyme & isoform).
  - **Cofactor:** nonprotein molecule necessary for enzyme activity
    - **Activator:** inorganic cofactor
    - **Coenzyme:** organic cofactor

Enzyme Classification and Nomenclature

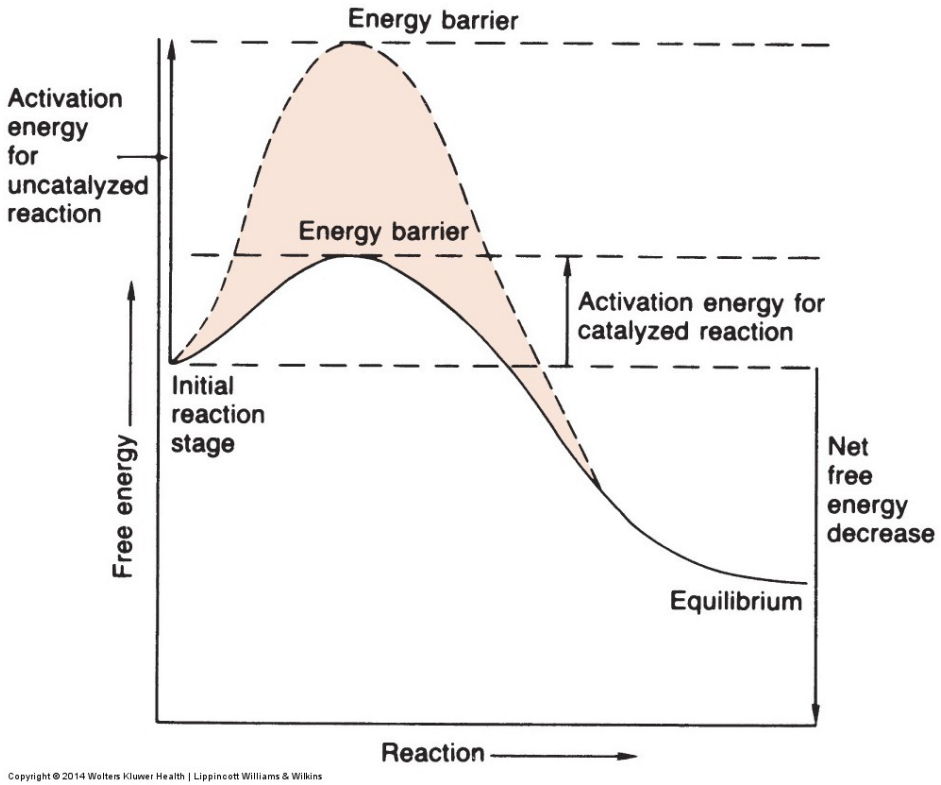
- IUB System assigns name & code to each enzyme
  - 1. **Oxidoreductases:** catalyze an oxidation–reduction reaction between two substrates
  - 2. **Transferases:** catalyze transfer of a group other than hydrogen from one substrate to another
  - 3. **Hydrolases:** catalyze hydrolysis of various bonds
  - 4. **Lyases:** catalyze removal of groups from substrates without hydrolysis; product contains double bonds
  - 5. **Isomerases:** catalyze interconversion of geometric, optical, or positional isomers
  - 6. **Ligases:** catalyze joining of two substrate molecules, coupled with breaking of pyrophosphate bond in ATP

Enzyme Kinetics

- Catalytic Mechanism of Enzymes
  - **Activation energy:** energy required to raise all molecules in 1 mole of a compound at certain temperature to transition state at peak of energy barrier
  - Enzymes catalyze reactions by lowering activation energy level.
  - Enzyme specificity
    - **Absolute:** specific to only 1 substrate
    - **Group:** specific to all substrates of a chemical group
    - **Bond:** specific to chemical bonds
    - **Stereoisometric:** specific to 1 optical isomer of a compound

Enzyme Kinetics (cont'd)

- Energy vs. progression of reaction, indicating energy barrier that substrate must surpass to react with and without enzyme catalysis



Enzyme Kinetics (cont'd)

- Factors That Influence Enzymatic Reactions
  - Substrate concentration
  - Enzyme concentration
  - pH
  - Temperature
  - Cofactors
  - Inhibitors

Enzyme Kinetics (cont'd)

- Measurement of Enzyme Activity
  - Common measurements
    - Increase in product concentration
    - Decrease in substrate concentration
  - Enzyme concentrations always performed in zero-order kinetics
  - Inhibitors must be lacking & other variables carefully controlled.
  - Types of measurement of enzymatic reactions: fixed-time & continuous monitoring (kinetic assay)

Enzyme Kinetics (cont'd)

- Calculation of Enzyme Activity
  - **International unit (IU):** amount of enzyme that will catalyze reaction of 1 µmol of substrate/min under specified conditions
  - Enzyme concentration usually expressed in units per liter (IU/L)
  - The SI systems uses the katal (mol/s)> activity expressed as katal/L
  - 1.0 IU = 17 nkatal
- Measurement of Enzyme Mass
  - Immunoassay methodologies
  - Risk of overestimating active enzyme due to possible cross-reactivity with inactive enzymes
- Enzymes as Reagents
  - Used to measure many nonenzymatic constituents in serum

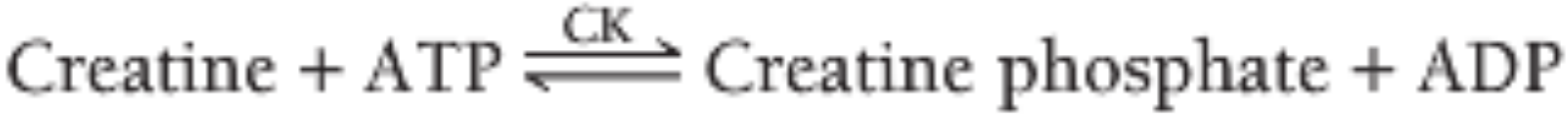
Measurement of enzyme activity

- Enzyme activity measurement is performed in zero-order kinetics, with the substrate in sufficient excess to ensure that no more than 20% of available substrate is converted to products
- Coenzymes are also in excess
- Coenzymes like NADH is often used, which absorbs at 340 nm while NAD^+ does not
- Coupled-enzyme assay: more than one enzyme is added excess as a reagent and multiple reactions are catalyzed

Enzymes of Clinical Significance

Creatine kinase

- A dimeric enzyme (82 kDa), two subunits B and M: BB (CK-1,; brain type), MB (CK-2; hybrid type) & MM (CK-3; muscle type)
- Catalyzes the following rxn:



- Optimal pH for forward and reverse rxns are 9.0 and 6.7, respectively
- As is true for all kinases, Mg^{+2} is a cofactor
- In muscle cells, it is involved in the storage of high-energy creatine phosphate. Every contraction cycle of muscle results in creatine phosphate use, with the production of ATP.

TABLE 13-3 CREATINE KINASE ISOENZYMES—TISSUE LOCALIZATION AND SOURCES OF ELEVATION

ISOENZYME	TISSUE	CONDITION
CK-MM	Heart	Myocardial infarction
	Skeletal muscle	Skeletal muscle disorder
		Muscular dystrophy
		Polymyositis
		Hypothyroidism
		Malignant hyperthermia
CK-MB	Heart	Myocardial infarction
	Skeletal muscle	Myocardial injury
		Ischemia
		Angina
		Inflammatory heart disease
		Cardiac surgery
		Duchenne-type muscular dystrophy
		Polymyositis
		Malignant hyperthermia
CK-BB	Brain	Central nervous system shock
	Bladder	Anoxic encephalopathy
	Lung	Cerebrovascular accident
	Prostate	Seizure
	Uterus	Placental or uterine trauma
	Colon	Carcinoma
	Stomach	Reye's syndrome
	Thyroid	Carbon monoxide poisoning
		Malignant hyperthermia
		Acute and chronic renal failure

CK: diagnostic significance

- CK levels are frequently elevated in disorders of:
  - Cardiac and skeletal muscle (myocardial infarction [MI], rhabdomyolysis, and muscular dystrophy).
  - Cerebral vascular accident, seizures, nerve degeneration, shock; hypothyroidism, malignant hyperpyrexia, Reye's syndrome
- CK level is considered a sensitive indicator of acute myocardial infarction (AMI) and muscular dystrophy, particularly the Duchenne type.
- CK levels are not entirely specific indicators as CK elevation is found in various other abnormal cardiac and skeletal muscle conditions.
- Levels of CK also vary with muscle mass and, therefore, may depend on gender, race, degree of physical conditioning, and age
- Normal serum contains mainly CK-MM, that constitute ~94-100% of total CK
- Mild to strenuous activity may contribute to elevated CK levels, as may intramuscular injections

CK: enzyme activity

$$\text{Creatine} + \text{ATP} \xrightleftharpoons{\text{CK}} \text{Creatine phosphate} + \text{ADP}$$

$$\text{ADP} + \text{phosphoenolpyruvate} \xrightleftharpoons{\text{PK}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LD}} \text{Lactate} + \text{NAD}^+$$

- CK is unstable in serum.
- It is stabilized by addition of N-acetylcysteine, mercaptoethanol and DTT
- Serum should be stored in a dark place, because CK is inactivated by light.
- Hemolysis of serum samples may elevate CK activity

CK & MI

- Demonstration of elevated levels of CK-MB, greater than or equal to 6% of total CK, is considered a good indicator for mayocardial damage, particularly AMI
- Troponins (I & T) may also elevate in AMI in absence of CK-MB elevations >> more specific
- Specificity of CK-MB for diagnosis of AMI can be increased if interpreted in conjunction with and LD isoenzymes and/or Troponins; or if measured sequentially over 48 hrs to show a typical rise and fall of enzyme activity

CK: reference range

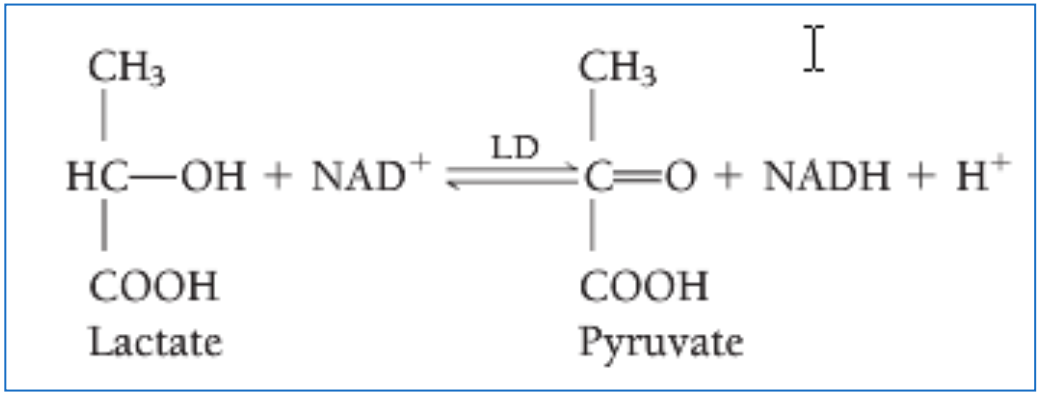
Reference Range

Total CK:

Males: 46 to 171 U/L (37°C) (0.8 to 2.9 µkat/L)
Females: 34 to 145 U/L (37°C) (0.6 to 2.4 µkat/L)
CK-MB: <5% total CK

The higher values in males are attributed to increased muscle mass. Note that enzyme reference ranges are subject to variation, depending on the method used and the assay conditions.

Lactate dehydrogenase (LD)



- **Source of error:** any degree of hemolysis; instability in any temperature
- **Reference range:** 125-220 U/L (37°C)

- Tissue source:
- LD is highly distributed in the body.
- High levels of LD are found in heart, liver, skeletal muscles, kidney and RBCs
- Less amounts of LD in lung, smooth and brain

LD: diagnostic significance

- Increased levels are found in cardiac, hepatic, and skeletal muscle and renal diseases, as well as in several hematologic and neoplastic disorders.
- The highest levels of total LD are seen in pernicious anemia and hemolytic disorders.
- Intramedullary destruction of erythroblasts causes elevation as a result of the high concentration of LD in erythrocytes.
- In the sera of healthy individuals, the major isoenzyme fraction is LD-2, followed by LD-1, LD-3, LD-4, and LD-5
- LD flipped pattern LD1>LD2 in AMI

TABLE 13-4 LACTATE DEHYDROGENASE ISOENZYMES—TISSUE LOCALIZATION AND SOURCES OF ELEVATION

ISOENZYME	TISSUE	DISORDER
LD-1 (HHHH)	Heart	Myocardial infarction
	Red blood cells	Hemolytic anemia
LD-2 (HHHM)	Heart	Megaloblastic anemia
	Red blood cells	Acute renal infarct
		Hemolyzed specimen
LD-3 (HHMM)	Lung	Pulmonary embolism
	Lymphocytes	Extensive
	Spleen	Pulmonary pneumonia
	Pancreas	Lymphocytosis
		Acute pancreatitis
		Carcinoma
LD-4 (HMMM)	Liver	Hepatic injury or inflammation
LD-5 (MMMM)	Skeletal muscle	Skeletal muscle injury

LD, lactate dehydrogenase.

CASE STUDY 13-1

A 51-year-old, overweight white man visits his family physician with a symptom of "indigestion" of 5 days' duration. He has also had bouts of sweating, malaise, and headache. His blood pressure is 140/105 mm Hg; his family history includes a father with diabetes who died at age 62 of AMI secondary to diabetes mellitus. An electrocardiogram revealed changes from one performed 6 months earlier. The results of the patient's blood work are as follows:

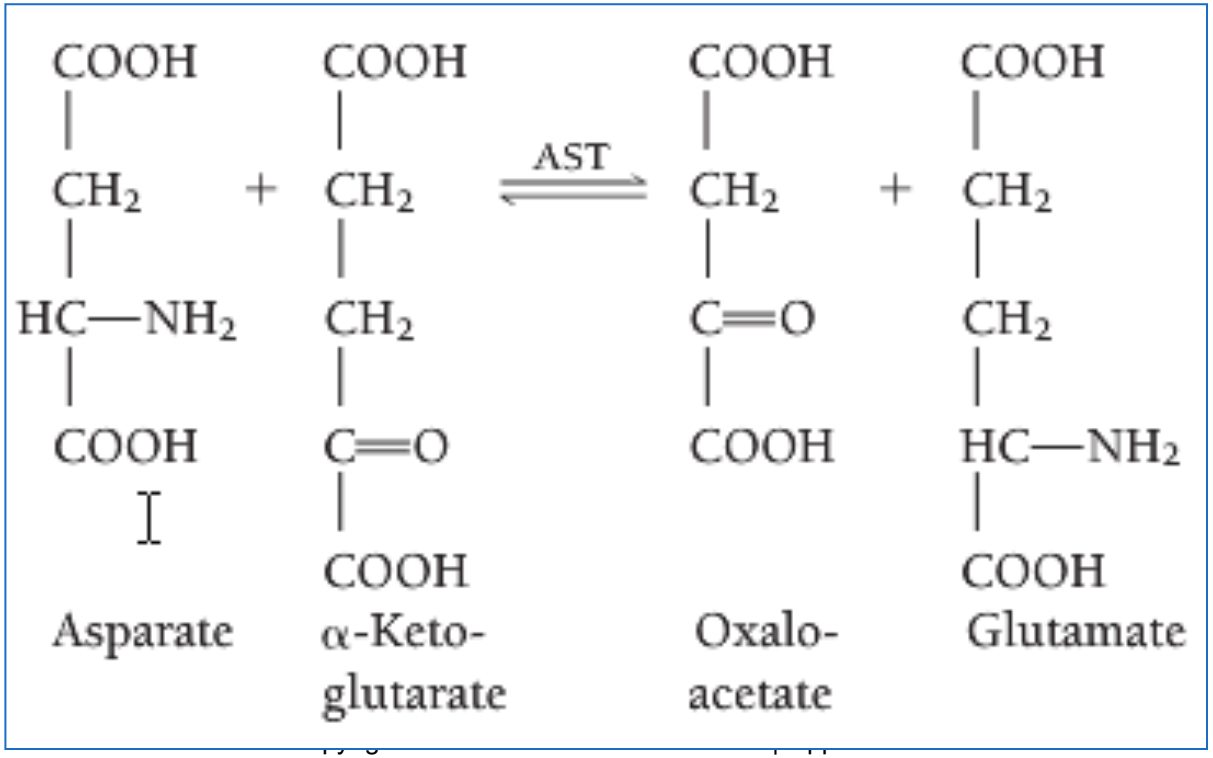
CK	129 U/L (30–60)
CK-MB	4% (<6%)
LD	280 U/L (100–225)
LD	Isoenzymes LD-1 > LD-2
AST	35 U/L (5–30)

Questions

1. Can a diagnosis of AMI be ruled out in this patient?
2. What further cardiac markers should be run on this patient?
3. Should this patient be admitted to the hospital?

Aspartate aminotransferase (AST)

- Older terminology: serum glutamic oxaloacetic transaminase (SGOT, or GOT),
- Rxn catalyzed by AST; transfer of an amino group:



Pyridoxal phosphate (PLP) functions as coenzyme

AST

- Tissue source:
  - AST is widely distributed in human tissue.
  - The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocytes

AST: diagnostic significance

- AST is mainly used to evaluate hepatocellular disorders & skeletal muscle involvement
- In AMI, AST begin to rise within 6-8 hrs, peak at 24 hrs & return to normal within 5 days
- AST is not specific for AMI
- AST is elevated in pulmonary embolism
- Half-life is ~16 hrs

AST: diagnostic significance/ cont'd

- AST levels are highest in acute hepatocellular disorders
- In viral hepatitis, levels may reach 100 times upper limit of normal (ULN)
- In cirrhosis, AST reaches ~4X ULN
- Skeletal muscle disorders & muscular dystrophy: 4-8X ULN

AST: cellular location & isoenzymes

- AST has 2 isoenzymes, but not routinely analyzed
- Intracellular conc of AST is 7000X higher than extracellular
- AST is located in cytoplasm & mitochondria,
- Cytoplasmic form is the predominant form

Assay for AST

- Karmen method:; optimum pH 7.3 – 7.8:

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{AST}} \text{Oxaloacetate} + \text{glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Malate} + \text{NAD}^+$$

Sources of error:

Hemolysis dramatically increases AST

Activity stable for 3-4 days at Refrig temp.

Ref Range:

AST: 5 – 35 U/: (37C) (0.1-0.6 ukat/L)

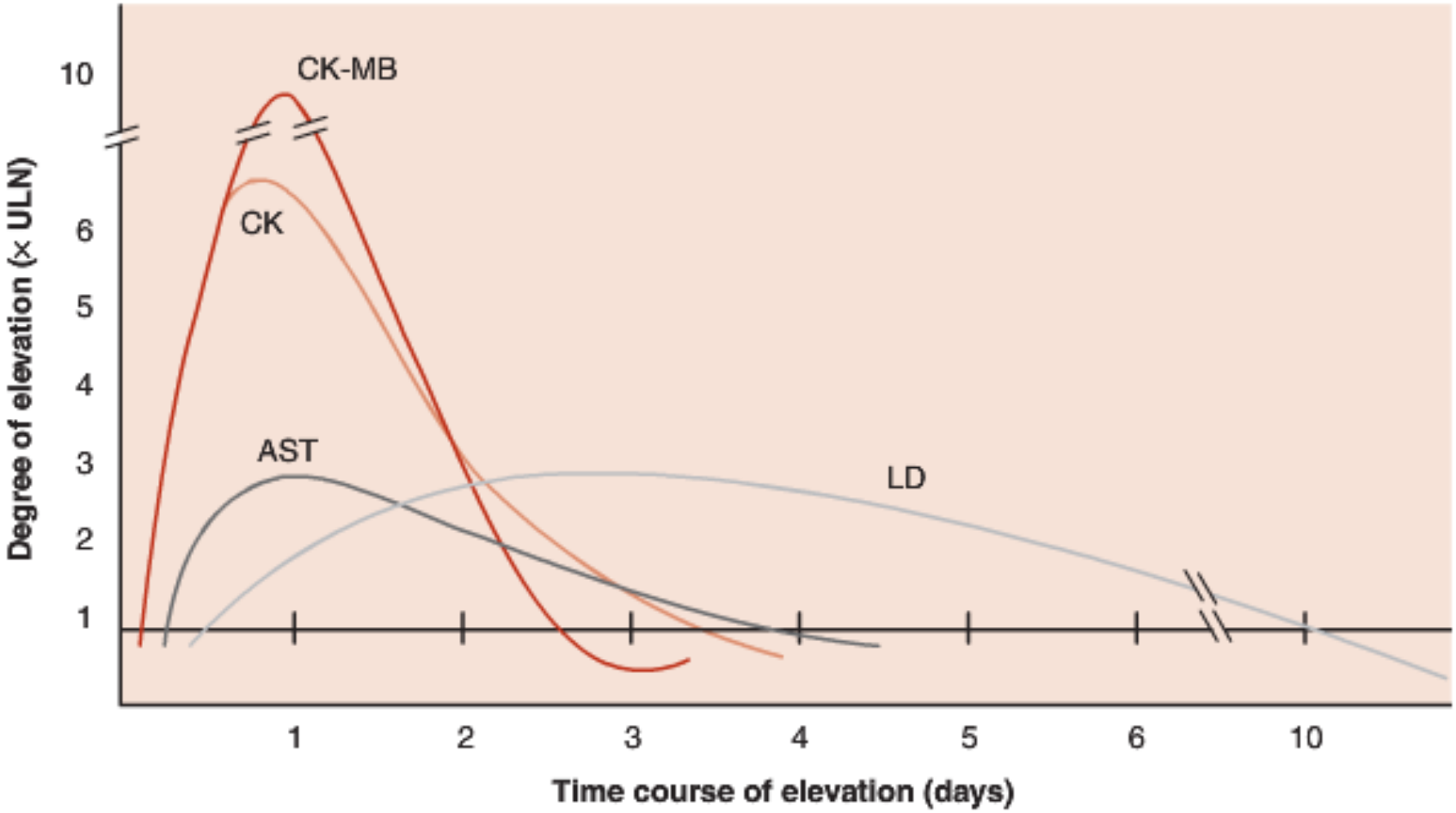
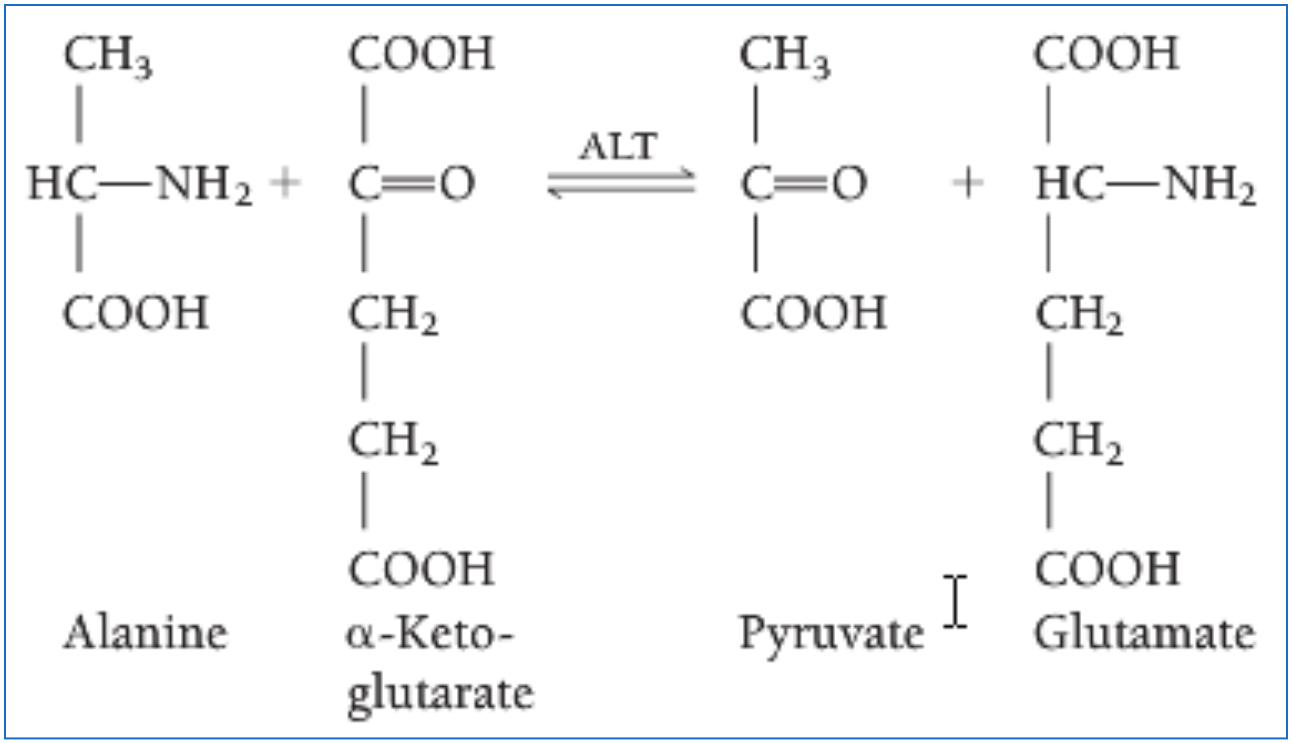


FIGURE 13-6 Time-activity curves of enzymes in myocardial infarction for aspartate aminotransferase (AST), creatine kinase (CK), CK-MB, and lactate dehydrogenase (LD). CK, specifically the MB fraction, increases initially, followed by AST and LD. LD is elevated the longest. All enzymes usually return to normal within 10 d. ULN, upper limit of normal.

Alanine aminotransferase (ALT)

- Formerly called serum glutamate-pyruvate transaminase (SGPT or GPT)
- Rxn catalyzed:



ALT: tissue source

- ALT is distributed in many tissues, with comparatively high concentrations in the liver.
- It is considered the more liver-specific enzyme of the transferases.
- Half life is 24 hrs
- Located in cytoplasm

Transaminase activities in human tissues, relative to serum as unity

	AST	ALT
Heart	**7800**	**450**
Liver	**7100**	**2850**
Skeletal muscles	5000	300
Kidney	4500	1200
Pancreas	1400	130
Spleen	700	80
Lungs	500	45
Erythrocytes	15	7
Serum	1	1

ALT: diagnostic significance

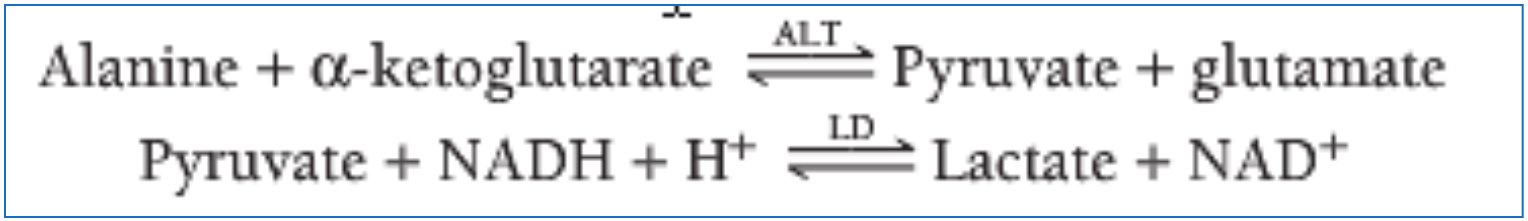
- ALT assays are confined mainly to evaluation of hepatic disorders.
- Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders
- In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain elevated longer

ALT: diagnostic significance/ cont'd

- Cardiac tissue contains a small amount of ALT activity, but the serum level usually remains normal in AMI unless subsequent liver damage has occurred

ALT assay

- Optimum pH 7.3-7.8



Source of error

ALT is stable for 3-4 days at 4C
Relatively unaffected by hemolysis

Ref Ranges:

ALT: 7-45 U/L, (37C) (0.1-0.8 ukat/L)

AST/ALT ratio

- The AST/ALT ratio was described by Fernando De Ritis in 1957 & hence known as De Ritis ratio

Table 1. Relative activity of transaminases in human tissues.*

	AST Activity	ALT Activity	AST/ALT Ratio
Liver	7,100	2,850	2.5
Kidney	4,500	1,200	3.8
Heart	7,800	450	17
Muscle	5,000	300	17
Serum	1	1	1.0

*Adapted from King J. Practical Clinical Enzymology, 1965.[3]

Ref: Botros & Sikaris (2013) Clin Biochem Rev 34:117-130.

De Ritis ratio

- De Ritis ratio or AST:ALT ratio is useful in differentiating between causes of liver diseases
- In healthy people, ALT>AST, when AST>ALT, then a disease is likely
- Ratio is less useful when liver enzymes are not elevated or multiple conditions exist
- The values of the ratio are not accepted as a reference range & it is difficult to define “healthy” limits of the ratio

Table 2. Clinical decision limits that can be applied to the De Ritis ratio. Healthy limits are derived from reference 162.

	De Ritis Ratio Decision Limit			
Condition	**<1.0**	**1.0 to <1.5**	**1.5 to <2.0**	**≥ 2.0**
Healthy	Women (up to 1.7) Men (up to 1.3)		Children	Neonate
Acute Viral Hepatitis	Resolving		Worsening	Fulminant
Alcoholic Hepatitis	Resolving		Alcohol Abuse	Acute Hepatitis
Chronic Liver Disease	Stable	Fibrosis risk		Other Causes
Muscle Disease	Chronic	Resolving		Acute

Ref: Botros & Sikaris (2013) Clin Biochem Rev 34:117-130.

CASE STUDY 13-2

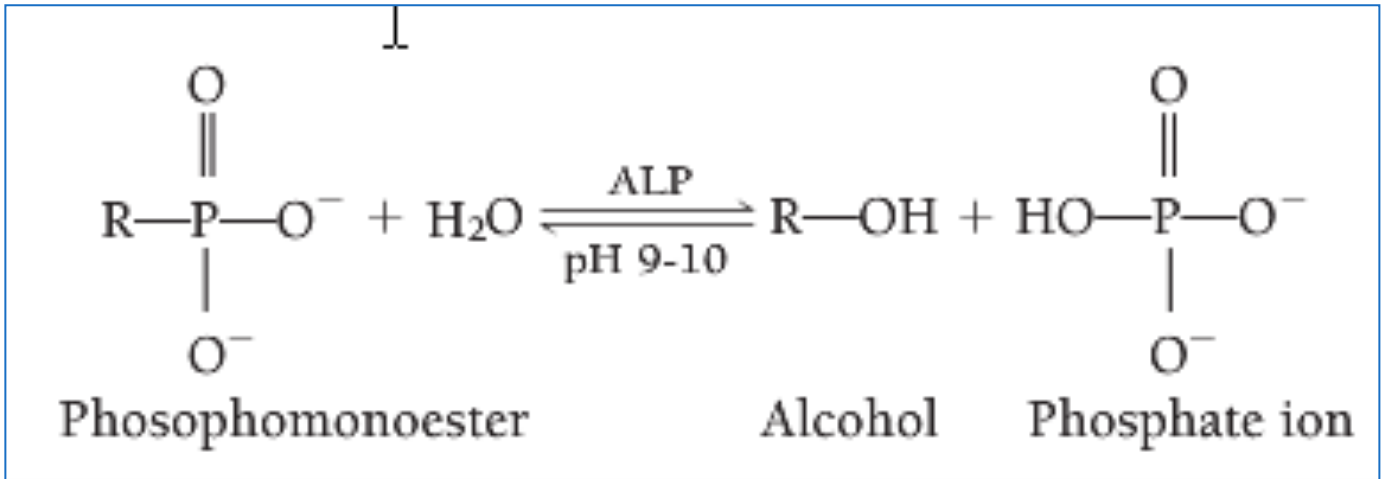
While a 71-year-old woman is walking home from a shopping center, she faints and falls. She is driven home by a friend. When home, she realizes that she is bleeding from her mouth and is slightly disoriented. She appears injured from the fall, but she does not remember tripping or falling. The woman is taken to a local emergency department. The examining physician determines that there was a loss of consciousness; to determine the reason, he orders a head CT and ECG and the following laboratory tests: CBC, PT, aPTT, CK, LD, AST, and troponin T and troponin I. All tests are within normal limits. The woman is sutured for the mouth injuries and admitted to a 24-hour observation unit.

Questions

1. What possible diagnoses is the physician considering?
2. What laboratory tests would be elevated at 6, 12, and 24 hours if this patient had an AMI?
3. What isoenzyme tests would be useful with this patient?

Alkaline phosphatase (ALP)

- ALP belongs to a group of enzymes that catalyze the hydrolysis of various phosphomonoesters at an alkaline pH.
- ALP is a nonspecific enzyme capable of reacting with many different substrates.
- Specifically, ALP functions to liberate inorganic phosphate from an organic phosphate ester with the concomitant production of an alcohol



ALP: tissue source

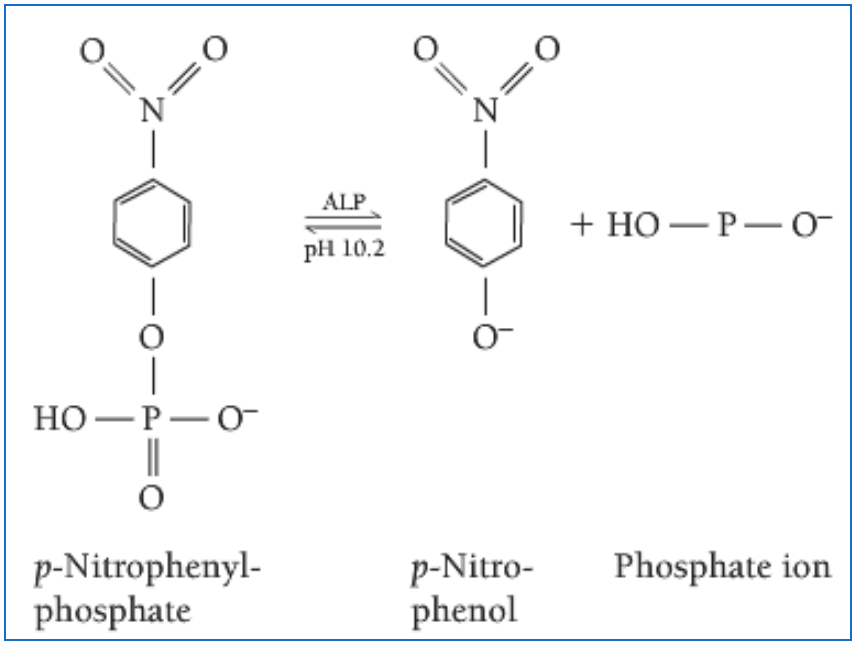
- ALP activity is present on cell surfaces in most human tissue.
- The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney.
- Hepatic ALP is most densely represented near the canalicular membrane of the hepatocyte. Thus, diseases that mainly affect hepatocyte secretion (e.g., obstructive diseases) will cause elevations of ALP levels.
- Bile-duct obstruction and primary biliary cirrhosis (PBC) are some examples of diseases in which elevated ALP levels are often predominant over transaminase level elevations.

ALP: diagnostic significance

- Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders.
- In hepatobiliary disorders, elevations are more predominant in obstructive conditions than in hepatocellular disorders;
- In bone disorders, elevations are observed when there is involvement of osteoblasts.
- In biliary tract obstruction, ALP levels range from 3 to 10 times the ULN.
- In hepatocellular disorders, such as hepatitis and cirrhosis, show only slight increases, usually less than three times the ULN.
- Due to overlapping of ALP elevations in different liver diseases, it assumes more diagnostic significance when evaluated along with other tests of hepatic function

ALP assay

- p-Nitrophenylphosphate (colorless) is hydrolyzed to p-nitrophenol (yellow), and the increase in absorbance at 405 nm, which is directly proportional to ALP activity, is measured.



Reference Range

ALP (total) (37°C)

Males/Females	4–15 y	54–369 U/L (0.9–6.3 µkat/L)
Males	20–50 y	53–128 U/L (0.9–2.1 µkat/L)
	≥60 y	56–119 U/L (0.9–2.0 µkat/L)
Females	20–50 y	42–98 U/L (0.7–1.6 µkat/L)
	≥60 y	53–141 U/L (0.9–2.4 µkat/L)

- Sources of error:
  - Hemolysis may cause slight elevations because ALP is 6x more conc in RBCs than in serum
  - ALP should be assayed as soon as possible, because its activity increases 3-10% upon standing at 25 or 4C for several hrs
  - Values may be 25% higher following ingestion of fat meal

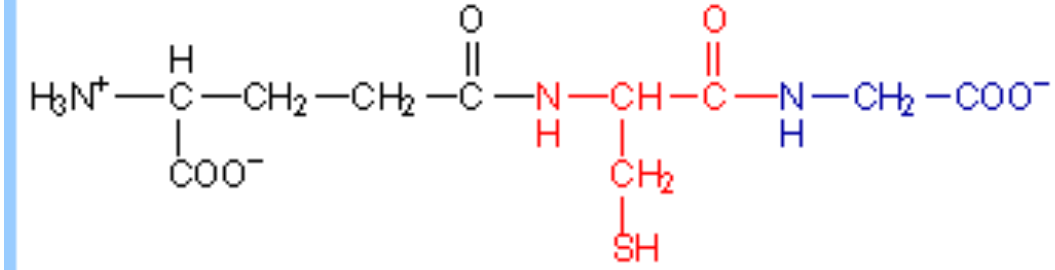
Category of liver disease by predominant serum enzyme abnormalities

Test	Liver disease category		
	Hepatocellular	Cholestatic	Infiltrative
AST, ALT higher than ALP level	Typical	--	--
ALP higher than AST, ALT levels	--	Typical	--
Elevation of ALP with near-normal AST, ALT levels	--	Typical	Typical

Cholestasis: a decrease in bile flow due to impaired secretion by hepatocytes or to obstruction of bile flow through intra-or extrahepatic bile ducts

Gamma-glutamyltransferase (GGT)

Glutathione + amino acid $\overset{\text{AST}}{\rightleftharpoons}$ Glutamyl peptide + L-cysteinylglycine



GGT: diagnostic significance

- GGT activity is found primarily in tissues of the kidney, brain, prostate, pancreas, and liver.
- In the liver, GGT is located in hepatic cells and particularly in the epithelial cells lining the biliary ductules.
- GGT is elevated in virtually all hepatobiliary disorders. But it's one of the first liver enzymes to rise in blood when any of the bile ducts become obstructed, for example, by tumors or stones. This makes it **one of the most sensitive of enzyme for detecting bile duct problems**

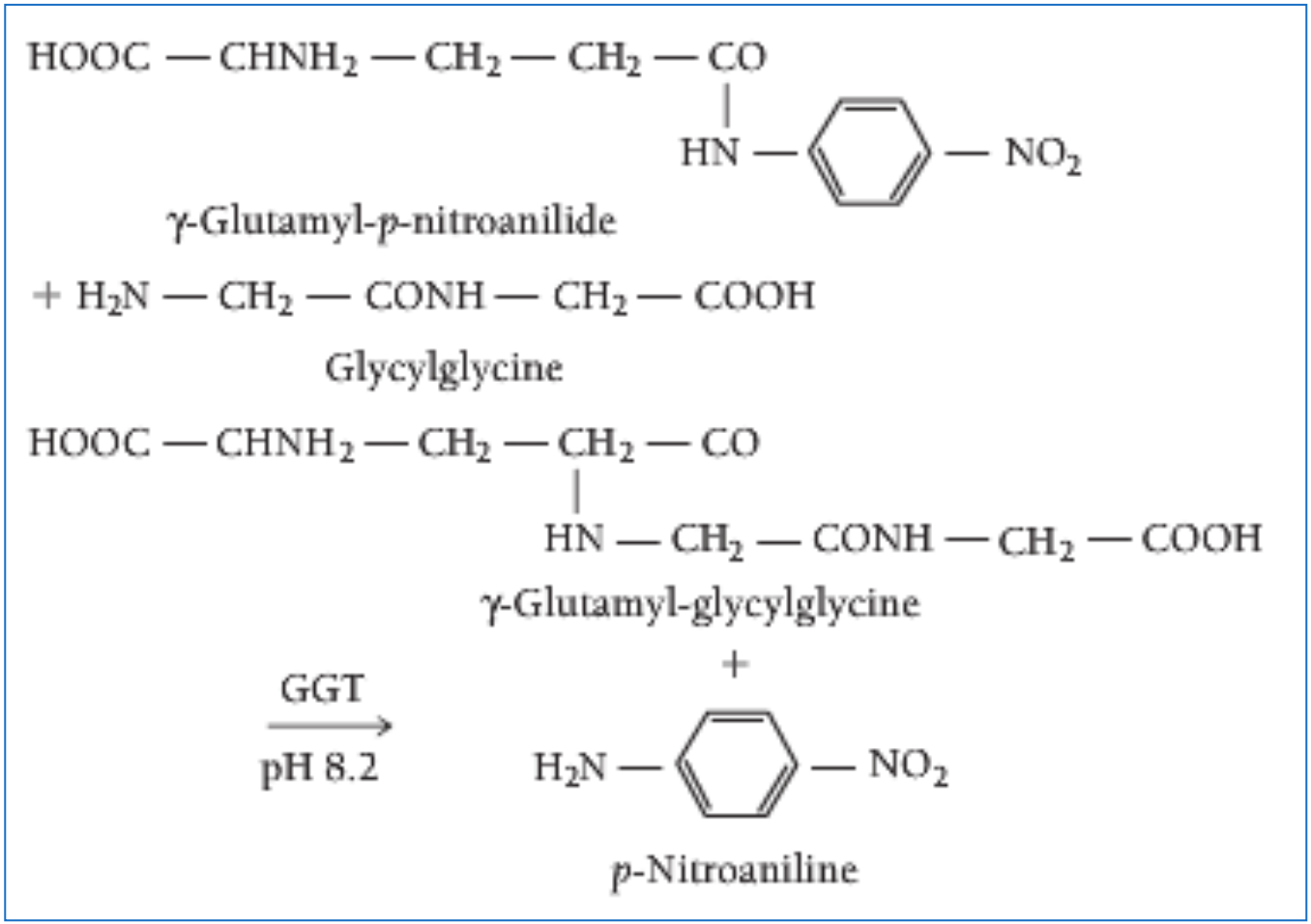
- GGT levels will be increased in patients receiving enzyme-inducing drugs such as warfarin, phenobarbital, and phenytoin.
- Enzyme elevations may reach levels four times the ULN.
- Heavy alcohol drinkers have GGT levels 2-3XULN
- Used to monitor alcohol abstention, where levels return to normal within 2-3 weeks of alcohol abstention

- GGT levels are elevated in acute pancreatitis, diabetes and MI, but their diagnostic values in these conditions is limited
- Both GGT and ALP are increased in liver diseases, but only ALP will be increased in diseases affecting bone tissue. Therefore, GGT can be used as a follow up to an elevated ALP to help determine if the high ALP result is due to liver or bone disease.
- GGT activity is useful in differentiating the source of an elevated ALP level because GGT levels are normal in skeletal disorders and during pregnancy.

How GGT is used?

- GGT test is used to determine the cause of elevated ALP. Both ALP and GGT are elevated in liver diseases, but only ALP will be elevated in bone disease.
- The GGT test is sometimes used to help detect liver disease and bile duct obstructions. It is usually ordered in conjunction with ALT, AST, ALP, and bilirubin.
- GGT can be used to screen for chronic alcohol abuse (it will be elevated in about 75% of chronic drinkers) and to monitor treatment for alcoholism or alcoholic hepatitis.

GGT assay



GGT: sources of error

- GGT is stable for 1 week at 4C
- Hemolysis does not affect GGT, because enzyme is lacking in RBCs

- Ref ranges:
- Male, 6-55 U/L (37C)
- Female, 5-38 U/L

Enzymes of Clinical Significance (cont'd)

- Acid Phosphatase (ACP)
  - **Tissue source:** prostate, bone, liver, spleen, kidney, erythrocytes, platelets
  - **Diagnostic significance:** prostatic carcinoma, hyperplasia of prostrate, prostatic surgery, osteoclasts, Paget's disease, breast cancer with bone metastases, Gaucher's disease
  - **Assay for enzyme activity:** same techniques as in alkaline phosphatase, except performed in an acid pH
  - **Source of error:** Serum should be separated from red cells as soon as blood has clotted; serum should be used immediately, frozen, or acidified.
  - **Reference range:** prostatic ACP: 0–3.5 ng/mL

Enzymes of Clinical Significance (cont'd)

- Amylase (AMS)
  - **Tissue source:** acinar cells of pancreas & salivary glands
  - **Diagnostic significance:** acute pancreatitis, disorders causing salivary gland lesions (mumps, parotitis), intraabdominal diseases
  - **Assay for enzyme activity:** four main approaches: amyloclast, saccharogenic, chromogenic, continuous monitoring
  - **Source of error:** stable for 1 week at room temperature & 2 months at 4°C; plasma triglycerides in acute pancreatitis with hyperlipemia; administration of morphine & other opiates
  - **Reference range:** serum: 28-100 U/L; urine: 1–15 U/hour

Enzymes of Clinical Significance (cont'd)

- Lipase (LPS)
  - **Tissue source:** primarily in pancreas; also in stomach & small intestine
  - **Diagnostic significance:** acute pancreatitis, other intraabdominal diseases (penetrating duodenal ulcers, perforated peptic ulcers, intestinal obstruction, acute cholecystitis)
  - **Assay for enzyme activity:** estimation of liberated fatty acids (triolein is substrate), turbidimetric methods, colorimetric methods
  - **Source of error:** stable in serum for 1 week at room temperature & 3 weeks at 4°C; hemolysis
  - **Reference range:** <38 U/L

Enzymes of Clinical Significance (cont'd)

- Glucose-6-Phosphate Dehydrogenase (G-6-PD)
  - **Tissue source:** adrenal cortex, spleen, thymus, lymph nodes, lactating mammary gland, erythrocytes
  - **Diagnostic significance:** G-6-PD deficiency (an inherited sex-linked trait), which can be clinically manifested in drug-induced hemolytic anemia; myocardial infarction, megaloblastic anemias
  - **Assay for enzyme activity:** A red cell hemolysate is used to assay for deficiency of enzyme; serum is used for evaluation of enzyme elevations.
  - **Reference range:** 7.9-16.3 U/g Hgb

Enzymes of Clinical Significance (cont'd)

- Drug Metabolizing Enzymes
  - Transform xenobiotics into inactive, water-soluble compounds for excretion through kidneys
  - Transform inactive prodrugs into active drugs, convert xenobiotics into toxic compounds, prolong elimination half-life
  - Catalyze addition or removal of functional groups through hydroxylation, oxidation, dealkylation, dehydrogenation, reduction, deamination, desulfuration (Phase I reactions)
  - Phase I reactions are often mediated by cytochrome P450 (CYP 450).