Biological Chemistry: Introduction to The Chemistry of Life

Amino Acids and Proteins

• Any time one deals with anything in Biology, one must also contend with proteins: the products of gene activation.
• To understand proteins, it is necessary to understand amino acids, to learn their structures and to learn a few of the functions and essentiality of the amino acids.
• There are 20 amino acids and 1 imino acid we will study:

<table>
<thead>
<tr>
<th>Glycine (gly)</th>
<th>Cysteine (cys)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine (ala)</td>
<td>Cystine (cys-cys)</td>
</tr>
<tr>
<td>Valine (val)</td>
<td>Threonine (thr)</td>
</tr>
<tr>
<td>Leucine (leu)</td>
<td>Methionine (met)</td>
</tr>
<tr>
<td>Isoleucine (ile or ileu)</td>
<td>Aspartic Acid (aspartate; asp)</td>
</tr>
<tr>
<td>Proline (pro)</td>
<td>Asparagine (asn)</td>
</tr>
<tr>
<td>Phenylalanine (phe)</td>
<td>Glutamic Acid (glutamate; glu)</td>
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<tr>
<td>Tyrosine (tyr)</td>
<td>Glutamine (gln)</td>
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<tr>
<td>Tryptophan (trp)</td>
<td>Histidine (his)</td>
</tr>
<tr>
<td>Serine (ser)</td>
<td>Lysine (lys)</td>
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<tr>
<td></td>
<td>Arginine (arg)</td>
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</table>
• The simplest amino acid is glycine (gly).
• It consists of two carbon atoms covalently bonded to each other.
• To one carbon atom, two oxygen atoms are bonded; to the other carbon atom, an amino group (NH$_3^+$) and 2 hydrogen atoms are bonded.
• The carbon that is directly attached to the CO$_2^-$ is called the $\alpha$-carbon.
• It is this carbon that makes all amino acids used by man the $\alpha$-amino acids.
• Gly is typically found in proteins where there are turns in the amino acid sequence, as it is very small and has a small "R" group (a hydrogen).
• R groups are radical groups, representative groups or reactive groups.
• In this case, and for the case of all the amino acids, we will use the second definition of R group to mean the rest of the amino acid molecule beyond the 2d carbon in the backbone of the amino acid.
• Gly is an amino acid with an uncharged polar R group.
Zwitterion: due to water solvation/ionization
Both positive (+) and negative (-) charges per molecule
Normal under physiological conditions
Another way to look at Glycine.

The same manner may be used with the other 19 amino acids.
• The next simplest amino acid is alanine. The difference between ala and gly is that the H in gly has been replaced by a CH$_3$ in ala. Ala is a small amino acid, especially suited for diffusing from muscle cells into the blood to be transported by the blood to the liver for utilization in gluconeogenesis.
• Valine (val), leucine (leu) and isoleucine (ile or ileu) are the next three simplest amino acids.
• These three amino acids are called branched chain amino acids (BCAA's) and are utilized for the synthesis of substrates for gluconeogenesis and for ketogenesis.
• Leu is the only purely ketogenic amino acid.
• Ketones are usually associated with someone who has diabetes mellitus and who is in diabetic coma.
• It is the ketones, or ketone bodies, that give the patient the sweet, fruity smelling breath of diabetic coma.
• Proline is actually an \textit{imino} acid.
• Note that it is a closed ring amino acid.
• Pro, like gly, is usually found in proteins where turns are required.
• A derivative of proline, hydroxyproline is found in connective tissue and helps make the tissue stronger.
Phenylalanine is alanine with a benzene ring attached to it ($C_6H_6$).

Phenylalanine is necessary for the synthesis of the catecholamines dopa, dopamine, norepinephrine and epinephrine.

Some people are born lacking an enzyme that regulates the catabolism of phe.

When this happens, a metabolite of phe, phenylpyruvic acid, builds up in nervous tissue and causes severe mental retardation.

This condition is known as phenylketonuria, or PKU.

The people who have PKU are generally blonde, blue-eyed and fair complected.

The reason for this is that phe is also necessary for the synthesis of a pigment called melanin that contributes to eye, hair and skin color.

People who have PKU must eat a diet low in phe the rest of their life.

Since phe is required by the body to initiate the synthesis of the catecholamines for neurotransmitter and hormonal functions, people who have PKU must add tyrosine to their diet -- the product of the hydroxylation of phe that doesn't occur in PKU.
Methionine is a sulfur containing amino acid.

Its necessity is to provide the methyl group (CH₃) to acceptor molecules in one-carbon metabolism.

One-carbon metabolism is important in the production of red blood cells, white blood cells and platelets.
• Tryptophan (trp) is the last of the amino acids with hydrophobic R groups.
• Trp is the precursor for the synthesis of serotonin (aka nature's downer).
• Serotonin from the health food store will NOT cross the blood brain barrier; trp is required for this to occur.
• Turkey and milk have high levels of trp.
• There seems to be some controversy as to whether or not there is enough trp in milk (especially warm milk) to render a person drowsy so that they will fall asleep when it is difficult for them to do so without assistance.
• In recent times, selective serotonin reuptake inhibitors have seen use in depression, eating disorders, obsessive compulsive disorder, to name a few, e.g. prozac, celexa
Amino Acids with Uncharged Polar R Groups

At physiologic pH, the R groups are not ionized as are the amino and carboxyl groups of the amino acids.

- Glycine has already been mentioned.
- Serine (ser) is alanine with an -OH group replacing a -H.
- As a rule, ser has a function similar to that of threonine (thr), another hydroxylated amino acid: it serves as an activation site in enzymes, i.e., when it is phosphorylated or dephosphorylated, the enzyme is turned on or off.
- The last hydroxylated amino acid is tyrosine (tyr – with phe discussion).
- It is, simply, hydroxy phenylalanine, with the -OH group straight across the benzene ring from the alanine moiety.
- Tyr has been discussed, previously, as well.
• Cysteine (cys) is a sulfur containing amino acid.
• It is found in most connective tissues.
• The most often thought about site of cys, though, is the hair.
• Hair maintains its shape by the presence of disulfide bonds (S-S).
• The disulfide bonds come from the loss of -H from the -SH group of two cys molecules in the hair which then bond to hold the hair in its appropriate shape to form cystine (cys-cys).
• Cosmetologists, beauticians utilize this property every day when they give perms.
• They first reduce the natural disulfide bonds in hair, then place the hair in the shape the customer asks, then finish the job with an oxidizing agent that forces the formation of the disulfide bonds and, voila!, a new style comes out from under the curlers, drier, etc.
Asparagine (asn) and glutamine (gln) are 4 and 5 carbons in length, respectively.

They are derivatives of the dicarboxylic amino acids aspartate and glutamate (coming up below).

Note that each has an extra NH$_2$ group on the carbon double bonded to an oxygen farthest from the $\alpha$-carbon.

These two molecules serve as ammonia transporters to the liver and kidney for urea synthesis.

Urea is a small, non-toxic compound (compared to ammonia's effects on the cell) that is excreted via the urine.
The next two amino acids under study are the acids aspartate (asp) and glutamate (glu)--the precursor amino acids of asn and gln, respectively.

Both are dicarboxylic amino acids, i.e., there is a COOH group on each end of the molecules.
• Arginine (arg) and lysine (lys) have positively charged R groups at physiological pH.

• Lysine is heavily involved in connective tissue biosynthesis.

• Children with low levels of arginine tend to be mentally retarded (hypoargininemia).

• Arginine is the last product of the urea cycle from which urea is clipped for excretion.
• Histidine (his) is positively charged at a pH of approximately 6 or below.

• His is the precursor molecule to histamine, the compound that causes many allergic reactions and which may be blocked by the use of anti-histamines.

• Histamine synthesis may be stimulated by the influence of norepinephrine or psychological stress.

• Because of this, many people who have itching-related health problems may be prescribed a drug like doxepin which has both histamine antagonistic properties and anxiolytic properties: both of which combat the health problem by reducing the anxiety felt by the patient which reduces the itching, which reduces the anxiety which reduces the itching, ad nauseam.
• Of these 20 amino acids,
  – 8 are essential (humans require them in their diets as humans lack the enzymes to synthesize them from scratch) and
  – 2 are semi-essential (required for growth by the young human).

• The essential amino acids are phe, val, trp, thr, ile, met, lys, leu.

• The semi-essential amino acids are his and arg.

• A helpful mnemonic to remember these is: PVT TIM HALL, where the first letter of each amino acid makes up this mnemonic.
Acid-Base Titrations

HA $\leftrightarrow$ H$^+$ + A$^-$
Or
HA + MOH $\leftrightarrow$ HOH + MA
Mono-protic Acid

H$_2$A $\leftrightarrow$ 2H$^+$ + A$^{-2}$
Or
H$_2$A + 2MOH $\leftrightarrow$ 2HOH + M$_2$A
Di-protic Acid
HOAc Titration – Mono-Protic Acid

Potentiometric Titration of HOAc -- Trial 1

Actual Student’s Data at WNC, 2008-01
Acid-Base Titrations

\[ H_3A \leftrightarrow 3H^+ + A^{-3} \]

Or

\[ H_3A + 3MOH \leftrightarrow 3HOH + M_3A \]

Tri-protic Acid

Histidine Titration

![Graph of pH vs. mL 0.1 N NaOH Added with dpH/dV and dp/dV axes]
Alanine Titration

Glutamate Titration

\[
pK_{\text{cooh}} + pK_{\text{NH}_2} = pI = pK where \text{ AA has a net charge 0}
\]

\[
pK_{\text{cooh}} + pK_R = pI
\]
Electrophoresis

• Defined: separation of particles in a gel by an electrical charge
• Each entity migrates to its point of electrical neutrality: its pI
Peptides and Peptide Bond

- Amino acids are the building blocks of proteins.
- In order for the amino acids to link together to form the numerous proteins necessary to keep a human functioning, they form a special bond between each other: the peptide bond.
- The peptide bond is formed between the carboxyl group of the first amino acid and the amino group of the second amino acid to form a dipeptide.
- The peptide bond is unique in that it appears to be a single bond, but has the characteristic of a double bond, i.e., it is a rigid bond.
- This kind of bond only occurs between amino acids.
- As the amino acid chain increases, the next amino acid adds onto the previous carboxyl group by its amino group.
Peptide Bond Synthesis
Peptide Bond and Peptides

\[ \text{AA} + \text{AA} = \text{Dipeptide} \]
\[ \text{AA} + \text{AA} + \text{AA} = \text{Tripeptide} \]
\[ \text{AA} + \text{AA} + \text{AA} + \text{AA} = \text{Tetrapeptide} \]
\[ 10 \text{ OR MORE AA’s} = \text{Polypeptide} \]
• By convention, the left amino acid is always #1; is the free amino end or the N-terminus.
• The farthest amino acid residue to the right is the amino acid in the protein that has the highest number and, as a general rule, is the free carboxyl end or the C-terminus.
• In some cases, the -OH may be replaced with an NH$_2$, making it an amide.
• When dealing with peptides, there is always one LESS peptide bond than there are amino acid residues in the protein, i.e., a tripeptide has 2 peptide bonds and three amino acids; a hexapeptide has five peptide bonds and six amino acids, _ad nauseum_.
• The sequence[s] of the amino acids held together by peptide bonds ONLY is called the primary structure of a protein.
Secondary Structure of Proteins

• The secondary structure of proteins is determined by how the amino acid sequence (primary structure) folds upon itself and bonds with hydrogen bonds, i.e., non-covalent attractive forces.

• There are, for this course, 3 secondary structures:
  – $\alpha$-helix
  – $\beta$-pleated sheet
  – Thermodynamic random coil
• Left Image: A protein that coils on itself in a right handed turn is called an $\alpha$-helix -- Green = N; black = C; red = O, blue = H
• Right Image: The $\alpha$-helix permits tissues to stretch a bit, like hair. Note that the H bonds are between the carbonyl oxygen and the amino hydrogen. Only a PORTION of a protein is in alpha-helix, NOT the whole protein. Black = C; red = O; green = N; orange’ish = R groups
The second of the secondary structures of proteins is called the pleated sheet or, sometimes, the \( \beta \)-pleated sheet.

The pleated sheet is in the anti-parallel organization, i.e., the peptide chains making up the sheet are running in opposite directions to each other.

Pleated sheets tend to make proteins that do not "give", e.g., silk, it doesn't seem to be of great importance to other proteins.
Thermodynamic Random Coil

- The last secondary structure about which we have interest is the thermodynamic random coil.
- Although we call this a random coil, nature tells us that there is a reason for every structure.
- We call it random as we have not worked out the "code" of this structure.
- In addition, if we denature this structure, the protein loses its function.
Tertiary Structure

• The tertiary structure of a protein is, for all intents and purposes the three dimensional shape of the protein brought about by interaction forces of ionic, hydrophobic and covalent disulfide links of the one protein chain.

• Two examples include the β-chain of hemoglobin and the myoglobin molecule.
• Tertiary structure, put another way, is the manner in which the R groups assist the protein in secondary structure formation to fold, twist, bend, kink, AGAIN, upon itself.
• Water soluble proteins fold so that hydrophobic R groups are tucked inside the protein and hydrophilic R groups are on the outside of the protein. WHY?
• This way, the protein may interact with the solvent (water) and not precipitate or otherwise be inactivated.
• Water insoluble proteins fold so that hydrophilic R groups are tucked inside the protein and hydrophobic R groups are on the outside of the protein. WHY?
• This is so that a protein, e.g., an ion channel in a cell membrane, may insert itself in a non-polar environment so that polar particles may be transported into or out of regions compartmentalized from each other.
• Ionic interactions also stabilize tertiary structures.
• Where, though, are the ionic groups?
• They are the R groups! The carboxyl groups on asp and glu; the $\varepsilon$-amino group on lys; the guanidino group on arg; the imidazole ring on his.
# pK Values

## Approximate Functional Group pK Values

<table>
<thead>
<tr>
<th></th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK\text{COOH} (COO\text{-})</td>
<td>2</td>
</tr>
<tr>
<td>pK\text{NH}_2 (NH_3^+) {EXCEPT Pro – see below}</td>
<td>9.5</td>
</tr>
</tbody>
</table>

**pK\text{R}**

<table>
<thead>
<tr>
<th></th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH (COO\text{-}) (Glu and Asp)</td>
<td>4</td>
</tr>
<tr>
<td>Imidazole (His)</td>
<td>6</td>
</tr>
<tr>
<td>SH</td>
<td>8</td>
</tr>
<tr>
<td>OH</td>
<td>10</td>
</tr>
<tr>
<td>Imino group (Pro)</td>
<td>10.6</td>
</tr>
<tr>
<td>NH\text{\textsubscript{2}} (NH\text{\textsubscript{3}}^+) (\varepsilon\text{-}amine -- Lys)</td>
<td>10.5</td>
</tr>
<tr>
<td>Guanidino (Arg)</td>
<td>12</td>
</tr>
</tbody>
</table>
R groups “cross-link” to form a “salt link”

- Top: 2 individual protein chains linked
- Bottom: an intra-chain link
• Disulfide bonds assist in tertiary structure by allowing the protein chain to interconnect itself and introduce a hair-pin into its structure -- just like how straight hair is curled and curly hair is straightened out
Quaternary Structure -- Hgb

- The last structure of proteins in which we have interest is called the quaternary structure: the organization of two or more protein chains to bind together in such a manner as to give the group of proteins a single function, e.g., the tetramer of hemoglobin.
- The 4 proteins are held together by salt links, hydrophobic and hydrophilic interactions.
- In Hemoglobin, disruption of these forces (to form deoxy hemoglobin) cause the hemoglobin molecule to become smaller than oxy-hemoglobin.

- Tetramer – salt-linked

- Each protein contains a heme group

- Each heme group binds Fe $^{2+}$

- NOT Fe $^{3+}$
How Does Hemoglobin (Hb or Hgb) Bind Oxygen and Act as a Buffer?
Oxygen Binding Curve Shift Factors: “Bohr Effect”

<table>
<thead>
<tr>
<th>Left Shift</th>
<th>Right Shift</th>
</tr>
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<tbody>
<tr>
<td>• Alkalosis</td>
<td>• Acidosis</td>
</tr>
<tr>
<td>• ↓ 2,3-BPG</td>
<td>• ↑ 2,3-BPG</td>
</tr>
<tr>
<td>• Hypothermia</td>
<td>• Fever</td>
</tr>
<tr>
<td>• Fetal Hb (&gt; affinity for oxygen than adult Hb)</td>
<td>• Anemia</td>
</tr>
<tr>
<td>• ACD-preserved blood (acid citrate dextrose: ↑ O₂ carrying capacity of RBC &gt;2-3 days old (in bag) with ↑ Hb – PROBLEM: doesn’t release O₂ to tissues for 18-24 hours after infusion)</td>
<td>• Hypoxia</td>
</tr>
</tbody>
</table>
Haldane Effect

- With $\uparrow \text{Hb(O}_2\text{)}_4$, at some $p\text{CO}_2 \rightarrow \downarrow \text{CO}_2$ content of blood
- With $\downarrow \text{Hb(O}_2\text{)}_4$ at some $p\text{CO}_2 \rightarrow \uparrow \text{CO}_2$ content of blood
- “Back side of Bohr effect” – greater effect than the Bohr effect on gas transport.
ACIDOSIS
pH go down, HCO₃⁻ go down, pCO₂ go up
The graphic at right illustrates some of the intracellular processes clinicians must be able to treat pharmacologically when a person is experiencing a myocardial infarction with concurrent intracellular metabolic acidosis.

- Grey thick bars are the intercalated disks;
- Thin blue bars are the striations found in myocardiocytes;
- Red thick bars represent the cell membrane;
- Purple oval a Na\(^+\) -- Ca\(^{2+}\) exchange transport protein;
- Green rectangle a \(\text{H}^+\) -- Na\(^+\) transport protein;
- Numbers in yellow indicate the direction of ionic transport)

One of the biggest concerns clinicians have regarding heart health during a myocardial infarction (MI; heart attack) is that the [\(\text{H}^+\)] may increase due to a build-up (and dissociation) of lactate and/or fatty acids, which may contribute to a metabolic acidosis in the heart muscle, which will thus kill more and more heart muscle.

This process is rendered even more critical in that as the H\(^+\) are exchanged OUT of the cardiac cells to compensate for the intracellular metabolic acidosis, Na\(^+\) and Ca\(^{2+}\) exchange occurs leading to excessively high levels of Ca\(^{2+}\) in the cells which may progress to further cell, and, hence, organ, death.
Compensatory Mechanisms for Alkalosis

- Alkalosis: pH goes up, HCO₃⁻ goes up, pCO₂ goes down

- Condition causing excessive elimination of CO₂ from blood to lung

- CO₂ blown off

- Decreased H₂CO₃ biosynthesized from the less CO₂ and H₂O via Carbonic Anhydrase

- Reduced H₂CO₃ dissociates to release LESS H⁺; Excess base accumulation (HCO₃⁻, e.g.)

- pH of ECF rises: RESPIRATORY ALKALOSIS

- Alkalosis intake or excessive loss of H⁺

- Increased base levels OR lowering of H⁺ levels in ECF

- pH of ECF rises: METABOLIC ALKALOSIS

- elevated pH turns off breathing centers

- Kidney reabsorbs protons into blood; excretes more bicar into urine (titration!)

- retain CO₂

- increases H₂CO₃ due to increased CO₂ present to react with H₂O

- more H⁺ from elevated H₂CO₃ dissociation

- pH of ECF drops: COMPENSATION!
Mendel’s Laws of Genetics

• There are many complicated ways in which to examine genetics, but the simplest manner is still that which Gregor Mendel developed in 1865.

• Mendel began his work by observing that pea plants had different characteristics from other pea plants.

• The same has been observed for a number of other plants, most notably the petunia.
Mini-Glossary

- **Genetic locus**: chromosomal location of the two copies of a gene.
- **Genotype**: the genetic information transmitted between generations
- **Phenotype**: the expression of the genetic material; what you actually “see”

To understand Mendel's work, we must accept that there is one genetic characteristic that is expressed, or is dominant, and one genetic characteristic that is not expressed, or is recessive.

Also remember that genes, as a general rule (and particularly as applied to humans) come in pairs. The idea here is that if a gene that is expressed (gives a dominant phenotype) is mixed with another gene that is expressed, then the phenotype is expressed.

If a gene that is not expressed is mixed with another like gene, then the phenotype is expressed.

If, however, a gene that is expressed is mixed with a gene that is not expressed, then the characteristic that is expressed is mostly the dominant characteristic (with some "leaking" of the recessive trait).

The only manner in which recessive traits may be observed is having both recessive traits combined.
• *Allele*: An allele is an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome. These DNA codings determine distinct traits that can be passed on from parents to offspring.

✓ Example: The gene for seed shape in pea plants (Mendel’s speciality) exists in two forms, one form or allele for smooth seed shape (S) and the other for wrinkled seed shape (w).

✓ Organisms have two alleles for each trait.

✓ When the alleles of a pair are heterozygous, one is dominant and the other is recessive. The dominant allele is expressed and the recessive allele is masked.

✓ Using the previous example, smooth seed shape (S) is dominant and wrinkled seed shape (w) is recessive. Smooth: (SS), Wrinkled: (ww), Crinkled: (Sw).
• **Pleitropism**: 1 gene that provides for 2 or more phenotypes.

• **Dominant**: refers to the phenotype, NOT to the genotype; tells us that the mutation in this type of gene presents clinically with only a single dose, i.e., heterozygous; this is at the gene level.

• **Recessive**: refers to phenotype, NOT to the genotype; tells us that the mutation in this kind of gene presents clinically with a double dose, i.e., homozygous; this is at the gene level.

• It is, therefore, inappropriate to refer to GENES as dominant or recessive: genes are either expressed or NOT expressed.
Dominant v Recessive: An Exception to The Rule

• **Sickle cell anemia:** recessive trait: homozygous.
• BUT, sickle cell gene is expressed with one dose, too, which produces carriers with hemoglobin S (HbS; $\alpha_2\beta_2s$) and HbA ($\alpha_2\beta_2$) that may cause sickling when exposed to low pO$_2$: heterozygous; this is expressed at the BIOCHEMICAL LEVEL.
• A recessive trait may, therefore, be termed **codominant** at the biochemical level of gene **product** (HbS and HbA) or **dominant** under changed environmental conditions (heterozygous sickling).
Mendel’s Laws

• During Mendel's work with plants, he developed three laws:

• 1. Law of Unit Inheritance: genetic factors keep their own identity and do not blend/merge/fuse in a hybrid, i.e., each gene has its own individual identity.

• 2. Law of Segregation: 2 alleles of one particular pair of genes are never found in the same reproductive cell, but always segregate between multiple gametes (1/2 to one cell and 1/2 to another).

• 3. Law of Independent Assortment: That different chromosomes conglomerate to reproductive cells in a manner that requires no dependence on other chromosomes (1/2 of chromosomes go to 1 cell and 1/2 to another BUT don't follow other chromosome halves in a dependent manner).

• If a patient has a disease that is demonstrable to follow Mendelian rules, in all probability, the disease -- regardless how involved the disease is -- comes from 1 gene.
**α-thalassemia**

- This disease is inherited in a manner consistent with autosomal recessive characteristics.
- It seems to be on 16p. With inactivation of 3 of the 4 α-globin chains, a form of hemoglobin known as HbH forms.
- This causes hemolytic anemia.
- It is caused by the formation of a tetramer of beta subunits.
- The new tetramer has very high oxygen affinity and, hence, doesn't want to release oxygen to the cells.
- This disorder causes jaundice, hepatosplenomegaly. Diagnostic testing includes reticulocyte counts, MCV (mean corpuscular volume) and detection of lots of hypochromia on a peripheral blood smear.
- Prenatal screening includes electrophoresing parental Hb to identify the presence of the thalassemic Hb.
- Therapy does not include iron: it is needless and it may be toxic -- increase folate intake.
- NOTE: inactivation of all 4 of the α-globin chains = a stillborn baby. This is called hydrops fetalis.
**β-thalassemia**

- This disorder is likewise inherited as α-thalassemia, but on 11p. The gene effected is the β-globin gene. There are two variations of this disorder: major and minor.

- **Major**: is the most common cause of transfusion-dependent anemia in childhood. The patients are normal at birth but develop anemia by their first year as fetal Hb (HbF) levels drop off. (HbF is a tetramer of $\alpha_2\gamma_2$; HbA$_2$ is a tetramer of $\alpha_2\delta_2$.) Without treatment, patient develops a massively enlarged spleen and liver, develops enlarged medullary cavity with thinned cortex, prominent forehead and maxilla and pathologic fractures. May cause RBC sickling. Diagnostic testing includes looking for reduced MCV, elevated HbA$_2$ OR F -- normal Hb, HbA, is a tetramer of 2 alpha sub-units and two beta subunits ($\alpha_2\beta_2$) -- and electrophoresing Hb. Therapy consists of blood transfusions with iron chelation, bone marrow transplants. Hb must be maintained at or above 11 mg%. Splenectomy reduces transfusions. Pneuimmune vaccine before, after or without splenectomy and PCN after splenectomy reduce infection by *Streptococcus pneumoniae*.

- **Minor**: usually asymptomatic. There seems to be no response to iron therapy.

- Genetic counseling needs to be approached sensitively
Protein Denaturation

• The denaturation of proteins includes anything that disrupts secondary, tertiary and/or quaternary structure of proteins:
  – heat,
  – alcohol,
  – salts,
  – heavy metals,
  – freeze/thaw,
  – acids/bases.

• All cause inactivation of proteins.
Groups of Proteins

- **Fibrous proteins** include:
  - Collagens: connective tissue; after it's boiled, the soluble part is called gelatin (Bill Cosby sells this as JELLO)
  - Elastins: in stretching tissues
  - Keratins: water-proofing proteins
  - Myosins: in muscle
  - Fibrin: blood clotting protein

- **Globular proteins** include:
  - Albumins: water soluble; transporters and increase blood osmotic pressure
  - Globulins: saline soluble; transporters and antibodies
  - Enzymes: biological reaction catalysts
Enzymes

Biological Catalysts
Enzymes Have Specific Functions

• Enzymes are categorized into one of 6 biological activities according to the Enzyme Commission:

  – Oxidoreductases: catalyze redox reactions -- involve NAD and FAD (E.C. 1.X.X.X)
  – Transferases: catalyze group transfers (E.C. 2.X.X.X)
  – Hydrolases: use water to lyse bonds (E.C. 3.X.X.X)
  – Lyase: nonhydrolytic and non-oxidative group removal (E.C. 4.X.X.X)
  – Isomerases: catalyse isomerization reactions (E.C. 5.X.X.X)
  – Ligase: catalyzes reactions requiring ATP hydrolysis (E.C. 6.X.X.X)
Enzyme “Add-On’s”

- Cofactors ≡ a molecule or ion of a non-protein nature that is required by an enzyme for complete catalytic capacity, e.g., Mn$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Cu$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Mo$^{2+}$
- Coenzyme ≡ a carbon-based molecule required by an enzyme for complete catalytic capacity, e.g., NAD$^+$, FAD, vitamins – bound loosely to the apoenzyme
- Apoenzyme ≡ active enzyme minus the cofactor; catalytically inactive
- Prosthetic group ≡ non-protein moiety tightly bound to apoenzyme
- Holoenzyme ≡ apoenzyme plus prosthetic group
- Zymogens ≡ immature enzymes that need “clipping” for activation – more later in course
• Enzymes are globular proteins
  – Exception: ribozymes
• Without enzymes, cellular reactions go too slowly to be conducive to life
• All enzyme names end in “ase”
Terminology

• Active site $\equiv$ 3-dimensional cleft in the enzyme caused by/coded by the primary structure of the protein; complimentary to the shape (geometry of the substrate)

• Specificity characteristics $\equiv$ due to the active site; crevice allows binding of 1) only one substrate or 2) 1 kind of R group

• Constitutive enzymes $\equiv$ always in the cell without regard to the availability of substrate

• Induced enzyme $\equiv$ present in the cell ONLY when substrate activates gene mechanisms causing intracellular release of active enzyme.
Practical Conditions

• To Study Enzymes:
• Substrate (S) must be converted to product (P) by the enzyme (E) under the following conditions:
  – The reaction is thermodynamically feasible
  – S goes through and above the appropriate $E_a$ for P to form
### Example

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Catalyst</th>
<th>Ea (@ RT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$</td>
<td>None</td>
<td>319.2 kcal/mol</td>
</tr>
<tr>
<td>$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$</td>
<td>I$^-$</td>
<td>239.4 kcal/mol</td>
</tr>
<tr>
<td>$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$</td>
<td>Catalase</td>
<td>33.6 kcal/mol</td>
</tr>
</tbody>
</table>
Efficiency of Enzymes

• Increases rate of reaction without being consumed themselves
• Lower the $E_a$; no effect on $K_{eq}$
• Permit reactions to reach equilibrium quicker
• Have pH and temperature requirements
• Cause reactions to go within seconds as opposed to lab reactions that may take years
• Necessity to/for life
  
  E.g., $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3$

• Catalyzed by carbonic anhydrase at a rate of $6 \times 10^5$ molecules of CO$_2$ condensed per second
Specificity of Enzymes

• In reaction types catalyzed
• In substance involved in the reaction (S)
  – Absolute specificity $\equiv$ catalyzes reaction with only one S
  – Relative specificity $\equiv$ catalyzes reaction of substrates with similar structures
  – Stereochemical specificity $\equiv$ D vs L – more later in class
Enzyme Regulation

• Cell regulates which enzymes function and when, i.e., not ALL enzymes are working at the same time
• Some catalyze uni-directional; some catalyze bi-directional
Enzyme Activity

• ≡ catalytic capacity of enzyme to increase reaction rate
• Turnover number ≡ # of S molecules acted upon by ONE enzyme molecule per minute
• Enzyme assays ≡ measure enzyme activity
Enzyme Activity

• International Units ≡ IU
  – The amount of enzyme that catalyzes 1 µmol of substrate to be altered to product per minute at a given pH, T and [S].
  – It measures the amount of enzyme present, therefore, an enzyme level of 150 IU = an enzyme concentration 150 times greater than the standard – useful in diagnosing diseases (more on this later).
Enzyme Models

• Of significance, of course, is the fact that the shape of the enzyme gives it its function (the shape of a protein gives it its function).
• Enzymes speed up the reaction rate in biological systems 100,000 - 1,000,000 fold!
• Some are known to increase the reaction rate > 10^{20}-fold!
• Enzymes have specific substrates (chemical group upon which the enzyme works), but can work on limited kinds of substrates.
• There are two generally accepted models for the functioning of enzymes: the lock and key model and the induced fit model.
• We will address the lock and key model first.
Model #1: Lock-n-Key

In this model, see graphic, above, the substrate (S) is complimentary to the binding/active site in the enzyme (E). This is likened to the lock and key, where the lock is complimentary to the key. As the E and S bind, they form the Enzyme-Substrate complex (ES). This is an intermediate in the reaction that will cause S to be changed into a product (P).

• The enzyme acts as a sort of scaffold, holding the substrate so that one specific reaction may occur.
• In this case, a bond (or bonds) is (are) broken as the enzyme changes its shape ever so slightly, causing the substrate to break exactly where it's supposed to, releasing the new products and the enzyme for use, again.
• Remember that the active sites (a, b, c) of the enzyme are complimentary to the SHAPE of the substrate.
Model #2: Induced Fit

- The second model is called the induced fit model.
- This means that as the S gets closer to the E, the E actually undergoes a conformational change (shape change) to fit the S, i.e., its shape is INDUCED to change by the presence of the substrate.

- Note that as S gets closer to E, the active site "a" (left site) changes shape to match the complimentary site on S.
- As S continues to get even closer, site "b" (middle site) shifts its shape, as does site "c" (right site) when S is all but bound to the enzyme.

Once ES is formed, this model conforms to the remainder of the lock and key theory of enzyme-substrate binding.
What Effects Enzyme Activity?

Easy Answer: [E], [S], T, pH and covalent bonding

Covalent bonding with enzyme (poisons):
1) CN⁻ with Cu²⁺ in IV and 2) CHON-SH with Hg²⁺, Pb²⁺, Cu²⁺, Ag⁺
[E] – longer answer

\[ 3 \, [E] = 3 \, V_{\text{rxn}} \]

Covalent bonding with enzyme (poisons):
1) CN with Cu\(^{2+}\) in IV and 2) CHON-SH with Hg\(^{2+}\), Pb\(^{2+}\), Cu\(^{2+}\), Ag\(^{+}\)
[S] – Longer Answer

- With increasing [S], causes S to bind at activation site causing conformational changes so that the active site binds S. At some [S], E is sat’d with S and will not work any faster. This rate is called the $V_{\text{max}}$. 

<table>
<thead>
<tr>
<th>$V_{\text{ran}}$</th>
<th>$V_{\text{ran}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[E]</td>
<td>[S]</td>
</tr>
</tbody>
</table>

Covalent bonding with enzyme (poisons):
1) CN$^-$ with Cu$^{2+}$ in IV and 2) CHON-SH with Hg$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Ag$^+$
T – Longer Answer

- Increases the rate of the reaction \( (V_{\text{rxn}}) \) to a point where activity drops off (denaturation)

Covalent bonding with enzyme (poisons):
1) CN\(^-\) with Cu\(^{2+}\) in IV and 2) CHON-SH with Hg\(^{2+}\), Pb\(^{2+}\), Cu\(^{2+}\), Ag\(^+\)
pH – Longer Answer

- pH optimum is where enzyme has greatest activity; at pH’s above and below this pH, still has some activity until pH extremes are reached. This causes enzyme denaturation at either end of extremes.

Covalent bonding with enzyme (poisons):
1) CN with Cu²⁺ in IV and 2) CHON-SH with Hg²⁺, Pb²⁺, Cu²⁺, Ag⁺
Covalent Bonding – Longer Answer

Covalent bonding with enzyme (poisons):
1) CN⁻ with Cu²⁺ in IV and 2) CHON-SH with Hg²⁺, Pb²⁺, Cu²⁺, Ag⁺
Anything Else?

• YES!!!!

• Effectors
  – Non-substrate that turns on E, e.g., calmodulin (in most cells) and troponin (in muscle cells {skeletal and cardiac}). To activate E’s, both must bind Ca$^{2+}$
Academic Aside:
Cellular [Calcium Ion]

BIG PROBLEM!!!

• Ca\(^{2+}\) HAS to stay outside the cell or separate from the cytosol until and when it is exactly needed. WHY???

1. Inside the cell [Ca\(^{2+}\)] = 0.0 M; [P\(_i\)] = 0.001 M
2. If you add high [Ca\(^{2+}\)] with this [P\(_i\)] it will form an intracellular Ca\(_3\)(PO)\(_4\) ppt.

• So, HOW do you get the Ca ion inside the cell or into the cytosol WITHOUT causing a precipitate?
• NERVE SIGNALS – a.k.a. neurotransmitters
NMJ - 1

1. no neurotransmitter bound to receptor to turn it on
2. When neurotransmitter binds to receptor, it turns on the calcium ion channel and increases calcium ion influx.
3. Calcium ion won’t ppt as calcium phosphate because this reaction is FAST!
4. When Ach is released from receptor calcium ion efflux is activated – is active transport
Anaerobic Myocardial Metabolism

- Elevated H⁺ due to a build up of lactate and/or fatty acids contributes to a metabolic acidosis in the heart muscle
  - Na⁺/Ca²⁺ Exchanger
  - H⁺/Na⁺ Exchanger
- As the H⁺ are exchanged OUT of the cardiac cells, Na⁺ and Ca²⁺ exchange leading to excessively high levels of Ca²⁺ in the cells which leads to cell death
Enzyme Inhibition

Descriptive Introduction
Normal

- The upper left graphic represents the normal ES complex.
Competitive Inhibition

- The lower left graphic represents competitive inhibition of an enzyme, i.e., an inhibitor specific to this enzyme COMPETES with the substrate for the active site of this enzyme.
- It is reversible; will block S from binding.
- One example of this sort of inhibition is carbamoyl choline that competitively inhibits acetylcholinesterase.
Uncompetitive Inhibition

• The lower right graphic represents uncompetitive inhibition.
• This sort of inhibition involves covalently bound inhibitor and inactivates the enzyme irreversibly.
• Two examples of this sort of inhibitor are nerve gas and organophosphates that inhibit acetylcholinesterase.
• Organophosphate poisoning may be reversed by injecting a drug called 2-PAM.
• Valium and atropine are useful to treat muscle spasms and breathing difficulties, as well.
Non-Competitive Inhibition

• The upper right graphic represents noncompetitive inhibition.
• Note that the inhibitor does NOT bind to the active site of the enzyme, rather it has its own unique binding site.
• When a noncompetitive inhibitor binds to an enzyme, it causes the enzyme to change shape and shuts off its activity reversibly by not allowing S to bind completely.
• This sort of inhibition is also referred to as allosteric inhibition and plays major roles in metabolic regulation.
• An example of a noncompetitive inhibitor is aspirin. Aspirin inhibits cyclo-oxygenase which is the main enzyme in prostaglandin biosynthesis.

Prostaglandins mediate pain, Inflammation, blood pressure, gastric mucous secretion, blood clotting, labor and delivery, to name a few.
Mixed Inhibitor Types

- An example of mixed inhibitor types is aspirin (ASA) and Ibuprofen (IBU).
- ASA is a UNcompetitive inhibitor of COX-1 (CycloOxygenase type 1).
- SA and IBU inhibit cyclo-oxygenase variants which is the main enzyme in prostaglandin biosynthesis.
- Prostaglandins mediate pain, Inflammation, blood pressure, gastric mucous secretion, blood clotting, labor and delivery, dysmenorrhea, to name a few.
- This inhibition is IR-reversible – unlike other NSAID’s (Non-Steroidal Anti-Inflammatory Drug’s).
- ASA acetylates COX-1 to inhibit it.
- The half life ($t_{1/2}$) of ASA varies by dosage: 250 mg dose $t_{1/2}$ = 2-4.5 hrs; 1 g dose $t_{1/2}$ = 5 hrs; 2 g dose $t_{1/2}$ = 9 hours; > 4 g $t_{1/2}$ = 15-30 hrs.
- ASA CHANGES COX-2 activity to produce anti-inflammatory lipoxins (“LX’s”; derived from $\omega$3 fatty acids (EPA) as well as $\omega$6 fatty acids such as $20:4\Delta5,8,11,14$), see image at right.
Mixed Inhibitor Types

- IBU is a **NON**competitive inhibitor of COX-2.
- It is reversible in its inhibition.
- IBU works primarily through COX-2 (Like Vioxx and Celebrex by reducing PGI₂).
- This permits “normal” TX(A₂ or B₂) production which increases the incidence of blood clots [IBU has lowest incidence of GI/Hematological Sx of the NSAID’s, by the way]).
- The half life for IBU is unique in that it’s about 1.8-2 hrs while its duration of action is about 2-4X the t½.
- The problem with these two medications is that IBU binds to COX-2 inhibiting the production of PGI₂ – the natural titrant of TX’s.
- What does this mean? Blood clots, potentially. If one is a cardiac patient and is taking low dose po ASA to prevent blood clot formation, yet needs IBU for pain control, what is one to do?
- Per http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm125222.htm, the patient needs to take their ASA first and wait 30 minutes to take their IBU (top of graphic at bottom of previous page) or take their IBU 8 hours before their ASA dose.
- If IBU is taken first or is not taken long enough before the ASA dose, IBU not only binds, it also blocks the binding of ASA, to COX-1 and COX-2 (graphic . Should this occur, the potential for a fatal MI due to thrombosis of [a] coronary arter[y]ies is elevated.
Reaction Kinetics

An Introduction
• A condition of equilibrium is reached in a system when 2 opposing changes occur simultaneously at the same rate.

• The rate of a chemical reaction may be defined as the # of mols of a substance which disappear or are formed by the reaction per unit volume in a unit of time.
Example

$I \rightarrow C$

$$Rate_{forward} = \frac{[I]_2 - [I]_1}{t_2 - t_1} = \frac{\Delta [I]}{\Delta t}$$
• The previous rate is for the DISAPPEARANCE of I, therefore:

\[
rate = - \frac{\Delta [I]}{\Delta t}
\]

where the negative sign means disappearing or "loss of"
Backwards Example

\[
\text{Rate}_{\text{backward}} = \frac{[C]_2 - [C]_1}{t_2 - t_1} = \frac{\Delta [C]}{\Delta t} = + \frac{\Delta [C]}{\Delta t}
\]

where the positive sign means for min g

when both reaction rates study I
More Complex Reactions

\[ X \rightarrow 3 \, Z \]

\[
\text{Rate} = - \frac{\Delta \, X}{\Delta \, t} \neq \frac{\Delta \, Z}{\Delta \, t}
\]

This is due to \( Z \) appearing 3 times as fast as \( X \) is disappearing.

\[
\therefore \text{Rate} = - \frac{\Delta \, X}{\Delta \, t} = \frac{1}{3} \frac{\Delta \, Z}{\Delta \, t}
\]
In General

For the Reaction:

\[ pP + qQ \rightarrow rR + sS \]
Reaction Order
In General

For the Reaction:

\[ pP + qQ \rightarrow rR + sS \]

\[ Rate = -\frac{1}{p} \frac{\Delta P}{\Delta t} = -\frac{1}{q} \frac{\Delta Q}{\Delta t} = \frac{1}{r} \frac{\Delta R}{\Delta t} = \frac{1}{s} \frac{\Delta S}{\Delta t} \]

and is proportional to

\[ [P]^n [Q]^m \]

or

\[ Rate = k \ [P]^n \ [Q]^m \]

where

\[ k = \text{proportionality constant or rate constant} \]
A reaction with an incredibly large rate constant is faster than a reaction with an incredibly small rate constant.
For the Reaction:

\[ pP + qQ \rightarrow rR + sS \]

The reaction is “n” order in \([P]\) and “m” order in \([Q]\)

OR

Is OVERALL “(n + m)” order
“n” and “m” DO NOT necessarily equal “p”, “q”, “r” or “s”
Example

\[2N_2O_5 (g) \rightarrow 4NO_2 (g) + O_2 (g)\]

\[\text{Rate} = - \frac{1}{2} \frac{\Delta [N_2O_5]}{\Delta t} = \frac{1}{4} \frac{\Delta [NO_2]}{\Delta t} = \frac{\Delta [O_2]}{\Delta t}\]

or

\[= k [N_2O_5]\]
• This reaction is **FIRST** order in $\text{N}_2\text{O}_5$, **NOT SECOND** order as one might intuit from the stoichiometry.
The Order of The Reaction

• = the specification of the empirical (experimentally-determined) dependence of the rate of the reaction on CONCENTRATIONS

• The order may = 0, a whole number or a non-whole number, e.g.,
  – 0
  – 1
  – 1½
  – 2

• We’ll focus on whole numbers and 0 (zero) for reactions of the type:

\[ \text{fF} + \text{gG} \rightarrow \text{Products} \]

• AND! The order of the reaction is defined in terms of REACTANTS not the products, therefore, products do not need to be specified
First Order Reactions

• Assume reaction is 1\textsuperscript{st} order in F and zero order in G:

\[
Rate = - \frac{1}{f} \frac{\Delta F}{\Delta t} = k'[F]^1 [G]^0
\]

\[
f \ k' = k \therefore \frac{\Delta F}{\Delta t} = k [F]
\]

Re\textit{arrange} : \( \frac{\Delta F}{F} = k \Delta t \) \textit{and integrate} :

\[
\ln \frac{[F]}{[F]_o} = - k t
\]

\[
OR
\]

\[
[F] = [F]_o e^{-kt}
\]
• Many radioactive decays fit 1st order reactions:
  • $^{226}\text{Ra}_{88} \rightarrow ^{222}\text{Rn}_{86} + ^4\text{He}_2$
  • $^{238}\text{U}_{92} \rightarrow ^{234}\text{Th}_{90} + ^4\text{He}_2$
• The rate is proportional to $[F]$
The Rate is Proportional to $[F]$ 

$$\frac{\Delta F}{\Delta t} = k [F]$$

<table>
<thead>
<tr>
<th>Double $[F]$</th>
<th>$k [F]$</th>
<th>$k [F]^2$</th>
<th>$k [F]^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate Change</td>
<td>↑ X 2</td>
<td>↑ X 4</td>
<td>↑ X 8</td>
</tr>
</tbody>
</table>
$Q_{10}$ Effect
Example

- If the rate of a chemical reaction doubles for every 10°C rise in temperature, how much faster would the reaction proceed at 55°C than at 25°C?
Q 10 Effect on Reaction Rate

Temperature (Celsius) vs. Relative Reaction Rate graph.
Solution

• Temperature increased 30°C, therefore, reaction rate increases 8-fold

Example:

• What if the temperature was increased to 105°C from 25°C?

• Temperature increased 80°C, therefore reaction rate increases 256-fold
Example

• How much faster would a reaction go at 100°C than at 25°C?
• For every 10°C increase in temperature, the reaction rate doubles. The change in temperature is 75°C. This is 7.5 10°C increases.

• Hence $2^{7.5} = 181$ times faster
Example

• In an experiment, a sample of NaOCl was 85% decomposed in 64 minutes. How long would it have taken if the temperature was 50°C higher?
• For every 10°C increase, the reaction rate doubles. 50°C increase is 5 10°C increases.

• Hence: $2^5 = 32$ times faster
• So: (64 minutes)/(32 times faster) = 2 minutes
There are 4 types of temperature dependence for reaction rates -- 1

- Rate increases with increasing temperature
- NORMAL
There are 4 types of temperature dependence for reaction rates -- 2

• Rate increases to a point, then reduces with increasing temperature
• E.g., enzymes being denatured
There are 4 types of temperature dependence for reaction rates -- 3

- Rate decreases with increasing temperature
- VERY RARE
- Known only for a few reactions that are multi-step reactions:
  - $A \rightarrow B$ Fast step
  - $B \rightarrow C$ Rate limiting step
There are 4 types of temperature dependence for reaction rates -- 4

- Rate increases with increasing temperature
- Odd behavior
- Explosive reaction when temperature shoots up
- Gradual rise in temperature due to chain reactions
Apply This to Enzymes

• Enzymes are, with a couple of exceptions, proteins
• Enzymes are biological catalysts
• Enzymes speed up biological reactions incredibly
• For this discussion:
  – E = enzyme,
  – S = substrate and
  – P = product
\[
E + S \xrightarrow{k_1} ES \quad \text{FAST}
\]
\[
ES \xrightarrow{k_{-1}} E + S \quad \text{FAST}
\]
\[
ES \xrightarrow{k_2} E + P \quad \text{SLOW}
\]

AND

\[
\frac{d}{dt} [ES] = -\frac{d}{dt} [ES]
\]
Short Method

\[ k_1 [E][S] = k_{-1} [ES] \]

Solve for \([ES]\):

\[ \frac{k_1}{k_{-1}} [E][S] = [ES] \]

Stop temporarily
• Rate limiting step is step 3
• Rate equation is: \( k_2 [ES] \)
• Substitute as before:

\[
\left( \frac{k_1 k_2}{k_{-1}} \right) [E][S] = k [E][S]
\]

**Write the overall reaction:**

\[ E + S \leftrightarrow ES \rightarrow E + P \]

*This is a Uni–Uni Rxn*
Uni-Uni Reaction
(Cleland Plot)
Bi-molecular Reactions

• An enzyme catalyzed reaction may utilize 2 substrates.
• This reaction is always SEQUENTIAL, however,
• May be
  – ORDERED or
  – RANDOM
E.g., Ordered Sequential Reaction

\[ E + X + Y \rightarrow E + R + S \]

E is still enzyme
X and Y are substrates
R and S are products
Putative Mechanism

\[ E + X \rightarrow EX \]
\[ EX + Y \rightarrow EXY \]

\[ EXY \rightarrow ERS \quad \text{SLOW STEP} \]

\[ ERS \rightarrow ER + S \]
\[ ER \rightarrow E + S \]

And:

\[ \frac{d}{dt} [ERS] = \frac{d}{dt} [EXY] \]
\[
\frac{d[ERS]}{dt} = k_1 [E][X] k_2 [EX][Y] \quad \text{and} \quad -\frac{d[EXY]}{dt} = k_3 [EXY]
\]

Equate

\[
k_1 [E][X] k_2 [EX][Y] = k_3 [EXY]
\]

Solve for [EXY]

\[
\left( \frac{k_1 k_2}{k_3} \right) [E][X][Y][EX] = [EXY]
\]

Stop here temporarily
• Rate Limiting Step is: \( k_3 [EXY] \)

• Substitute:

\[
k_3 \frac{k_1 k_2}{k_3} [E][X][Y][EX] = k_1 k_2 [E][X][Y][EX]
\]

\[
= k [E][X][Y][EX]
\]
Kinetic Data Tells us:

• The following sequence MUST be taking place:

\[ E + X + Y \rightarrow EX \text{ (FIRST!)} \rightarrow EXY \text{ (SECOND!)} \rightarrow ERS \rightarrow ES + R \rightarrow E + S \]

• And is an Ordered Bi Bi Reaction
Ordered Bi Bi Reaction
• In the case where separate experiments about the same system give 2 different rate equations, e.g.,

\[ k [E] [X] [Y] [EX] \]

And

\[ k [E] [X] [Y] [EY] \]
Mechanism = Random Sequential Bi-Bi
• What, though, if an enzyme catalyzed a reaction that bound one substrate, released its product, then binds a SECOND substrate and releases ITS product?

   Overall Reaction is:  \( E + X + Y \rightarrow E + R + S \)
Mechanism

\[ E + X \rightarrow EX \]

\[ EX \rightarrow ER \quad 1^{st} \text{ rate limiting step} \]

\[ ER \rightarrow E + R \]

\[ E + Y \rightarrow EY \]

\[ EY \rightarrow ES \quad 2^{nd} \text{ rate limiting step} \]

\[ ES \rightarrow E + S \]
• Note: while written unidirectionally, in many cases the reactions are reversible
• With 2 rate limiting steps, this reaction and its kinetics get ugly fast.
• This sort of reaction between 2 substrates and the 1 enzyme act like a ping pong game.
Ping Pong Mechanism
Function of Kinetics

• To Determine Reaction Mechanisms
Enzyme Inhibition

An Introduction to Quantitative Enzymology

• From this point on, the arithmetic manipulations to study enzymes are all based on the following reaction sequence:

\[ E + S \leftrightarrow ES \rightarrow EP \rightarrow E + P \]
Inherent to this sort of study are 3 assumptions:

1. \([E] \ll [S]\)

2. \(P \neq S\)

3. \(ES\) forms either
   1. \(E + S\) OR
   2. \(E + P\)
We can use the above sequence to write a statement about the rate of the reaction (V) which is proportional to [E], [S], and $k_1$, $k_2$ and $k_3$ (these latter three rate constants are “fudge factors” – more on them in CHEM 122 – they can also be used to determine $K_{eq}$ as we’ll see in a bit).
Catalytic Velocity

\[ V = k_3 [ES] \]

≡ product of \([ES]\) and \(k_3\) (rate limiting step is rate of P formation and, hence, ES loss) or
• Can we write equation[s] to tell us about [ES] in terms of measurable [E], [S] and [P]? YES!!!!!!

\[ V_{\text{formation}} [\text{ES}] = k_1 [\text{E}] [\text{S}] \]

\[ V_{\text{loss of}} [\text{ES}] = (k_2 + k_3) [\text{ES}] \]

!!! ES = INTERMEDIATE in reaction sequence!!!
• When $V_{\text{formation}} = V_{\text{loss}}$,

– $[\text{ES}]$ is a constant as $[S]$ decreases and $[P]$ increases.

• We may write that arithmetically as

$$k_1 [E] [S] = (k_2 + k_3) [\text{ES}]$$
Steady State Approximation

• This approximation is so called because the [intermediate] does not change.

• Let’s solve for \([ES]\) since it DOES remain constant:

\[
[ES] = \frac{k_1 [E][S]}{(k_2 + k_3)} \iff \frac{[E][S]}{(k_2 + k_3)} = \frac{k_1}{(k_1)}
\]
• Remember that equilibrium constants express what is going on in a reaction in terms of products and reactants.
• They can also be expressed in terms of rate constants. In this case, the equilibrium constant is given a special name and icon: $K_M$ – the Michaelis-Menton constant.
$K_M$ Equals

$$K_M = \frac{k_2 + k_3}{k_1}$$

$\therefore$

with substitution

$$[ES] = \frac{[E][S]}{K_M}$$
Is There A Way to Determine [E]?

• YES!!!!!!

\[ [E] = [E_T] - [ES] \text{ which is the UNCOMBINED } E \]

• By substituting \([E_T] - [ES]\) for \([E]\), we can obtain the following equation:

\[ [ES] = \frac{([E_T] - [ES])[S]}{K_M} = \frac{[E_T][S]}{K_M} - \frac{[ES][S]}{K_M} = \frac{[E_T][S] - [ES][S]}{K_M} \]
By Rearranging, We Get

\[ K_M [ES] = [E_T] [S] - [ES] [S] \]

\[ [ES] K_M + [ES] [S] = [E_T] [S] \]

\[ [ES] (K_M + [S]) = [E_T] [S] \]
And

\[
[ES] = \frac{[ET][S]}{K_M + [S]}
\]
Remember, now

- The rate limiting step equation is:
  \[ V = k_3 \, [ES] \]

So let’s substitute for [ES]

On the next slide
\[ V = \frac{k_3 [E_T][S]}{K_M + [S]} \]

When \([S] \gg\gg K_M\),

\[ \frac{[S]}{K_M + [S]} \rightarrow 1 \]
This means

- E is saturated with S at AND causing $V_{\text{max}}$ and

$$V_{\text{max}} = k_3 \ [E_T]$$

- When we substitute on the next slide, we get:
Michaelis-Menton Equation

\[ V = \frac{V_{\text{max}} [S]}{K_M + [S]} \]
Application #1 – when \([S] = K_M\)

\[
V = \frac{V_{\text{max}} [S]}{K_M + [S]} = \frac{V_{\text{max}} K_M}{2 K_M} = \frac{V_{\text{max}}}{2}
\]

\(\text{hence}\)

\[
K_M = [S] \ @ \ \frac{V_{\text{max}}}{2}
\]
Cont’d

Find $V_{\text{max}}$ and go to y axis

Go to $V_{\text{max}}/2$ on the y axis, read over to the curve and down to the x axis: $K_m$ is at that point

This is in the form of a rectangular hyperbola -- not very useful to the enzymologist.
Lineweaver & Burke Modification

- They took double reciprocals (the other name of this method) and obtained the following equation:

\[
\frac{1}{V} = \frac{1}{V_{\text{max}}} + \frac{K_M}{V_{\text{max}} [S]}
\]
Graphically

This is very useful! -- more to come later.
Application #2

- When $k_2 \gg k_1$ (for most – not all – enzymes)

$$K_M = \frac{k_2}{k_1}$$
Cont’d

• When \(K_M\) is high, \(k_2\) is increased and/or \(k_1\) is decreased and favors \(ES \rightarrow E + S\)
• High \(K_M\) = weak bonding of \(S\) with \(E\) to form \(ES\)
• When \(K_M\) is low, \(k_2\) is reduced and/or \(k_1\) is increased and favors \(ES \rightarrow E + P\)
• Low \(K_M\) = strong bonding of \(S\) with \(E\) to form \(ES\)
Cont’d – E.g.

\[ K_M = 1 \times 10^{-9} \text{ M} \leftarrow \text{low } K_M \]

vs

\[ K_M = 1 \times 10^{-3} \text{ M} \leftarrow \text{high } K_M \]

- E binds with S 1,000,000-fold tighter with the E that has the low \( K_M \) than with the E with the high \( K_M \).
Lineweaver-Burke and Inhibitors
Competitive Inhibitors

- Inhibitor drives no change in $V_{\text{max}}$
- $K_M$ changes with inhibitor
- Inhibition can be overcome by very high [S], i.e., “swamping out” the inhibitor
- Reversible; “temporary”
- Competitive inhibitor completely or partially identical to S molecular shape
- If effects slope and $K_M = \text{competitive inhibitor}$
- NO effect on y-intercept
Un-Competitive Inhibitors

- $V_{\text{max}}$ changes – decreases with inhibitor
- $K_M$ changes
- Inhibition can not be overcome with high [S] since inhibition is covalent at active site
- Is overcome only 1) with proper drug therapy or 2) with synthesis of new enzyme
- Irreversible; “permanent”
- If effects x and y intercepts = un-competitive inhibition
- No effect on slope
Non-Competitive Inhibitors

- $V_{\text{max}}$ changes (decreases with inhibitor)
- No change in $K_M$
- Inhibition can not be overcome by high $[S]$ since inhibition does not occur at active site or E, but at surface site
- Reversible; “temporary”
- aka feedback inhibition; allostERIC inhibition
- If effects slope and y intercept = non-competitive inhibition
- No effect on x-intercept
Isoenzymes/Isozymes

• \equiv \text{multiple forms of enzymes in different tissues with the same activity;}
• Have identical cofactors but slightly different apoenzymes.
• Two best examples: LDH and C[P]K
# LDH – Lactate Dehydrogenase

<table>
<thead>
<tr>
<th>LDH</th>
<th>LD₁</th>
<th>LD₂</th>
<th>LD₃</th>
<th>LD₄</th>
<th>LD₅</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₄</td>
<td>H₃,M</td>
<td>H₂M₂</td>
<td>HM₃</td>
<td>M₄</td>
</tr>
<tr>
<td>Heart</td>
<td>Heart (†); Brain = Kidney</td>
<td>Brain = Lung</td>
<td>Lung (†); Skeletal muscle</td>
<td>Liver = Skeletal Muscle</td>
<td></td>
</tr>
</tbody>
</table>

H = heart sub-units; M = muscle sub-units
**C[P]K – Creatine [phospho] kinase**

<table>
<thead>
<tr>
<th>C[P]K</th>
<th>BB</th>
<th>MM</th>
<th>MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1° Brain</td>
<td>1° Skeletal muscle</td>
<td>1° Heart</td>
<td></td>
</tr>
</tbody>
</table>
Medical Uses of Enzymes and Enzyme Assays

• When cells die or are injured, they dump some or all of their E’s into the blood. Assays are used to make diagnoses, e.g.,
  – C[P]K, LDH 2° MI (myoglobins and troponins are being used, as well)
  – GPT (ALT) 2° liver problems
  – GOT (AST) 2° MI or liver
  – Ratios
    • GPT:GOT – normal = 0.75; viral hepatitis = 1.6
    • LD₁:LD₂ – normally < 1; 48° after MI, > 1 and is called the LD₁-LD₂ “flip”

• Calcium ion channel blockers – block calcium ion influx via channel which leads to reduced calcium ion inside the cell which leads to reduced muscle contraction which makes it easier for the heart to beat to reduce the risk of MI or death after MI.
Physiological Enzymology
Pepsin hydrolyzes proteins at the C-terminus of

<table>
<thead>
<tr>
<th></th>
<th>The aromatic amino acids</th>
<th>Met</th>
<th>Sulfur-containing amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td></td>
<td>Leu</td>
<td>BCAA</td>
</tr>
<tr>
<td>Tyr</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pepsin Activity -- #1

- Note disulfide bond
- Note pepsin cleavage site
  - Note products
Pepsin Activity -- #2

- Note disulfide bond
- Note cleavage sites
  - Note products
Proteases from Small Bowel

• Aminopeptidase – removes N-terminal amino acid from peptide:

  Asp-Gly-Pro-Lys-Arg-Cys-Phe + aminopeptidase

Yields

  Asp + Gly-Pro-Lys-Arg-Cys-Phe

• If repeated one AA at a time, would disassemble peptide slowly
Proteases from Small Bowel

• Dipeptidase: a final protease that hydrolyzes dipeptides to free amino acids:

\[
\text{Pro-Met} + \text{dipeptidase} \\
\text{Yields} \\
\text{Pro} + \text{Met}
\]
Pancreatic Proteases

<table>
<thead>
<tr>
<th>Trypsin</th>
<th>Chymotrypsin</th>
<th>Carboxypeptidase</th>
<th>Elastase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaves at the C-terminus of Arg and Lys</td>
<td>Cleaves at the C-terminus of Phe, Trp, Tyr</td>
<td>Removes the C-terminal amino acid - one at a time</td>
<td>Cleaves at the C-terminus of Ser, Thr, Tyr, Asn, Gln and Cys</td>
</tr>
</tbody>
</table>

• Note that chymotrypsin has some of the same activity of pepsin. Pepsin, though, functions BEST at a pH of around 2. It STILL has SOME function at higher pH's, although it "prefers" the acidic conditions for its "pH optimum".
• The pH optimum is the pH at which optimal activity is attained.
• Chymotrypsin functions best at an alkaline pH, as is found in the small bowel.
Trypsin

- Secreted as trypsinogen and activated by enterokinase
- Cleaves at the C-terminus of Arg and Lys – the positively charged amino acids
A quick and dirty lab test to detect the potential for a newborn patient to develop cystic fibrosis (CF). Although we usually think of CF as a pulmonary disease, it has multiple ramifications, including bowel disorders. This disorder comes about because the pancreas gets plugged by this disease in its process, rendering digestion difficult, to say the least. Since the pancreas gets plugged, it can not secrete digestive enzymes like trypsin.

To perform this easy screening procedure, you need the materials in Figure, left. Place the bottom of a Petri dish flat on a lab surface, break one of the applicator sticks in two and place them in the dish on top of a moistened paper towel. With another applicator stick, smear a little baby poop on the piece of x-ray film and mix it with normal saline.

Place the film on the applicator sticks and cover with the top of the Petri dish. Incubate at 37°C. After incubation, rinse off the film and examine it.
Examine Film

Plain film
- No trypsin in poop
- Plugged pancreas
- Test further for CF

“Hole” in film
- Trypsin in poop
- Pancreas ok
- No further tests for CF
Cystic fibrosis

- This disease is inherited autosomal recessive on 7q31-32.
- The protein involved is the cystic fibrosis transmembrane regulator (CFTR). This protein works with a chloride channel, but it is uncertain as to how.
- Although most of us are familiar with this disorder causing lung problems, it also causes GI disturbances (bulky, greasy stools), lots of gas and infertility in more than 95% of effected males (no vas deferens develops).
- Cor pulmonale develops in advanced cases; this is of poor prognosis.
- To some degree these symptoms may be treated with enzyme capsules to replace those not secreted by the pancreas. This patient may also need antacids.
- Diagnostic testing includes sweat chloride testing, probing for CFTR and a fecal test to test for presence of pancreatic enzymes. NOTE: 1 in 22 is a carrier of this disorder.
Chymotrypsin[ogen] – Activated by Trypsin

- Cleaves at C-terminus of Trp, Phe, Tyr – the aromatic amino acids

![Diagram showing cleavage of a peptide by chymotrypsin](image-url)
Elastase

- Cleaves at the C-terminus of the neutral amino acids: Ser, Thr, Tyr, Cys, Asn, Gln
Elastase Aside

• Elastase is present in high quantities in lung tissue
• Elastase activity is inhibited under normal conditions by $\alpha_1$-PI – alpha one-protease inhibitor
• This allows lungs to remain pliable and “stretchy-able”
• Smoking inhibits $\alpha_1$-PI – alpha one-protease inhibitor
• Elastase is activated and the lungs lose pliability and the patient is working on “getting” COPD
α₁-antitrypsin (α₁-AT; aka A1PI) Deficiency

- This disorder is inherited autosomal recessive from 14q. The protein effected is α₁-AT.
- The lack of this protein increases the risks of premature COPD in smokers. (α₁-AT is produced in the liver and travels to the lungs where it inhibits elastase activity -- if elastase is not inhibited, this causes small airway destruction; with smokers, it causes COPD.)
- For those lacking α₁-AT secondary to the inherited disorder, there is commercially available protein available for replacement therapy.
- To determine whether one has this disease, fetal DNA testing may be performed, RFLP's may be used, fetal blood levels of α₁-AT may be taken by periumbilical blood sampling (PUBS) in the last half of gestation and arterial blood gases may be utilized, as well.
- The best therapy is to quit smoking, provide symptomatic treatment and treat infections to any part of the respiratory system aggressively.
Carboxypeptidase

• Removes the C-terminal amino acid from a peptide

\[
\text{Cys-Pro-Leu-Arg-Gly-Lys} + \text{Carboxypeptidase}
\]

Yields

\[
\text{Cys-Pro-Leu-Arg-Gly} + \text{Lys}
\]
Proteases

- If each enzyme were to work one at a time, this process would take forever
- Therefore
- All these enzymes (small bowel and pancreas) work at the same time to disassemble proteins
Protease Perspective

- Note back-up enzymes and specificity
Carbohydrate digestion is pretty straight-forward: it starts in the mouth, as mentioned, above. α-Amylase hydrolyzes amylose from starch to a disaccharide, maltose. Figure, above, illustrates carbohydrate digestion in the small bowel.

- MaltAse (remember, if it ends in "ase" it's an enzyme) hydrolyzes maltOse to two molecules of glucose.
- SucrAse hydrolyzes sucrOse to a molecule of glucose and a molecule of fructose.
- LactAse hydrolyzes lactOse to a molecule of glucose (for rapid, short-term energy) and a molecule of galactose (for longer term energy; stored in the liver in glycogenesis).
- Note, too, that the pancreas secretes amylase into the small bowel.
- Like pepsin and chymotrypsin, the two amylase's are sort of back-ups to each other.
Nucleases, Nucleotidase and Nucleosidase
Elements of Amino Acid Metabolism
A Narrow View of Essential Amino Acid Metabolism

Aromatic Amino Acids
- Phenylalanine (phe)
- Tryptophan (trp)

Branched Chain Amino Acids
- Valine (val)
- Leucine (leu)
- Isoleucine (Ile)

Positively Charged Amino Acids
- Arginine
- Lysine
- Histidine (his)

Neutral Amino Acids
- Threonine (thr)
- Methionine (met)

Examine as groups where possible, then separate when needed.
Essential vs Semi-Essential

- Essential Amino Acids
  - Phe
  - Val
  - Thr
  - Trp
  - Ile
  - Met
  - Lys
  - Leu

- Semi-Essential Amino Acids
  - His
  - Arg
His Catabolism
Histamine Synthesis
3-Methylhistididine

- Catabolite of muscle contraction
- NOT further metabolized, but excreted
- Useful in determining muscle protein turnover ACCURATELY
Phe and Trp Catabolism – First Step
Inborn Error of Metabolism: PKU
Phenylketonuria (PKU)

- PKU is inherited as an autosomal recessive disorder. Classical PKU is on 1p; atypical PKU is on chromosome 4.
- Classic PKU is caused by a deficiency in phenylalanine hydroxylase.
- PKU causes mental retardation, hyperactivity, eczema; it is associated with blond, blue-eyed and fair-skinned individuals.
- The urine of patients with untreated PKU smells like a "mouse".
- Blood tests are now mandated within 2-3 days after a child is born.
- Urinary testing may also be undertaken.
- Therapy is to reduce phe in the diet. If the patient follows the diet, there will be normal development; if the patient does not follow the diet, mental retardation will set in.
- It is important to remember to titrate the phe levels carefully: phe is necessary to regulate the fever centers of the brain. Too little and the child has a fever constantly; too much and the child becomes irreversibly mentally retarded.
Phe and Trp Catabolism – Second Step
Inborn Error of Metabolism: Alkaptonuria
Alkaptonuria

- Alkaptonuria is inherited as an autosomal recessive disorder. It has been mapped to chromosome 3 and is a disorder of homogentisate oxidase. Its classical diagnosis is based upon the fact that the urine turns brown/black on standing -- particularly if the urine is alkaline or has alkali added to it. It leads to a dark pigmentation of ligaments, cartilage, fat, skin and urine called "ochronosis". Ochronosis is a dark blue discoloration that is easiest observed in regions of skin that overly cartilage. This discoloration usually is present in/by the 3d and 4th decades of life. Alkaptonuria also causes degenerative joint disease (arthritis) of the spine and peripheral joints. Although the disease makes one miserable, there does not seem to be a reduced life expectancy.

- Since this disease is, relatively speaking, benign, it is probably not necessary to reduce the amounts of phe and trp in the diet. Large doses of vitamin C seem to reduce oxidation/polymerization of homogentisate (in the test tube). Arthritis is treated with anti-inflammatory drugs.

- Recently (Feb. 2006), there have been some studies that have suggested that, while NSAID’s work nicely to reduce inflammation, they may also be hindering prostaglandin-mediated osteoblastic repair of the bony surfaces – some are now advocating the use on non-NSAID’s to mediate the pain of arthritis.

- 2009: some clinicians and researchers are suggesting that a combination (unstandardized as of yet) of acetaminophen and ibuprofen will provide narcotic levels of pain relief without addiction and other side effects of narcotics.
Phe and Trp Catabolism – Third Step
Catecholamines - Synthesis
S-Adenosylmethionine – “SAM”

1 carbon metabolism
Catecholamines: Catabolism and VMA
Trp and Nicotinic Acid and Nicotinamide
Serotonin Synthesis
BCAA
Metabolism
Maple Syrup Urine Disease (MSUD)

• This disease is inherited autosomal recessive; 5 forms are known.
• The most severe form involves the protein α-keto acid decarboxylase/acyl CoA dehydrogenase. The urine smells like maple syrup or burned sugar.
• Diagnostic testing examines the levels of branched chain amino acids (BCAA) and alloisoleucine in urine.
• Therapy includes dietary restrictions on BCAA.
• If caught within 10 days after birth, the child will undergo normal growth/development. BCAA's need to be monitored regularly.
<table>
<thead>
<tr>
<th></th>
<th>PKU</th>
<th>Diet Restriction</th>
<th>MSUD</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>YES</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td>Lifetime</td>
<td>Time</td>
<td></td>
<td>Lifetime</td>
</tr>
<tr>
<td>Messes with neurological tissue forever</td>
<td>Comment</td>
<td>Messes with neurological tissue forever</td>
<td></td>
</tr>
<tr>
<td>Blonde, blue-eyed</td>
<td>Pigment</td>
<td></td>
<td>No obvious characteristics</td>
</tr>
<tr>
<td>?</td>
<td>Acute Therapy</td>
<td></td>
<td>Thiamin at doses 100-500X normal</td>
</tr>
<tr>
<td>?</td>
<td>Crisis</td>
<td></td>
<td>Peritoneal dialysis</td>
</tr>
</tbody>
</table>
BCAA Metabolism -- 2
Inborn Error of Metabolism: Methyl Malonic Acid - Uria and -Osis

**Methyl malonic aciduria**
- B$_{12}$ deficiency with a secondary lack of methyl-B$_{12}$ complex formation
- Responds to B$_{12}$ dose > 500 µg

**Methyl malonic acidosis**
- Responds to dose > 250 µg
- B$_{12}$ deficiency with a secondary lack of adenosyl-B$_{12}$ complex formation
- Leads to elevated blood [Me-malonyl CoA] – since has H$^+$, increases [H$^+$] or reduces pH = acidosis
Methionine Catabolism
Inborn Error of Metabolism: Homocystinuria

- Elevated levels of both homocysteine and homocystine excreted in the urine.
- Due to cystathionine synthetase impaired activity.
- Therefore, cysteine BECOMES an essential amino acid.
- Causes fragile long bones, spontaneous dislocation of lens of eye, premature death.
- ALL seem due to collagen cross-linkage inhibition which causes weaknesses for “blowouts”
Homocystinuria

- This disease is inherited autosomal recessive. There is a deficiency in cystathionine-β-synthetase. The life expectancy of a patient with this disorder is reduced in the untreated patient and in the pyridoxine (B₆)-unresponsive patient. It causes retardation, arachnodactyly (spider fingers -- long, slender, curved), osteoporosis, dislocated optic lenses, high risk to throw clots (idiopathic), may have seizures, MI, CVA and PE.

- Diagnostic testing is to detect homocystinuria and cyanocobalamin levels. Therapy is aimed at two groups:
  - Group 1 is vitamin responsive and their urinary homocysteine excretion is reduced with doses at or greater than 200 mg of B₆ every day;
  - Group 2 is vitamin-unresponsive and must be treated by dietary modifications: reduce met in diet and increase cys in diet.
Two Forms of Homocystinuria

- First form
  - Mental retardation
  - May be increased risk of CVA
  - Tx: reduce excretion of homocystine with ≥ 200 mg B₆ qd
  - VITAMIN therapy

- Second form
  - Not responsive to B₆ tx
  - Therefore must feed cys and decrease met
  - DIET therapy
Threonine Catabolism

Primary function of thr is for phosphorylation to activate/inactivate various enzymes
Lysine Catabolism
Lys Significance

• Provides crosslinking in collagen
• Provides crosslinking in elastin
• When crosslinking is inhibited
  – By severe Cu deficiency or
  – Sweet pea toxin (β-aminopropionitrile)
• Leads to
  – Increased solubility of collagen
  – Increased rigidity of elastin
  – Death due to aortic rupture from the lack of elasticity at the aortic root
Arginine Catabolism
The Urea Cycle