CLINICAL ENZYMES

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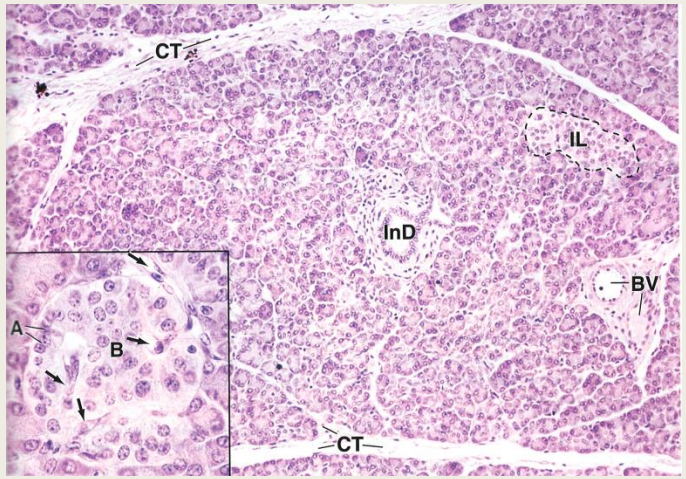
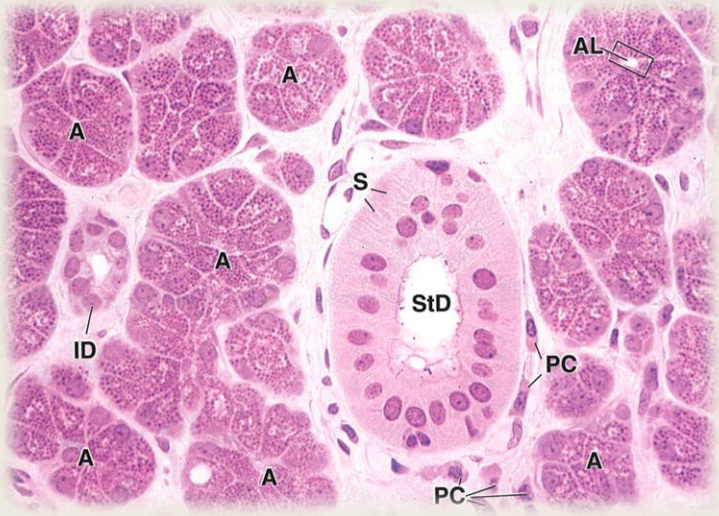
Amylase (AMY/AMYL)

- Hydrolase that breaks down starch and glycogen
  - *Requires Cl^- and Ca^{2+} activators*
  - *Acinar cells of pancreas and salivary glands are sources of amylase*
    - Smallest enzyme, found in urine
    - Salivary amylase deactivated by stomach
  - *Acute pancreatitis- AMY rises in 5-8 hours, peaks @ 24, and normal at 3-5 days*





Amylase

- Somogyi Units
  - *Specifically amount of reducing sugar liberated (multiple products) We don't use.*
  - *U/L with our own ref. range*
- Measurement Methods
  - *Separate out salivary with wheat germ lectin*
  - *Immunoassay for specific*

  Isoenzymes
- Macroamylasemia: benign

TABLE 13-6	AMYLASE METHODOLOGIES
Amyloclastic	Measures the disappearance of starch substrate
Saccharogenic	Measures the appearance of the product
Chromogenic	Measures the increasing color from production of product coupled with a chromogenic dye
Continuous monitoring	Coupling of several enzyme systems to monitor amylase activity

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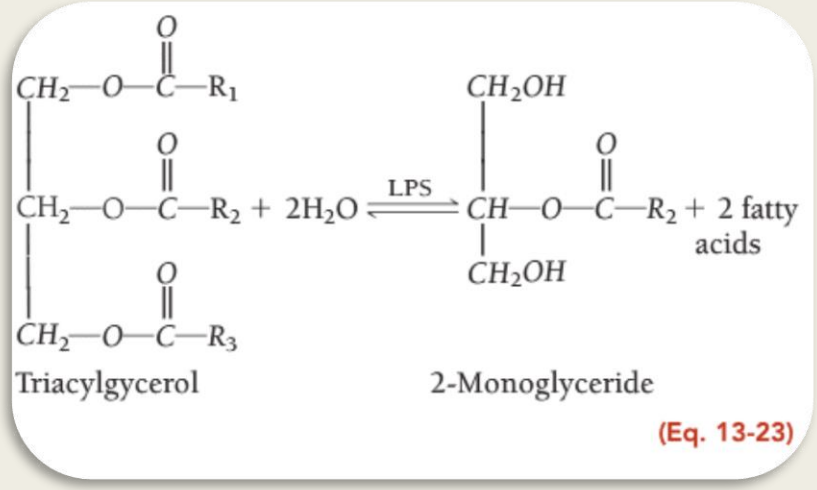
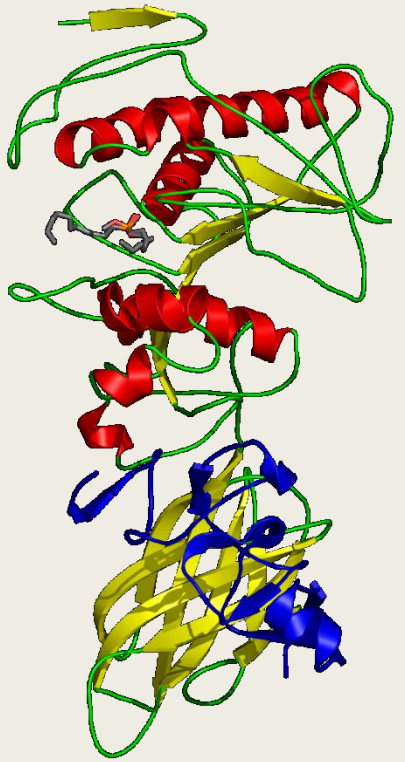
Lipase (LIPA/LPS)

- Hydrolyzes ester bonds to produce alcohol and fatty acids
  - *Specifically partially hydrolyzes triglyceride into 2-monoglyceride and 2 fatty acids*
  - *Pancreatic lipase is specific for the fatty acids at positions 1 & 2*
    - Substrate must be emulsified to occur, bile salts and colipase accelerate



Lipase



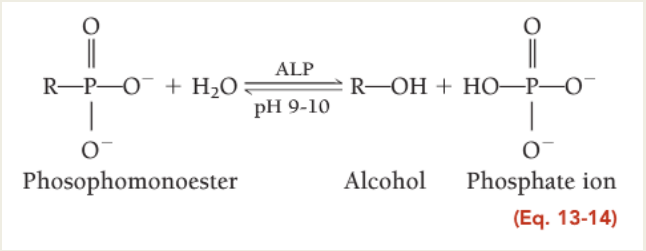
- Lipase is primarily found in pancreas
  - *Specific for pancreas!*
  - *Increases 4-8 hours after acute pancreatitis*
  - *Peaks at 24 hours*
  - *Normal at 8-14 days*
    - Longer lasting marker for pancreatitis
- Assay
  - *Early method Cherry-Crandall used olive oil to measure*
  - *Turbidimetric assay sees decrease in turbidity caused by fats*
  - *Colorimetric also based on glycerol kinase coupled reaction*

Pancreatitis

- Exocrine secretions digest foods
  - *Safeguards prevent activation of enzymes*
  - *Intrapancreatic secretion and activation*
    - “autodigestion”
- Precipitated by alcohol abuse, gallstones, trauma, [extremely] high triglycerides
  - *Treatments usually supportive*
  - *TPN only has given way to EN (Enteral Nutrition)*

Alkaline phosphatase (ALp)

- Group of enzymes that catalyze hydrolysis of phosphomonoesters at alkaline pH
  - *Liberates inorganic phosphate from organic molecule*
  - *Production of alcohol as a result*



  - *Optimal pH 9-10 but varies with substrate*
    - Requires Mg^{2+} as activator

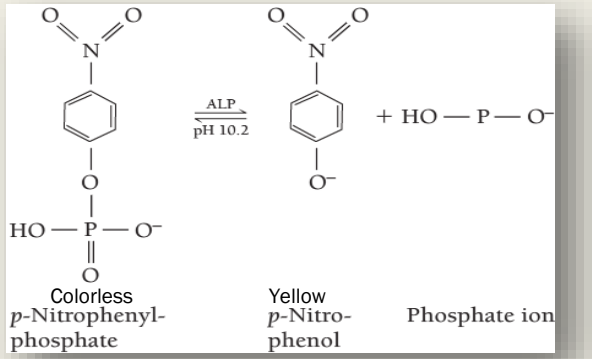
- Present on most cell outer surfaces
  - *Liver, bone, spleen, intestine, placenta and kidneys are highest*

Alkaline phosphatase

- Diagnostic Significance (specificity?)
  - *Liver and Bone*
    - Biliary tract obstruction- inducible enzyme will rise high
    - Bone disorders
      - *Paget's, osteomalacia & rickets, hyperparathyroidism, osteogenic sarcoma*
      - *Also present after fracture and during bone growth*
  - *Pregnancy- rises significantly from placental origin*

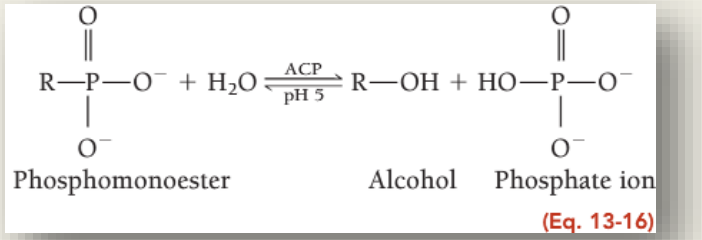
Alkaline phosphatase

- *Isoenzymes*
  - Electrophoresis to separate, Liver fastest, then bone, placental, intestinal
  - Heat stability, measure before and after 56ºC for 10’
    - *Liver lives if <20% left, bone is source if >20% liver is source*
      - Placental even more, will resist 65ºC for 30’
  - Chemical inhibition
    - *Phenylalanine inhibits intestinal and placental ALP more than liver and bone*
  - Regan and Nago isoenzymes- carcinoplacental ALP
    - *Occur in 3-15% of cancer patients, ectopically produced*
- Assay: leverage nonspecificity
  - *Bower & McComb*



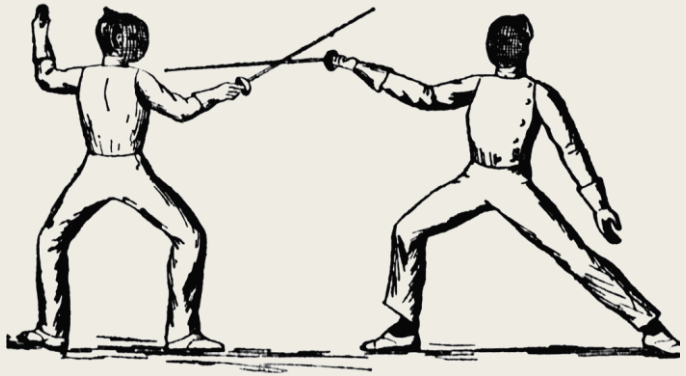
Acid Phosphatase (ACP)

- Hydrolase that catalyzes similar reactions to ALP
  - *Ideal pH is approx. 5.0*



  - *Found in prostate, bone, liver, spleen, kidney, RBCs, platelets*
    - Prostate is largest source
    - Insensitive- Only detects prostate cancer after metastasis
      - *Replaced by PSA*
    - Inhibition: Prostatic ACP inhibited by tartrate
  - *Other usage? Rape kit test for ACP + up to 4 days*

Phosphatases Side-by-Side



Alkaline Phosphatase

- Optimal pH 9-10
- Tissue source: Liver, bone, spleen, intestine, placenta, kidneys
- Undemanding preanalytics
- Routine part of CMP, HFP

Acid Phosphatase

- Optimal pH ~5
- Tissue source: prostate
- Special storage (buffered, frozen, NO HEMOLYSIS)
- Esoteric test, rarely performed

Aspartate aminotransferase (AST)

- Transferase/transaminase
  - *Moves amino group from aspartate to α-keto acid*
    - May be called SGOT (old name)
  - *Requires pyridoxal phosphate coenzyme*
    - Ketoacids formed are eventually used in TCA cycle
  - *Mostly found in heart, liver, skeletal muscle, with little in kidney, pancreas, RBCs*
    - Largely raised in liver disorders and skeletal muscle issues
      - *Also seen in cases of pulmonary emboli*
      - *In cirrhosis some elevation 4xULN vs. hepatitis 100xULN*
- Assay:

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

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

(Eq. 13-11)

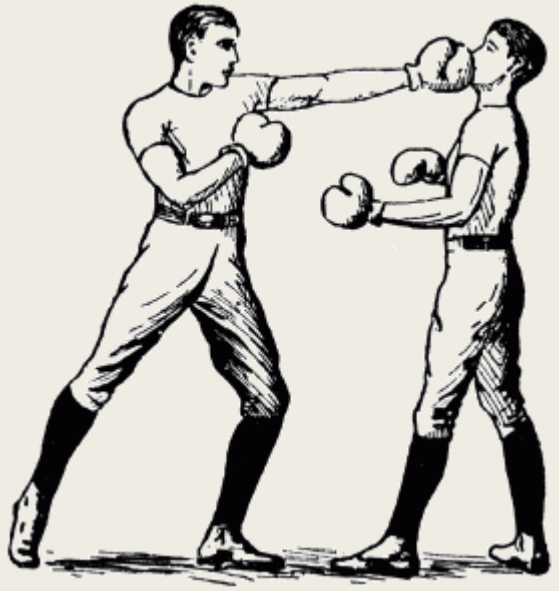
Alanine aminotrasferase (alt)

- Another transferase similar to AST
  - *Also requires pyridoxal phosphate coenzyme*
  - *May also be called SGPT*
- Distributed widely by highly concentrated in liver
  - *More specific for hepatic disorders*
  - *ALT often rises higher than AST, remains high longer*
- Assay: occurs best at pH 7.3-7.8

$$\text{Alanine} + \alpha\text{-ketoglutarate} \underset{}{\overset{\text{ALT}}{\rightleftharpoons}} \text{Pyruvate} + \text{glutamate}$$
$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \underset{}{\overset{\text{LD}}{\rightleftharpoons}} \text{Lactate} + \text{NAD}^+$$

(Eq. 13-13)

Transferases Side-by-Side



AST

- Found in liver, heart, skeletal muscle, RBCs
- Forms oxaloacetate and glutamate
- Requires pyridoxal phosphate coenzyme
- Rises in cases of pulmonary emboli, cirrhosis (mild), hepatitis (steep)

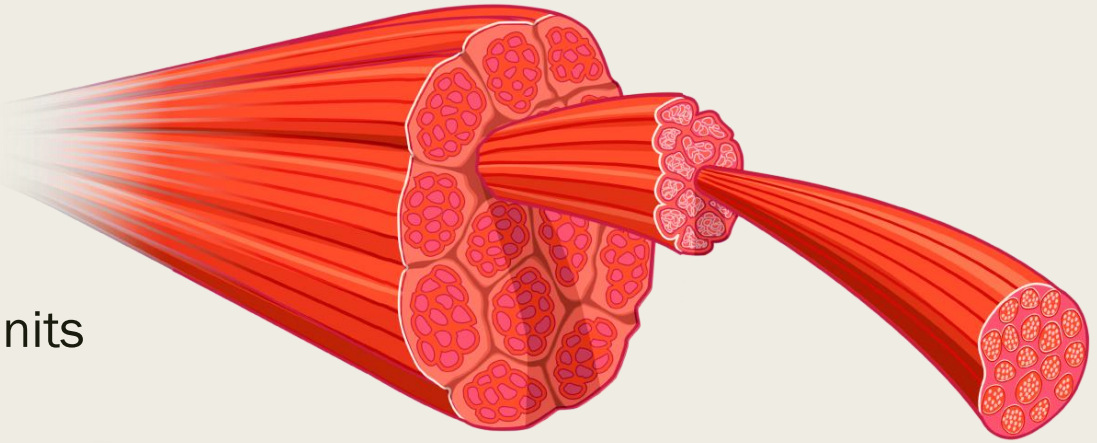
ALT

- Very liver specific
- Forms pyruvate and glutamate
- Requires pyridoxal phosphate coenzyme
- Rises higher than AST, but in only from liver disorders

γ-Glutamyltransferase (GGT)

- Transfers γ-glutamyl group from peptides to amino acids
  - *Clinically only important for the liver and biliary tract*
- Very sensitive to hepatobiliary disorders
  - *High elevations largely seen in blockage of biliary tract*
- Inducible enzyme
  - *Also raised by some drugs (warfarin, phenobarbital, phenytoin)*
  - *May indicate chronic alcoholism, monitor compliance*
- Assay conversion to a colored compound
  - *γ-glutamyl p-nitroaniline (GGPNA)*

Creatine Kinase (CK)



- ATP regeneration in muscle
- Dimeric enzyme composed of M and/or B subunits
  - *CK1 (BB)- Brain, most anodal*
    - Short half-life doesn't contribute to total
  - *CK2 (MB)- Heart*
    - Predominantly in heart, but also skeletal muscle
  - *CK3 (MM)- Muscle, most cathodal*
    - Predominantly skeletal, but also cardiac muscle

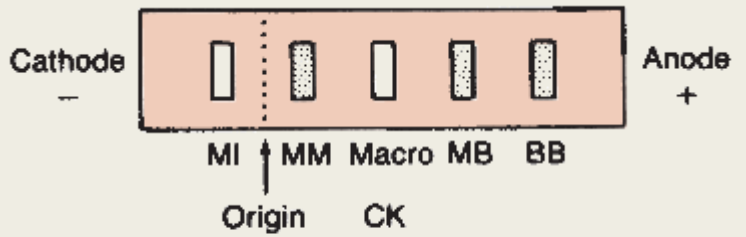
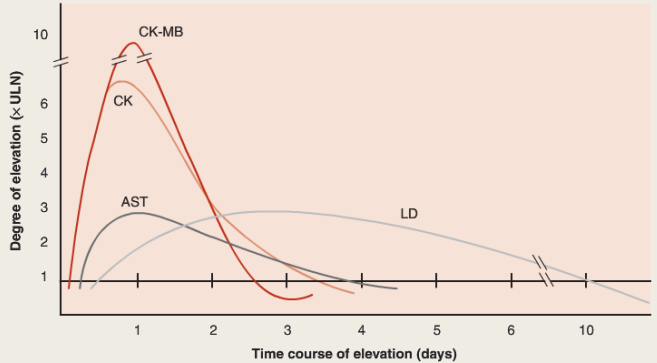


FIGURE 13-5 Electrophoretic migration pattern of normal and atypical creatine kinase (CK) isoenzymes.

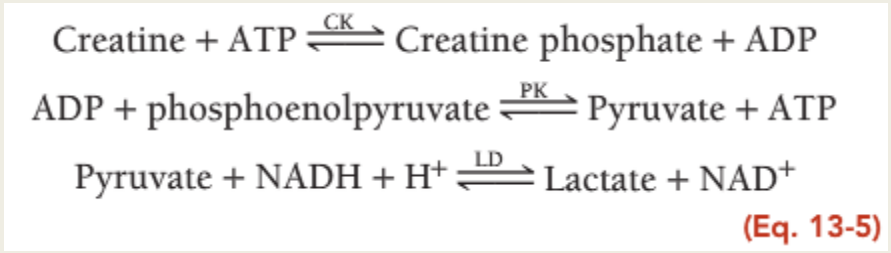
Creatine Kinase

- Significance
  - *Frequently seen in disorders of cardiac and skeletal muscle*
    - MI, rhabdomyolysis, muscular dystrophy
      - *Extremely high in Duchenne MD 50-100x ULN*
  - *Not very specific for MI, also seen in strokes, seizures, nerve degeneration, CNS shock etc…*
  - *Separation into isoenzymes improves specificity*
    - In healthy person <6% of total is MB, mostly MM ~ 94%
    - CK-MB is high in cardiac tissue
      - *Begin to rise after 4-8 hours, peak at 12-24 hours, normal at 48-72 hours*
    - Replaced by *troponin*, released earlier and lasts longer

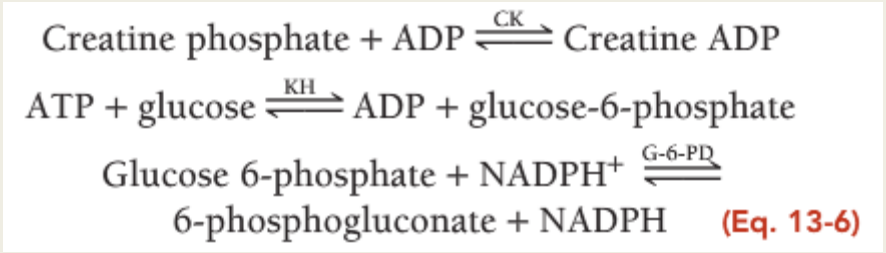


Creatine Kinase

- Assay: can go either way with PK & LD

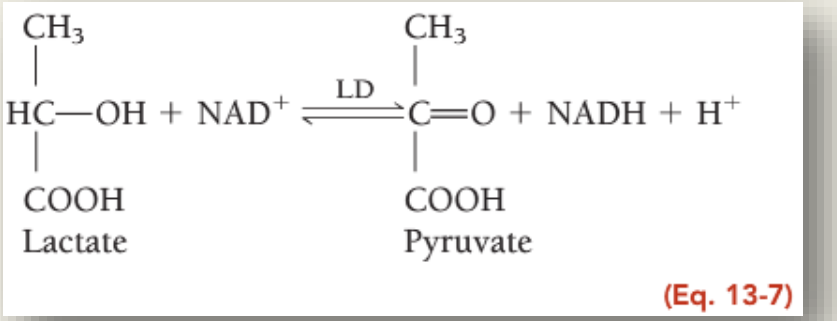


- Or with Hexokinase and G6-PDH



Lactate Dehydrogenase (LD)

- Converts lactic acid to pyruvic acid and generates NADH



- High amounts found in the heart, liver, muscle, kidney, and RBCs
  - *Elevated in numerous disorders*
    - Cardiac, hepatic, skeletal muscle, renal, hematological, and neoplasms
    - Highest total levels from anemias

Lactate dehydrogenase

- Viral hepatitis, cirrhosis
  - *Minimal increase*
- AMI & pulmonary infarct
  - *Same minimal increase*
    - LD rises within 12-24 hours, peaks at 24-48 hours, stays up for 10 days
- Leukemia
  - *Marked elevation in ALL possible*
- Isoenzymes allow greater specificity

TABLE 13-5 LACTATE DEHYDROGENASE (LD) ISOENZYMES AS A PERCENTAGE OF TOTAL LD

ISOENZYME	%
LD-1	14–26
LD-2	29–39
LD-3	20–26
LD-4	8–16
LD-5	6–16

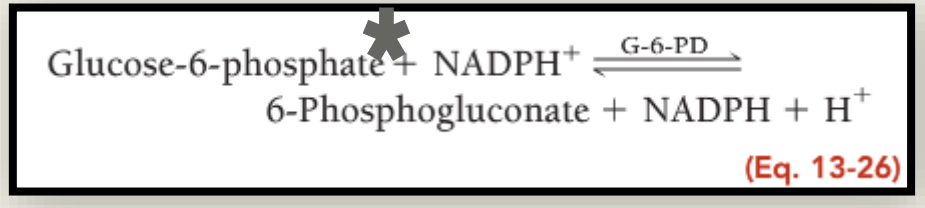
Source: Lott JA, Stang JM. Serum enzymes and isoenzymes in the diagnosis and differential diagnosis of myocardial ischemia and necrosis. *Clin Chem*. 1980;26:1241.

Lactate Dehydrogenase

- Each enzyme consists of 4 peptide chains
  - *LD-1 (HHHH)- Heart & RBCs*
  - *LD-2 (HHHM)- Heart and RBCs*
  - *LD-3 (HHMM)- Lung, WBCs, spleen, pancreas*
  - *LD-4 (HMMM)- Liver*
  - *LD-5 (MMMM)- Skeletal muscle*
- In AMI and intravascular hemolysis LD-1 > LD-2
  - *This is not the normal state, so this is known as the LD flipped pattern*
- LD-6?
  - *Alcohol dehydrogenase that migrates to LD-5 spot*

Glucose-6-Phosphate dehydrogenase (G-6-PD)

- Oxidoreductase that is part of the pentose phosphate shunt glucose metabolism
- Protects cells from oxidative damage
  - *Tissue sources: adrenal cortex, spleen, thymus, lymph nodes, mammary gland, and RBCs*
    - Little activity in normal serum
  - *Most of its importance is in the RBC*
    - Maintains NADPH in reduced form
      - *Insufficiency results in low NADPH, exposure to oxidizing agents, cells will burst from damage*
  - *Deficiency is Sex-Linked trait*
    - Drug induced hemolytic anemia



* Sample is hemolysate

Cytochrome Oxidase

- Cytochrome p450 oxidase (CYP 450)
  - *Superfamily of enzymes (absorb light at 450 nm)*
    - Metabolize more than 50% of all drugs
    - More than 500 different enzymes
  - *Different alleles allow different speeds of drug metabolism*
  - *Also found in steroid forming tissues*
    - Synthesize steroid hormones from cholesterol
- Pharmacogenomics depends upon these enzymes to determine effective therapies

Cholinesterases

- Break down choline neurotransmitters
- Target of organophosphate pesticides and some therapeutic drugs
  - *Acetylcholinesterase (AChE) intracellular*
  - *Pseudocholinesterase or Serumcholinesterase (SChE) extracellular*
    - Butyrylcholinesterase (BChE) preferred, new name
      - *Butyrylcholine a synthetic choline used to distinguish from AChE*
- Sarin, Tabun, Soman, VX
  - *Chemical weapons, nerve gas*
- Chronic toxicity- wide neurological symptoms
- Ref: 4-12k U/L
  - *Symptoms with a decrease of 40%*