

# BIOCHEMISTRY, MOLECULAR BIOLOGY & GENETICS REVIEW

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## MOLECULAR BIOLOGY

### 1. Nucleic Acid Structure

- Nucleotides: Base: Purine (A,G) or Pyrimidine (U,C,T)  
Sugar: Ribose (RNA) or Deoxyribose (DNA)  
Phosphate(s) (Absent in nucleosides)
- Polynucleotides: 3', 5' phosphodiester linkages between nucleotides  
Chain polarity based on sugar: 3' --> 5' or 5' --> 3'  
Complementary base pairing: A = T or U, G  $\equiv$  C  
Negatively charged phosphates
- DNA: B-form, right-handed double helix (Watson & Crick)  
Other forms: A (RNA-RNA or RNA-DNA), Z-DNA (left-handed)  
Antiparallel strands, complementary base pairing  
Melting temperature depends on GC/AT ratio  
Buoyant density (CsCl centrifugation) depends on GC/AT content  
Repetitive as well as unique sequences in eukaryotic genomes  
Human genome size: 3 billion base pairs/haploid, about 1000X > E. coli  
Nucleosomes and histones in eukaryotes
- RNA: Three major kinds: Ribosomal (rRNA)  
Transfer (tRNA)  
Messenger (mRNA)  
Hydrolyzed to nucleotides by alkali (2'-OH), unlike DNA; U instead of T  
Generally single stranded, but may have secondary structure

### 2. Nucleic Acid Analysis (Tools & Techniques)

- Restriction enzymes (palindromic recognition sites, defense role in bacteria)  
DNA sequencing (4 lane gel ladder)  
Electrophoresis (NAs have negative charge, smaller move faster through gel)  
Hybridization detection methods (complementary probe)  
Southern blot -- DNA analyzed  
Northern blot -- RNA analyzed  
(Western blot -- Protein, antibody detection)  
Reverse transcriptase (to make cDNA from mRNA)  
Polymerase Chain Reaction (PCR)  
Molecular cloning: plasmid & virus vectors, transfection into host cells, selection and screening for desired clones  
Restriction Fragment Length Polymorphisms (RFLP): Use in allelic analysis to detect inherited defects

### 3. DNA Replication

Semi-conservative

Bidirectional with many origins in eucaryotic chromosomes (multiple replicons)

Polymerization 5' --> 3' for each new strand, antiparallel copying of template

Discontinuous (Okazaki fragments) on lagging strand

RNA primer required (at origin and on lagging strand)

PPi released from each dNTP added (PPi --> 2 Pi)

Enzymes/proteins involved: Helicase (unwindase)

Topoisomerases

Single-strand binding proteins

Primase (RNA polymerase that makes primer)

Replicative DNA polymerase (E. coli: Pol III)

Processivity, Both strands, Trombone model

3'-exonuclease (proof reading)

5'-exonuclease (primer removal, E. coli: Pol I)

Ligase (ATP)

Occurs in S phase of cell cycle, complex controls

23 pairs of chromosomes in humans, linear DNA

### 4. DNA Damage and Repair

Thymine dimers, chemical adducts, deamination, nicks, ds breaks, etc.

Nucleotide excision repair -- replacement of damaged strand in local area

DNA glycosylase pathways (base excision repair) -- base removal followed by  
cleavage at abasic site and gap filling

Mismatch repair — defective in hereditary nonpolyposis colorectal cancer

Xeroderma pigmentosum -- defective excision repair, increased risk of skin cancer

### 5. RNA Synthesis (Transcription)

Three eukaryotic RNA polymerases:

Pol I -- major rRNA gene (nucleolus)

Pol II -- mRNAs

Pol III -- tRNAs and 5S rRNA

RNA polymerase binds at promoter sites to initiate transcription

Complex interaction with protein transcription factors, enhancer sites

No primer is needed; NTPs are used as substrates; One strand of the DNA copied

### 6. Processing of RNA Transcripts

Major rRNA: Transcribed from tandemly repeated genes, in nucleoli

Methylation of the RNA

Cleavage to 28, 18, and 5.8 S forms (5S from separate genes)

tRNA: Multiple gene copies

Transcripts cleaved and in some cases spliced; Many bases modified

CCA added to 3'-end (for a.a. attachment)

mRNA: Generally from single-copy genes

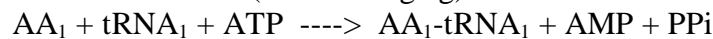
5'-capping with methyl-G structure (eukaryotic)

Some 3' cleavage followed 3'-poly A addition (eukaryotic); Splicing  
Intervening sequences (introns): Very common in higher eukaryotic genes  
Introns removed by splicing at the RNA level  
(Spliceosome, snRNAs and RNPs)  
RNA transport to cytoplasm and eventual degradation

## 7. Protein Synthesis (Translation)

The genetic code: Triplet nature, 64 codons total, 3 stop codons  
Universality (mitochondrial exceptions)  
Degeneracy, Wobble

Activation of amino acids (tRNA charging):



Important role of the synthetases

Events on the ribosome:

Initiation: Small ribosomal subunit, Met-tRNA\*, GTP, Initiation factors  
mRNA binding (5'-cap important, ATP used in eukaryotes)  
AUG initiation codon; Large subunit added last

Elongation: Binding of next AA-tRNA at A site (GTP, elongation factor)  
Peptide bond formation (rRNA catalysis)  
Translocation to P site (GTP and second elongation factor)

Termination: Requires stop codon, releasing factor, and GTP (eukaryotes)

Post-translational events:

Protein folding (chaperones), targeting (signal sequences), processing  
Protein degradation or turnover (lysosomes, proteasomes & ubiquitin)

Some inhibitors:

Puromycin: Inhibits translation by causing premature termination due to  
resemblance to AA-tRNA

Antibiotics that selectively inhibit bacterial protein synthesis:

Streptomycin, Tetracycline, Chloramphenicol, Erythromycin

Diphtheria Toxin: ADP-ribosylates and inhibits eucaryotic elongation  
factor involved in translocation

Actinomycin D: Inhibits transcription by binding to DNA template

## 8. Regulation of Gene Expression

Transcriptional control in bacteria: Operon model; Induction of the lac operon by  
lactose; inducer blocks ability of repressor protein to bind to operator site

Eucaryotic transcriptional control:

Importance of multiple transcription factors (proteins) that interact with  
DNA at enhancer sites, with each other, and with RNA polymerase

Importance of chromatin structure (euchromatin vs. heterochromatin)

Role of DNA methylation in gene silencing -- methylation of C in CpG sites in  
higher eucaryotes

Controls involving changes in gene number or arrangement in eucaryotes

Gene amplification: Can be drug-induced (Methotrexate, DHFR);  
Also seen in some cancers

- Gene rearrangements: Variable and constant regions of immunoglobulins
- Post-transcriptional controls:
  - Alternative mRNA splicing
  - Other RNA processing steps
  - Cytoplasmic transport
  - RNA degradation
- Controls of translation
  - Heme regulation of globin synthesis
  - Inhibition of host protein synthesis by some viruses
- Controls of post-translational events
  - Targeting to appropriate subcellular site (signal sequences, etc.)
  - Processing & modification (glycosylation, phosphorylation, etc.)
  - Protein degradation (ubiquitin pathway)

## HUMAN GENETICS

### 1. Patterns of Inheritance

- Mendelian patterns: Autosomal recessive and dominant  
X-linked recessive and dominant
- Non-Mendelian: Mitochondrial inheritance  
Genomic imprinting  
Penetrance and variable expressivity  
Anticipation (Triplet repeat diseases)  
X-inactivation in females  
Multifactorial inheritance

### 2. Human Chromosomes

- Autosomes: 22 pairs in diploid state
- Sex chromosomes: Females: XX                      Males: XY
- Chromosomal abnormalities:
  - Numerical: polyploidy, aneuploidy, trisomy
  - Structural: translocations, deletions, inversions

### 3. Genetic Testing, Mapping, Fingerprinting

Review nucleic acid tools and techniques above

### 4. Gene Therapy

- Somatic vs. Germline
- Therapeutic approaches: Ex vivo vs. in vivo delivery of DNA
- Delivery methods: Viral vectors, nonviral approaches
- Problem of gene targeting
- Production of therapeutic proteins by recombinant DNA technology

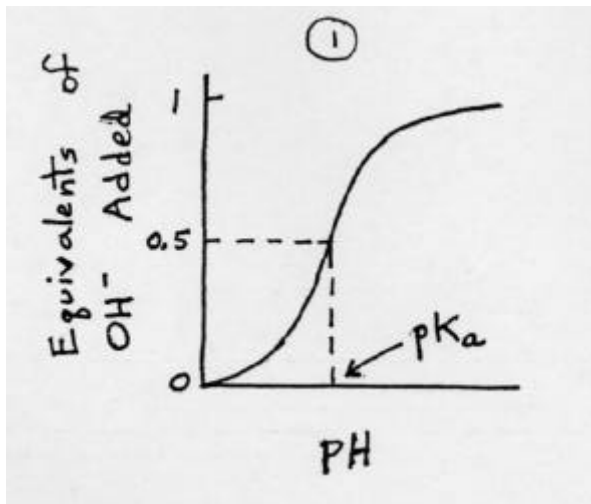
# PROTEIN STRUCTURE

## 1. General Features of the 20 Amino Acids

Acidic: Asp, Glu      Amides: Asn, Gln  
Basic: Lys, Arg, His  
Branched chain: Leu, Ile, Val  
Aromatic: Tyr, Trp, Phe  
Sulfur-containing: Cys, Met  
Hydroxy: Ser, Thr, (Tyr)  
Others: Gly (no asym.C), Ala, Pro (iminoacid)

## 2. Ionizations and pH

Acid: Proton donor  
Base: Proton acceptor  
pH:  $-\log[H^+]$   
Buffer: Mix of acid and its conjugate base (salt)  
pI: Isoelectric point, net charge = 0  
Henderson-Hasselbalch Equation:  $pH = pK_a + \log\{[salt]/[acid]\}$   
Titration curve (Figure 1)



## 3. Levels of Protein Structure

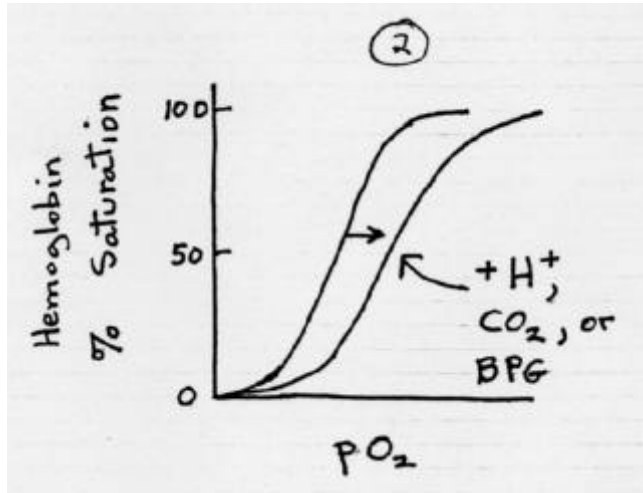
Primary: A.A. sequence, peptide bond (planar)  
Secondary: alpha helix, beta sheets (H-bonding)  
Tertiary: H-bonding, ionic interactions, hydrophobic forces; disulfide bond  
Quaternary: subunits

#### 4. Properties of Hemoglobin

Alpha<sub>2</sub>Beta<sub>2</sub> Tetramer; 4 Hemes; 4 Ferrous irons

Cooperative oxygen binding, sigmoid curve (unlike monomeric myoglobin)

Curve shifts to the right (oxygen release) due to CO<sub>2</sub>, H<sup>+</sup> (Bohr effect), or 2,3-Bisphosphoglycerate (2,3-BPG) (Figure 2)



Sickle cell anemia: point mutation, polymer formation in deoxygenated state

Methemoglobinemia: Ferric iron

Thalassemias: Reduced amounts of alpha or beta chains, defect in gene expression

#### 5. Properties of Collagen

Triple helix

Rich in Pro; Gly every third A.A.

Hydroxylation of Lys and Pro (iron, vitamin C -- scurvy)

Crosslink formation involving oxidized Lys/OH-Lys (Lysyl oxidase, copper)

### ENZYMES

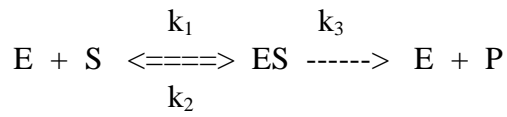
#### 1. General Properties

Nature of catalysis: lowers activation energy; increases rate of reaction in both directions; doesn't change equilibrium position, or  $\Delta G$

Most biological catalysts are proteins, but some are RNA (Ribozymes)

Coenzymes/cofactors -- provide additional catalytic groups (vitamins & minerals)

#### 2. Michaelis-Menten Kinetics



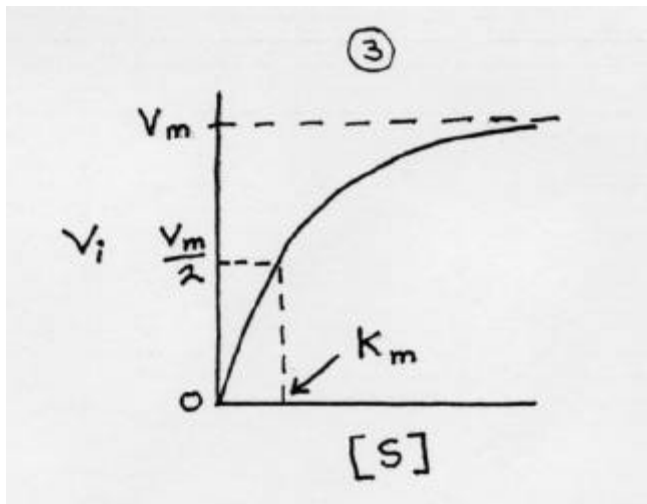
$$v_i = V_m[S]/\{K_m + [S]\}$$

$$K_m = (k_2 + k_3)/k_1$$

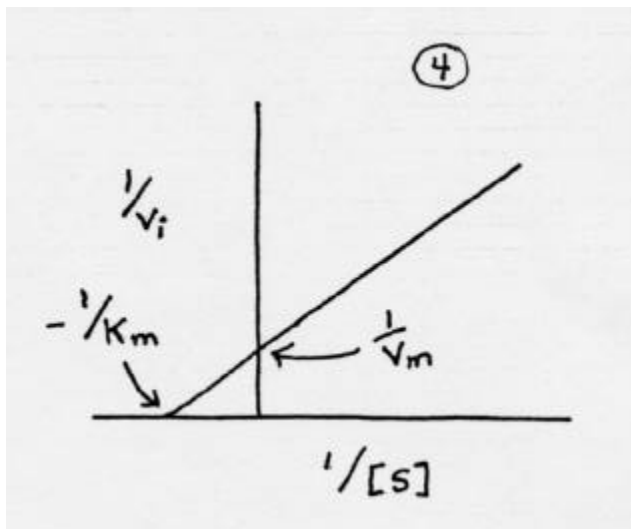
$$V_m = k_3 E_t$$

$$E_t = [E] + [ES]$$

Michaelis -Menten Plot  $\{v_i \text{ vs. } [S]\}$ , hyperbolic, shows saturation (Figure 3)

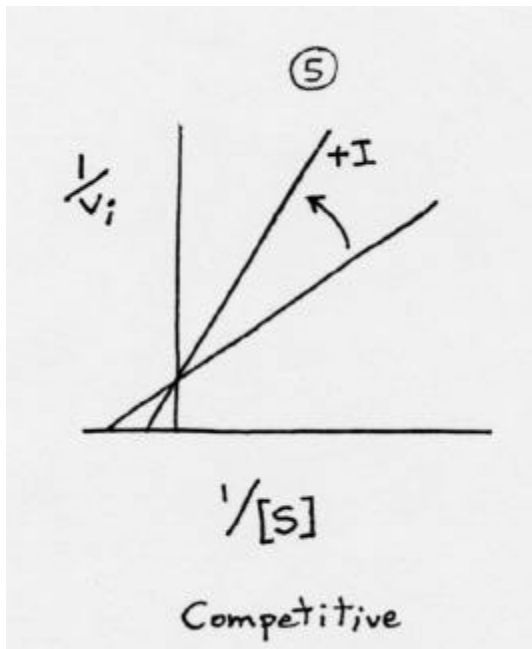


Lineweaver-Burk Plot  $\{1/v_i \text{ vs. } 1/[S]\}$ , linear (Figure 4)

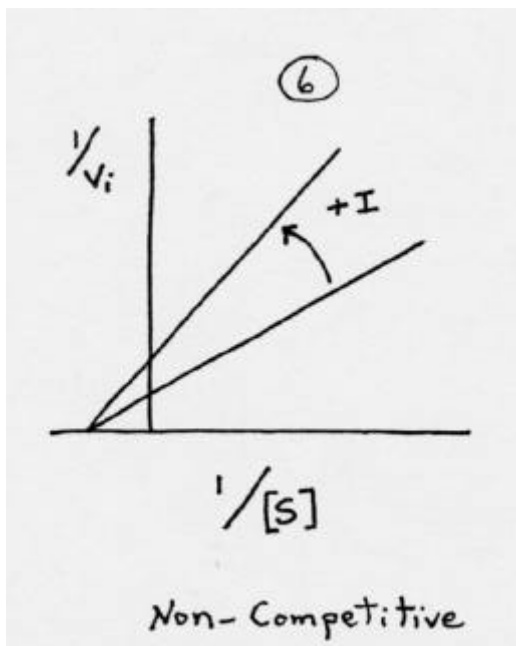


Effects of inhibitors:

Competitive:  $K_m$  changes (Figure 5)

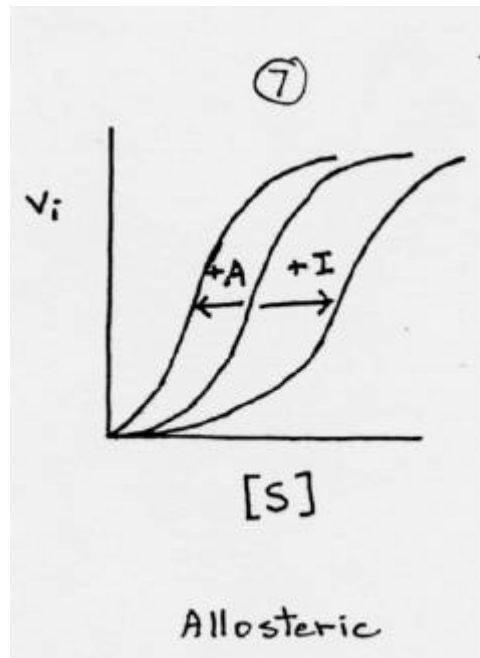


Noncompetitive:  $V_m$  changes (Figure 6)



### 3. Regulation of Enzyme Activity

Allosteric effects: (Figure 7)



Substrate cooperativity: sigmoid  $v_i$  vs.  $[S]$  curve

Activators: shift curve to left

Inhibitors: shift curve to right

Peptide cleavage (zymogens)

Phosphorylations (via protein kinases) -- Ser/Thr, Tyr

## ENERGY METABOLISM

### 1. General Concepts

Central role of mitochondria: matrix, inner membrane

Importance of ATP and high-energy phosphate bonds

Thermodynamic principles:

First law: Energy conservation

Second law: Entropy of universe increases for spontaneous processes

Free energy relationships:

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = \Delta G^0 + RT \ln \{[\text{Products}] / [\text{Reactants}]\}$$

$\Delta G < 0$  Exergonic reaction

$\Delta G > 0$  Endergonic reaction

$\Delta G = 0$  Equilibrium;  $\Delta G^0 = -RT \ln K_{eq}$

## 2. Tricarboxylic Acid (TCA) Cycle (Citric Acid or Krebs Cycle)

Uses 2 carbons from acetyl group of acetyl CoA to produce:

2 CO<sub>2</sub>

3 NADH (each worth about 2.5 ATPs)

1 FADH<sub>2</sub> (each worth about 1.5 ATPs)

1 GTP

Roles in catabolism of amino acids, fatty acids, sugars

Role in gluconeogenesis (energy, OAA)

Roles in amino acid synthesis (OAA & αKG) and fatty acid synthesis (citrate)

## 3. Mitochondrial Shuttle Systems

Malate - Aspartate (reducing equivalents in, OAA carbons out)

Glycerol phosphate (cytoplasmic reducing equivalents in)

Citrate shuttle (acetyl units out for fatty acid synthesis)

## 4. Formation of Acetyl CoA from Pyruvate

Pyruvate dehydrogenase complex (3 enzymes); Reaction:



Cofactors (vitamins): CoA (pantothenic acid), NAD<sup>+</sup> (niacin), TPP (thiamin), FAD (riboflavin), Lipoic acid

## 5. Electron Transport Chain (ETC)

Located in inner mitochondrial membrane; Complexes I, II, III, IV

Electrons from mitochondrial NADH or FADH<sub>2</sub> go to O<sub>2</sub> (producing H<sub>2</sub>O):



Chemiosmotic coupling: Protons are pumped out by complexes I, III, and IV

ATP synthesis is driven by the electrochemical gradient

ATP leaves mitochondrion in exchange for cytoplasmic ADP

Inhibitors:

Rotenone, Amytal: Inhibit NADH Dehydrogenase (Complex I)

Antimycin A: Inhibits Cytochrome b<sub>1</sub>/c (Complex III)

Cyanide, Azide, CO: Inhibit Cytochrome  $a_3$  (Complex IV)  
Atractyloside: Blocks ATP/ADP antiport  
Uncouplers (dinitrophenol, DNP; ionophores): Dissipate ion gradients  
Oligomycin: Inhibits ATP Synthase)

## CARBOHYDRATE METABOLISM

### 1. Classes of Carbohydrates

Monosaccharides: Glucose, Fructose, Galactose

Disaccharides: Sucrose (Glu + Fru);

Lactose (Gal + Glu)

Maltose (2 Glu)

Polysaccharides: Glycogen (alpha 1,4 and alpha 1,6 linkages)

Starch (less branched than glycogen)

Cellulose (beta 1,4 linkage)

### 2. Glycolysis

Cytoplasmic process (aerobic or anaerobic)

Regulated irreversible steps:

A. Hexokinase/glucokinase: Glucose + ATP ----> G-6-P + ADP

B. Phosphofructokinase-1: Fructose-6-P + ATP ----> F-1,6-bisP + ADP

Inhibitors: ATP, citrate

Activators: AMP, F-2,6-bisP (decreased by cAMP)

C. Pyruvate kinase: PEP + ADP ----> Pyruvate + ATP

Inhibited by phosphorylation due to cAMP in liver

Genetic defect in red cell form can produce hemolytic anemia

Other important steps:

Aldolase: Interconverts 6C and 3C compounds:

F-1,6-bisP <====> Glycerald-3-P + DHAP

Glycerald-3-P DH: NADH production

Phosphoglycerate kinase: reversible, ATP production:

Glycerald-1,3-bisP + ADP <====> 3-P-Glycerate + ATP

Lactate DH:  $\text{NAD}^+$  regeneration in anaerobic glycolysis

Pyruvate + NADH ---> Lactate +  $\text{NAD}^+$

Heart and muscle isoenzymes

Net energy produced:

Anaerobic:  $4 - 2 = 2$  ATPs

Aerobic:  $4 - 2 = 2$  ATPs + 2 NADHs (worth about 1.5 ATPs each) = 5

### 3. Gluconeogenesis

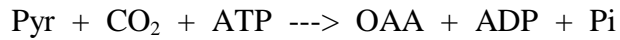
Occurs in liver (major) and kidney (minor)

Regulated by glucagon (+) and insulin (-)

Increased in kidney by acidosis (from glutamine)

Irreversible regulated steps:

A. Pyruvate carboxylase (biotin, mitochondrial):



Required activator: Acetyl CoA

B. PEP Carboxykinase:



Induced by glucagon via cAMP

Repressed by insulin

C. Fructose-1,6-bisPase:  $\text{F-1,6-bisP} + \text{H}_2\text{O} \rightarrow \text{F-6-P} + \text{Pi}$

Inhibitors: AMP, F-2,6-bisP

Activators: ATP, citrate

D. Glucose-6-Pase:  $\text{G-6-P} + \text{H}_2\text{O} \rightarrow \text{Glucose} + \text{Pi}$

Substrates for gluconeogenesis: Lactate; Alanine and other glucogenic amino acids; Glycerol (enters at DHAP);

*Not* most fatty acids nor acetyl CoA

Energy: Mainly from fatty acid oxidation in liver

#### 4. Glycogen Metabolism

Synthesized from UDPG by glycogen synthase (alpha 1,4 linkages):

Inhibited by phosphorylation due to cAMP (glucagon)

Activated by dephosphorylation (insulin)

Broken down by phosphorylase (phosphorolysis of  $\alpha$ 1,4 linkages to give G-1-P)

Activated by phosphorylation due to cAMP (glucagon)

Inhibited by dephosphorylation (insulin)

Branching and debranching enzymes (alpha 1,6 branch point cleavage yields glucose, not G-1-P)

Especially important in liver and muscle -- liver glycogen is an important source of blood glucose during early fasting state; muscle glycogen is a source of internal energy for contraction

Glycogen storage diseases

Von Gierke's -- defect in G-6-Pase (liver)

Other defects can occur in phosphorylase, phosphorylase kinase, branching or debranching enzymes, etc.

#### 5. Pentose Phosphate Pathway (hexose monophosphate shunt)

Glucose-6-phosphate dehydrogenase: First enzyme; Defect leads to hemolytic anemia -- Drugs that can act as oxidizing agents increase the problem

Functions of Pentose-P Pathway:

Production of NADPH (needed for fatty acid synthesis, maintaining reduced glutathione, etc.)

Production of ribose-5-P (needed for nucleotide & nucleic acid synthesis)

Interconversion of sugars via the transaldolase & transketolase reactions

## LIPID METABOLISM

### 1. Categories of Lipids

Fatty acids: Saturated -- Palmitic (C<sub>16</sub>), Stearic (C<sub>18</sub>)  
 Monounsaturated -- Palmitoleic, Oleic  
 Polyunsat. -- Linoleic & Linolenic (essential); Arachidonic

Triacylglycerols: Glycerol + 3 fatty acids

Phosphoglycerols: Glycerol + 2 fatty acids + phosphate + X  
 X = Serine, Ethanolamine, Choline, Inositol, Glycerol

Sterols: Cholesterol, Steroid hormones, Bile acids, Vitamin D (sterol derivative)

Sphingolipids: Sphingomyelin, Cerebrosides, Sulfatides, Globosides, Gangliosides  
 (sphingosine + fatty acid + sugars or other components)

Eicosanoids: Prostaglandins, Thromboxanes, Leukotrienes

## 2. Lipid Transport in Blood (as protein particles)

Chylomicron -- from intestine to organs, initially via the lymphatic system  
 VLDL -- triacylglycerols from liver to other organs  
 LDL -- cholesterol from liver to other organs  
 HDL -- cholesterol back to liver  
 Albumin -- free fatty acids from adipose tissue to other organs

## 3. Fatty Acid Oxidation (beta oxidation)

Occurs in mitochondria; Carnitine involved in f.a. transport from cytoplasm  
 Fatty acyl CoA derivative is oxidized to give FADH<sub>2</sub>, NADH and Acetyl CoA  
 from successive 2-C units: Acetyl CoA enters TCA Cycle; FADH<sub>2</sub> and  
 NADH donate electrons to ETC

Odd-carbon fatty acids (rare) also yield one propionyl CoA, which is converted to  
 succinyl CoA and enters TCA cycle (the only part of fatty acids that is  
 glucogenic)

## 4. Fatty Acid Synthesis

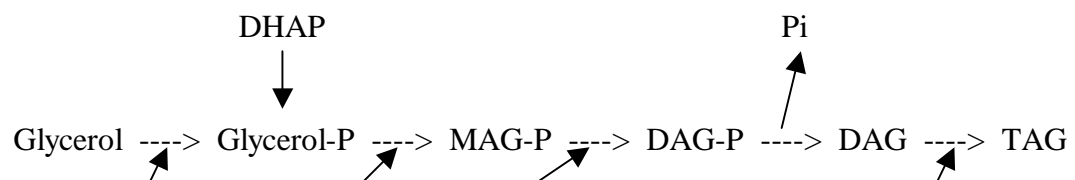
Cytoplasmic process; Citrate shuttle brings acetyl groups out from mitochondrion:  
 Citrate cleavage in cytoplasm: Citrate + CoA --> OAA + AcCoA  
 Completing cycle: OAA --> Malate --> Pyruvate + CO<sub>2</sub> (malic enzyme /  
 MDH decarboxylating); Pyruvate returns to mitochondrion

Need for NADPH (from pentose phosphate pathway and malic enzyme reaction)

Regulated enzyme: Acetyl CoA Carboxylase (biotin):  
 Acetyl CoA + CO<sub>2</sub> + ATP ---> Malonyl CoA + ADP + Pi  
 Increased by insulin, decreased by glucagon in liver

Fatty Acid Synthetase:  
 Acetyl CoA + 7 Malonyl CoA + 14 NADPH ---->  
 Palmitate + 14 NADP + 8 CoA + 7CO<sub>2</sub>

## 5. Triacylglycerol (TAG) Formation



ATP

FACoA

FACoA

FACoA

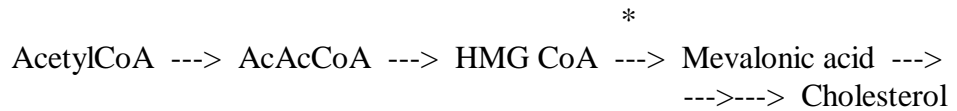
Mobilization of FAs from TAGs in adipose tissue involves hormone-sensitive lipase, which is stimulated by glucagon and inhibited by insulin

### 6. Phospholipid Synthesis

Involvement of CDP derivatives such as CDP-DAG and CDP-choline  
DAG-P is a common precursor of both TAGs and phosphoglycerols

### 7. Cholesterol Synthesis

Cytoplasmic pathway:

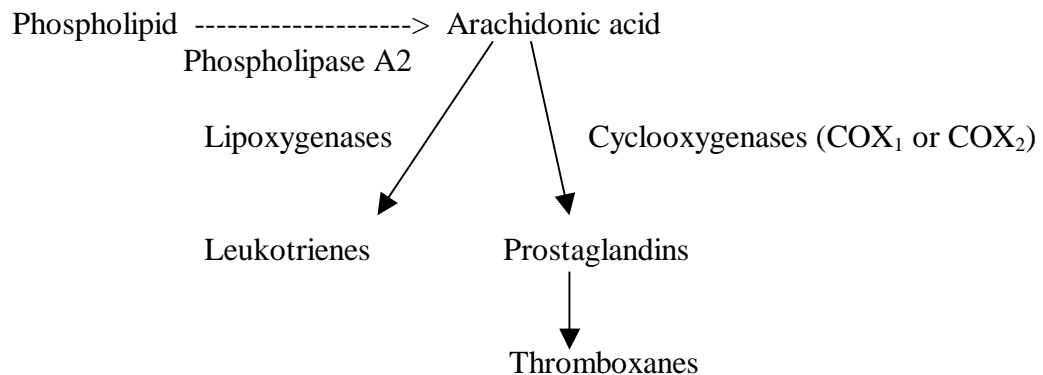


HMG CoA reductase (\*) is key enzyme; it can be inhibited by drugs used to treat elevated serum cholesterol (HMG = hydroxy methyl glutaryl)  
Cholesterol is a precursor of steroid hormones, vitamin D, and bile acids  
Bile acids function in the emulsification of fats and serve to eliminate cholesterol from the body via the feces

### 8. Ketone Bodies

Acetoacetate, β-Hydroxybutyrate, Acetone  
Formed in liver mitochondria from acetyl CoA; Used by non-hepatic tissues  
Formation increases under conditions of increased fat breakdown, as in starvation, diabetes, etc.  
Role as energy source for muscle, etc. and for brain during prolonged starvation

### 9. Eicosanoid Metabolism



Aspirin and other non-steroidal anti-inflammatories inhibit cyclooxygenase (more specific inhibitors are becoming available for COX<sub>1</sub> and COX<sub>2</sub>)  
Anti-inflammatory steroids lead to inhibition of phospholipase A2

## 10. Lipid Diseases

Hypercholesterolemia due to LDL receptor defect: Elevated serum LDL cholesterol and increased risk of atherosclerosis; Receptors deficient in number or unable to internalize LDLs, resulting in reduced feedback inhibition of intracellular cholesterol synthesis.

Lipid storage diseases: Deficiency in one of the enzymes that catabolize sphingolipids. Examples: Gaucher's, Nieman-Pick, Fabry's, Tay-Sachs, etc.; The resulting lipid accumulates to harmful levels, often having effects on nerve and muscle.

I-Cell Disease: A generalized lysosomal storage disease, due to a defect in adding the mannose-6-P targeting signal which directs enzymes to the lysosomes during their processing in the Golgi.

## SIGNAL TRANSDUCTION PATHWAYS

### 1. Cyclic AMP

Hormone -----> Cell Surface Receptor -----> G Protein ----->

Adenylate Cyclase -----> cAMP -----> Protein kinase A ----->

Protein phosphorylations (on ser/thr)

G proteins can be stimulatory or inhibitory; They are active when GTP is bound and inactive after GTP is hydrolyzed to GDP by internal GTPase.

G proteins are targets for ADP-ribosylation by cholera toxin and pertussis toxin, interfering with their ability to function as GTPases.

Phosphodiesterases that breakdown cAMP are inhibited by methylxanthines like caffeine. (cAMP = a second messenger)

Protein phosphorylations are reversed by Phosphoprotein Phosphatases.

Phosphorylated proteins can be more or less active, can be enzymes or transcription factors.

### 2. Cyclic GMP

Membrane-associated guanylate cyclase: A transmembrane protein with external receptor domain and internal cyclase domain (Atrial Natriuretic Factor receptor)

Cytoplasmic guanylate cyclase: A heme protein activated by nitric oxide (NO)  
Cyclic GMP activates Protein Kinase G (ser/thr)

### 3. Phosphoinositide Pathway

Hormone ---> Cell Surface Receptor ---> G Protein ---> Phospholipase C

Phosphatidylinositol bis phosphate (PIP<sub>2</sub>) -----> IP<sub>3</sub> + DAG

Second Messengers:

IP<sub>3</sub> ---> Releases Ca<sup>2+</sup> from ER

Ca<sup>2+</sup> ---> Stimulates calmodulin-dependant protein kinases and other enzymes

DAG ---> Activates Protein Kinase C  
Ca<sup>2+</sup>

Phorbol ester tumor promoters stimulate Protein Kinase C

LiCl (used to treat manic-depression) inhibits a phosphatase involved in IP<sub>3</sub> recycling

#### 4. Tyrosine Protein Kinase Receptor Pathways (Insulin, EGF, PDGF, etc.)

Hormone ---> Transmembrane Receptor Tyr Kinase ---> Autophosphorylation of the Receptor on Tyr ---> Downstream Events (via docking of proteins, other phosphorylations, etc.)

For insulin, downstream events include phosphorylation of insulin receptor substrate 1 (IRS-1), other phosphorylations, eventual activation of phosphoprotein phosphatases, and dephosphorylation of key enzymes.

#### 5. Steroid / Thyroid / Vitamin D / Retinoic Acid Pathways

Hormone ---> Intracellular receptor (in cytoplasm or nucleus)

Activated receptor binds to a hormone response element (HRE) in DNA and alters the rate of (usually activates) transcription for specific genes.

## AMINO ACID METABOLISM

### 1. Catabolism of Amino Acids

Conversion of carbon skeletons to ketogenic or glucogenic precursors and amino groups to ammonia and urea

Strictly ketogenic: Lys and Leu (Carbons to acetyl CoA or ketone bodies)

Glucogenic (totally or partially): All other 18 amino acids (Carbons to αKG, succinyl CoA, fumarate, OAA, or pyruvate; then to glucose)

Reactions involved in amino/amido group elimination:

Transamination: Pyridoxal phosphate coenzyme, always involve glu/αKG, no free ammonia

Deamination: Oxidative (redox cofactor): Glu, Gly

Non-oxidative: Ser, Thr, Cys

Deamidation: Gln, Asn

Role of Gln in transport of "ammonia" in blood:

In most tissues:  $\text{Glu} + \text{NH}_3 \rightarrow \text{Gln}$  (ATP required)

In kidney, intestine, and liver:  $\text{Gln} \rightarrow \text{Glu} + \text{NH}_3$

Role in elimination of acid in kidney

## 2. Urea Cycle

Liver mitochondria (steps 1 & 2) and cytoplasm (steps 3, 4, & 5)

Known genetic defects occur in each of the 5 enzymes, lead to hyperammonemia

Acetyl Glu regulates Carbamoyl-P synthetase I (step 1)

Other enzymes: Orn transcarbamoylase, Arg-succinate synthase,

Arg-succinate lyase, Arginase

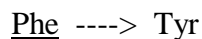
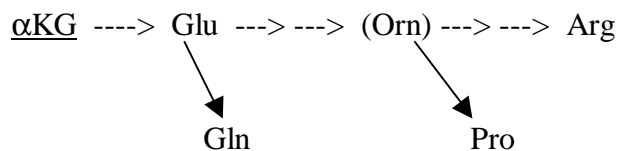
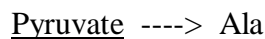
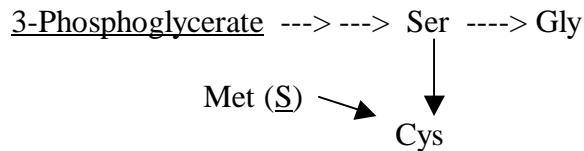
Nitrogens of urea come directly from  $\text{NH}_3$  and aspartate but indirectly from all amino acids via transaminations and deaminations, etc.

## 3. Amino Acid Biosynthesis

Essential amino acids (required in diet): Leu, Ile, Val, Phe, Try, Met, Thr, Lys, His, and sometimes Arg (in children)

Nonessential amino acids (can be made in humans in adequate amounts): Ala, Asp, Asn, Glu, Gln, Pro, Ser, Gly, Cys, Tyr

Metabolic sources of the nonessential amino acids:



## 4. One-carbon Metabolism

Conversion of folic acid to coenzyme THFA: Dihydrofolate reductase (inhibited by antifolates, like methotrexate, used to treat cancer)

Sources of one-carbon fragments for THFA pool: Ser, Gly, His, Trp (minor)

Metabolic uses of the one-carbon THFA pool:

Permits conversion of glycine to serine and synthesis of glycine

Synthesis of purines (2 carbons)



## AMP, and GMP

### Salvage:

Phosphoribosyl transferases (PRTases)  
HGPRTase -- missing in Lesch-Nyhan Syndrome;  
low in some forms of gout

### Degradation:

Uric acid end product; accumulates in joints to cause gout (can treat with allopurinol which inhibits xanthine oxidase)  
Adenosine Deaminase (ADA) or Purine Nucleoside Phosphorylase (PNP) defects cause immunodeficiency diseases

## 2. Pyrimidines (U, C, T)

### De novo synthesis:

From Asp, Gln, and CO<sub>2</sub>; Involves formation of carbamoyl-P by cytoplasmic Carbamoyl-P Synthetase II  
Regulated via feedback inhibition of Carbamoyl-P Synthetase II by UTP and activation by PRPP  
CTP formed from UTP  
Orotic aciduria -- accumulation of an intermediate of the pathway; treated with uridine to bypass the block

### Deoxynucleotide formation:

Ribonucleotide reductase -- uses both purine and pyrimidine NDPs;  
Thioredoxin the immediate source of reducing power;  
Complex feedback regulation by dNTPs  
Inhibited by hydroxyureas  
Thymidylate Synthetase:  
 $dUMP + CH_2-THFA \rightarrow dTMP + DHFA$   
Inhibited by antifolates and 5-FdUMP (from fluorouracil)

### Salvage:

Kinases used for nucleosides and PRTases for bases

### Degradation:

Ring cleavage occurs, unlike purines; simple products, no uric acid

## TOPICS NOT COVERED IN ABOVE REVIEW

Aspects of nutrition and digestion  
Neurochemistry  
Blood clotting  
Heme and iron metabolism

