BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
Electron transport
Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
Fatty acid Catabolism
Mobilization from tissues (mostly adipose)
Activation of fatty acids
Transport; carnitine
Oxidation: β-oxidation, 4 steps:
Protein Catabolism
Amino-Acid Degradation
Dealing with the nitrogen; Urea Cycle
Dealing with the carbon; Seven Families
Nucleic Acid & Nucleotide Degradation

Exam-3 material

ANABOLISM I: Carbohydrates

PHOTOSYNTHESIS:

Exam-4 material

Overview;; Key experiments:
Light Reactions
Reaction center
Photosystems (PSII & PSI - NADPH)
Proton Motive Force - ATP
Carbon Assimilation - Calvin Cycle
Overview and regulation
C4 versus C3 plants
Kornberg cycle - glyoxylate
Carbohydrate Biosynthesis in Animals
Gluconeogenesis
Glycogen Synthesis
Pentose-Phosphate Pathway
oxidative-NADPH
non-oxidative-Ribose 5-P
Regulation of Carbohydrate Metabolism
Anaplerotic reactions

ANABOLISM II: Lipids

Fatty Acids
contrasts
location & transport
Synthesis: ACC & fatty acid synthase
Control of fatty acid metabolism
Diversification of fatty acids
elongation
desaturation
Eicosanoids
Prostaglandins and Thromboxane
Triacylglycerides
Membrane lipids
Glycerophospholipids
Isoprene lipids:
Ketone body synthesis
Cholesterol

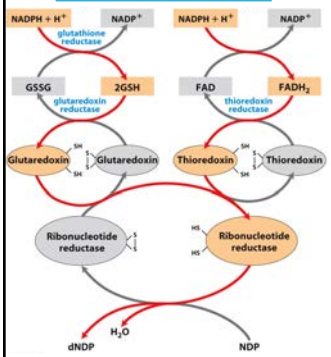
ANABOLISM III: Nitrogen (Amino Acids & Nucleotides)

Nitrogen cycle - Nitrogen fixation
nitrogenase
Nitrogen assimilation
Plants
Nitrate/nitrite reductases
Animals
Glutamine synthetase
Glutamate synthase
Amino-acid Biosynthesis
non-essential
essential
Nucleotide Biosynthesis
RNA precursors
Denovo vs. salvage
Purines
Pyrimidines
DNA precursors
Control of nitrogen metabolism
Secondary products of amino acids

Exam-5 material

Biosynthesis Amino Acids & Nucleotides

Source of Reducing Electrons for Ribonucleotide Reductase

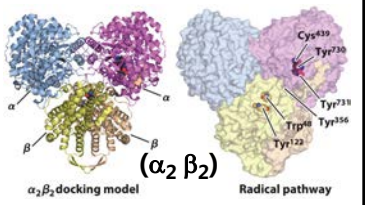


- NADPH serves as the electron donor.
- Funneled through glutathione or thioredoxin pathways

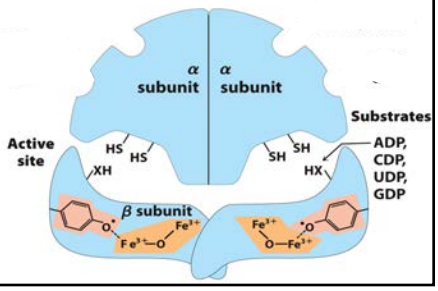
Structure of Ribonucleotide Reductase

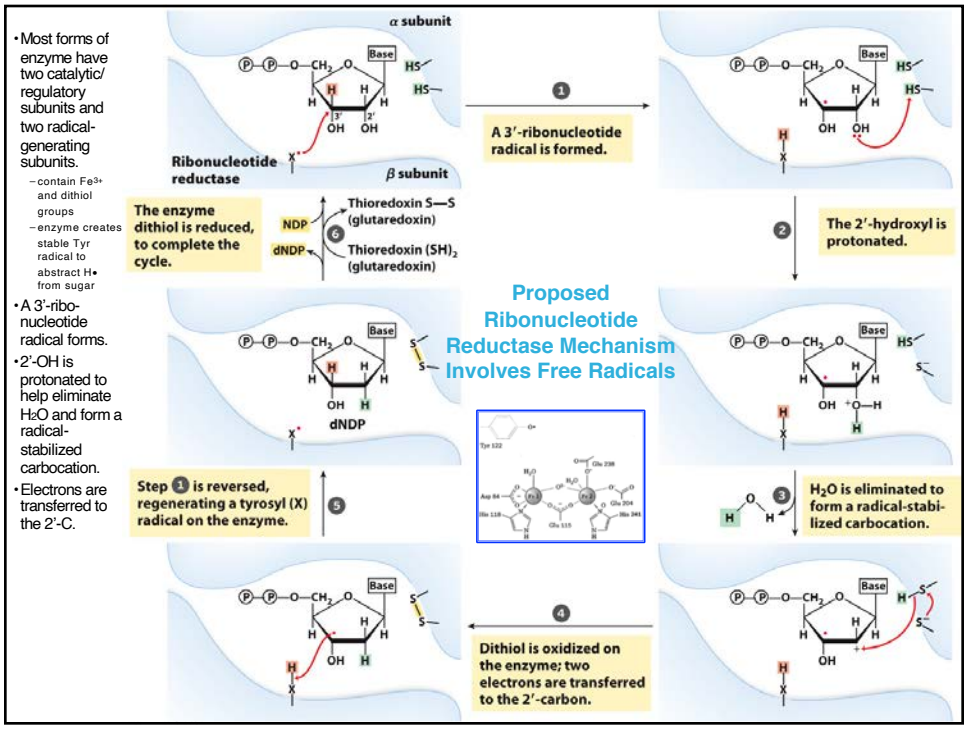
α_2 are regulatory and half the catalytic site; need to be reduced.

β_2 are the other half of the active site, and the free-radical generators



JoAnne Stubbe (1946–)

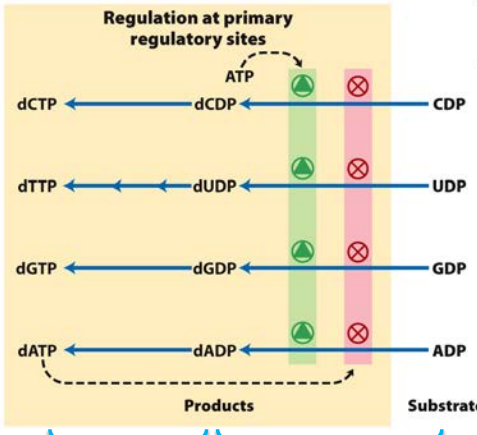


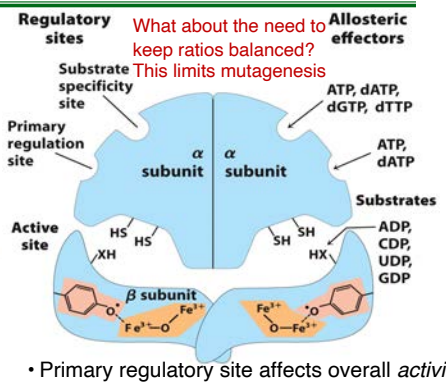


Biosynthesis Amino Acids & Nucleotides

On/off Regulatory Site of Ribonucleotide

Feedback allosteric inhibition by dATP & activation by ATP





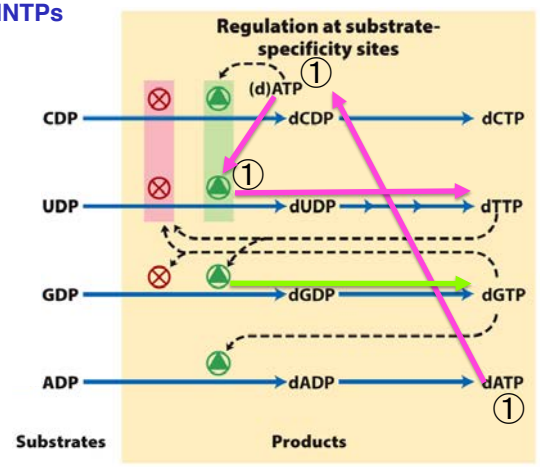
- Primary regulatory site affects overall *activity*
  - –ATP activates
    - If energy sufficient, get ready for DNA replication
  - –dATP inhibits
    - If too much dATP, don't make any of the others as well
- Enzyme ***oligomerizes*** to accomplish this change.

Biosynthesis Amino Acids & Nucleotides

Specificity Regulatory Sites of Ribonucleotide Reductase

Feedback allosteric inhibition by dNTPs

- The other site affects *substrate specificity* in order to maintain balanced pools of nucleotides.
- THREE LEVELS:
  - **A:T**: If dATP high → MORE specificity for UDP (A:T)



Biosynthesis Amino Acids & Nucleotides

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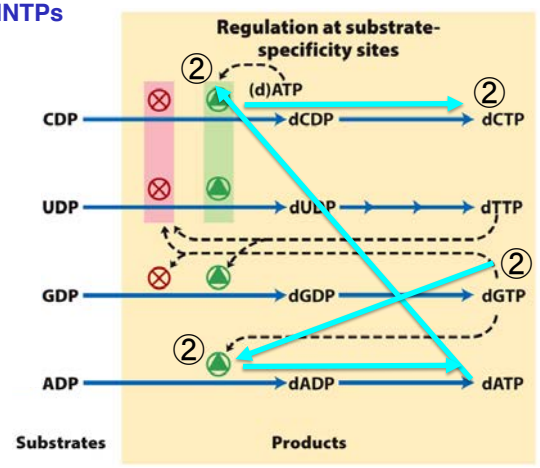
  - **G:C**: if dGTP high, more dATP→ more specificity for CDP



Biosynthesis Amino Acids & Nucleotides

Specificity Regulatory Sites of Ribonucleotide Reductase

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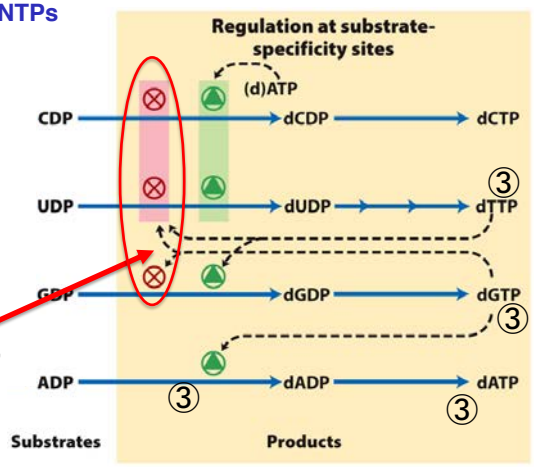
- THREE LEVELS:
  - **A:T**: If dATP high → MORE specificity for UDP (A:T)
  - **G:C**: if dGTP high, more dATP→ more specificity for CDP
  - **Inhibition**: If dTTP or dGTP high, UDP, GDP (classic feedback) **&** CDP inhibited (balance G:C).



Biosynthesis Amino Acids & Nucleotides

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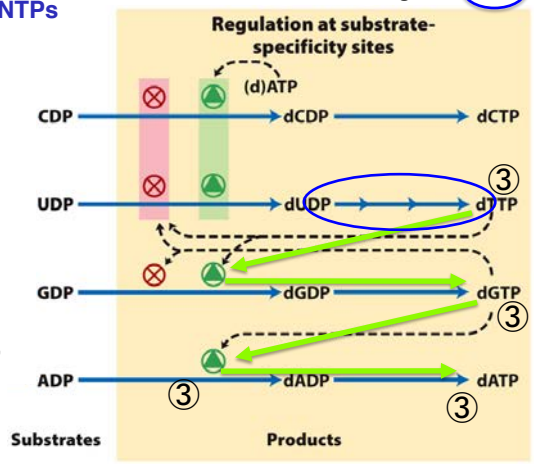
  - **G:C**: if dGTP high, more dATP→ more specificity for CDP
  - **Inhibition**: If dTTP or dGTP high, UDP, GDP **&** CDP inhibited.
    T:A: If dTTP high, it will indirectly cause MORE specificity for ADP→more dATP

Example:
dGTP inhibits making less dCTP
dATP activates making more dTTP



Biosynthesis Amino Acids & Nucleotides

dTTP is made from dUMP

Ribonucleotide reductase

Non-specific *nucleoside diphosphate kinase*

GDP→dGDP→dGTP

ADP→dADP →dATP } DNA

CDP→dCDP→dCTP

UDP→dUDP→dUTP

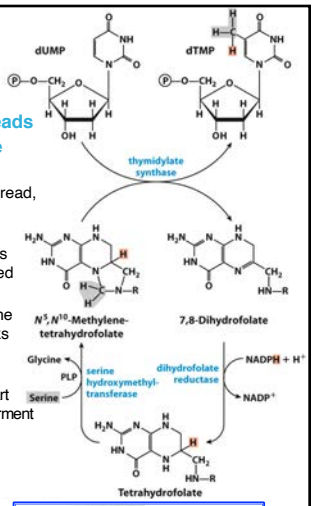
dUMP ← *dUTPase*

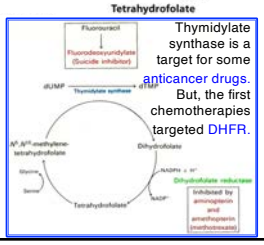
dUMP → dTMP →dTDP→dTTP

Thymidylate Synthase

1. dUDP is made by *ribonucleotide reductase*
2. dUTP is made by *nucleoside diphosphate kinase*
3. dUMP is made by *dUTPase*, with PP_i as product
4. dUMP → dTMP by *thymidylate synthase*
   - adds a methyl group from *N^5,N^{10}-methylene-tetrahydrofolate*
5. dTMP → dTDP → dTTP

- **Folic Acid Deficiency Leads to Reduced Thymidylate Synthesis**
- Folic acid deficiency is widespread, especially in nutritionally poor populations.
- Reduced thymidylate synthesis causes uracil to be incorporated into DNA.
- Repair mechanisms remove the uracil by creating strand breaks that affect the structure and function of DNA.
  - associated with cancer, heart disease, neurological impairment





Thymidylate synthase is a target for some anticancer drugs. But, the first chemotherapies targeted DHFR.

ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

1) Nitrogen fixation: N_2 → NH_4
2) Nitrogen assimilation: incorporation of ammonia into biomolecules
3) Biosynthesis of amino acids
   a) non-essential
   b) essential
4) Biosynthesis of nucleotides and deoxynucleotides
5) Control of nitrogen metabolism
6) Biosynthesis and degradation of heme; other 2° products of amino acids

Biosynthesis Amino Acids & Nucleotides

Regulation of Nucleotide Metabolism (Review):

Purines: Feedback inhibition by AMP, GMP, IMP at committed steps
Pyrimidines: Feedback inhibition by CTP at committed step
PRPP synthesis: Feedback inhibition by GDP, ADP, UMP, CTP
RNA/DNA precursors: GTP activates CTP synthetase from UTP; GTP used for AMP and ATP used for GMP; Ribonucleotide reductase

Regulation of Overall Nitrogen Metabolism and Amino Acid Biosynthesis

Multilayered approach: Often, more than one mechanism of feedback regulation is utilized. There are FOUR modes of Feedback control

De novo purine-GMP

Sequential – every committed product feeds back

homoserine dehydrogenase in Thr & Ile

Concerted – all end-products must be present to inhibit at all

Glutamine Synthetase

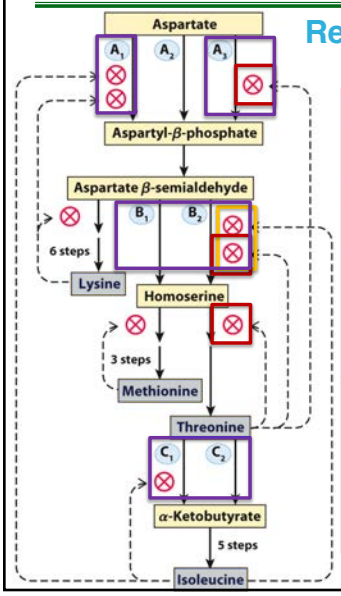
Cummulative – all end-products inhibit the same percentage

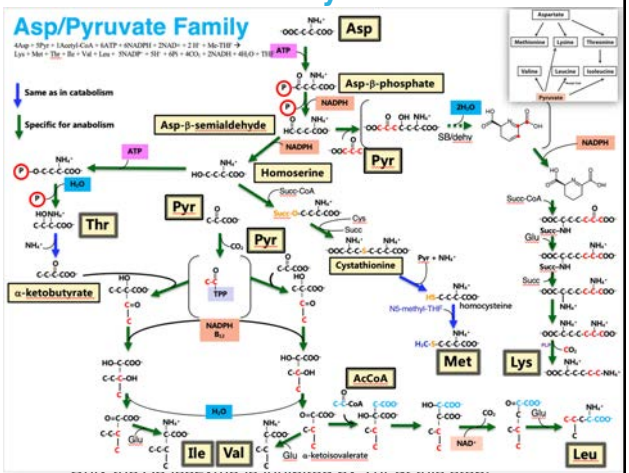
Aromatic amino acids

Isozymes – use of isozymes for regulation of specific pathways, each end-product inhibits a specific isozyme

Biosynthesis Amino Acids & Nucleotides

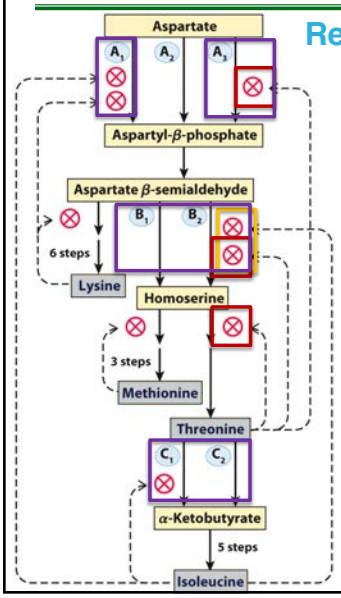
Regulation of Aspartate/Pyruvate-Derived Pathways





What is a good example of Cumulative feedback inhibition?

Biosynthesis Amino Acids & Nucleotides



Regulation of Aspartate/Pyruvate-Derived Pathways

Classic Feedback Inhibition in Lys, Met, Thr, and Ile Biosynthesis

Sequential Feedback Inhibition in Thr Biosynthesis

Concerted Feedback Inhibition of Homoserine Dehydrogenase in Ile and Thr Biosynthesis

Use of Isozymes Is Another Important Means of Regulation:

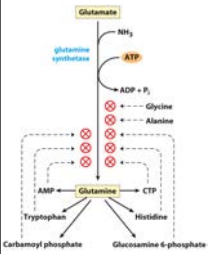
Homoserine dehydrogenase (B)
Serine/Threonine dehydratase (C)
Aspartate Kinase (A)

Asp Kinase initiates biosynthsis of Lys, Met, Thr, and Ile. Use of isozymes, all regulated by different effectors, allows *E. coli* to produce the amino acids when needed.

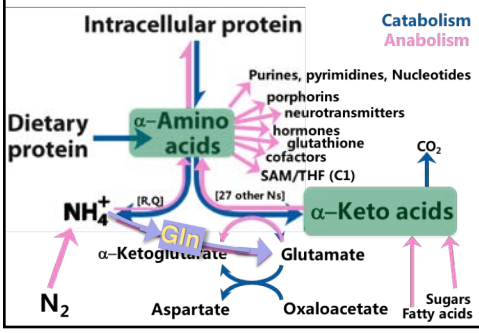
Example: *Asp Kinase* isozyme A1 is inhibited if Ile or Lys is high, but not if Met or Thr are high.
Only the A3 isozyme is inhibited by Thr at this step.

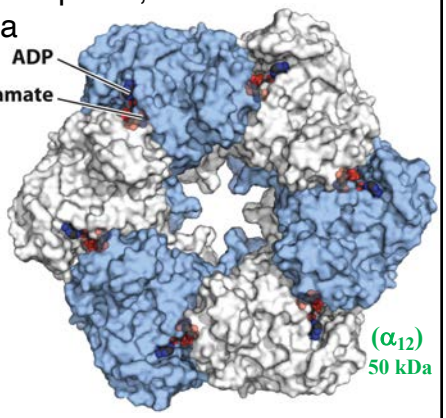
What is a good example of Cumulative feedback inhibition?

Biosynthesis Amino Acids & Nucleotides



Cumulative allosteric inhibition by:
CTP, AMP, His,
Trp, Carbamoyl- phosphate, Glc-amine-6-P, Gly, Ala





Glutamate + NH_4^+ + ATP → glutamine + ADP + P_i + H^+

Biosynthesis Amino Acids & Nucleotides

Adenylylation of Glutamine Synthetase

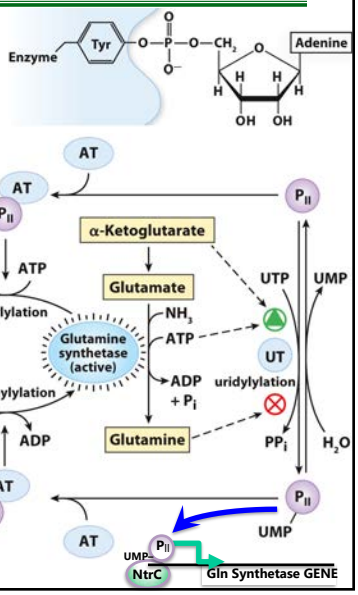
Adenylylation (attachment of AMP) to Tyr-397 assists in inhibition.

- Increases sensitivity to feedback inhibitors
- Part of complex cascade that is dependent on [Glu], [α-ketoglutarate], [ATP], and [P_i]
- Activity of *adenylyltransferase (AT)* regulated by binding to regulatory protein P_{II}
- ***Adenylylation*** inhibits *Gln synthetase*.

When P_{II} is uridylylated by *uridylyltransferase (UT), which* *adenylyltransferase* stimulates deadenylylation of Gln synthetase (increasing the latter's activity).

- Uridylyation controlled by Gln (inhibits) and α-KG & ATP (activates).

ALSO, uridylylated P_{II} upregulates transcription of Gln synthetase.



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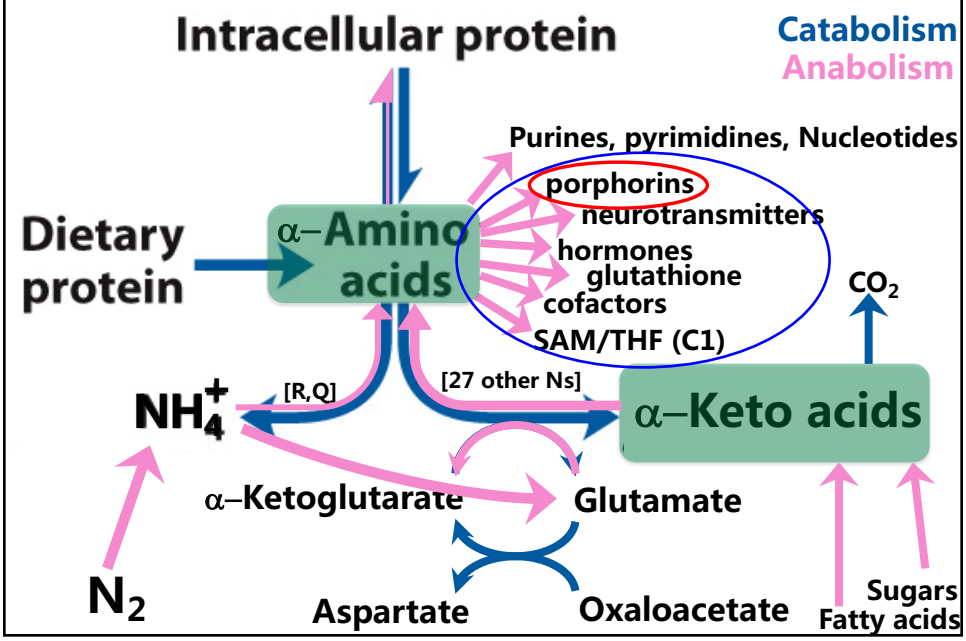
Biosynthesis Amino Acids & Nucleotides



Biosynthesis Amino Acids & Nucleotides

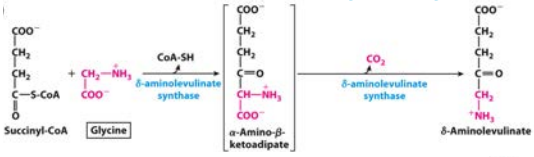
Many Important Metabolites are Derived from Amino Acids

- Porphyrin rings (e.g., heme, cytochromes, chlorphylls, etc.)
- Phosphocreatine
- Glutathione
- Cofactors; niacin, biotin, folic acid
- Neurotransmitters (serotonin, GABA, adrenalin, DOPA, histamine)
- Signaling molecules
  - Hormones; melatonin, adrenaline
  - Paracrine signals; NO, leukotrienes
- Cell-wall constituents; Peptidoglycan, Lignin

Biosynthesis Amino Acids & Nucleotides

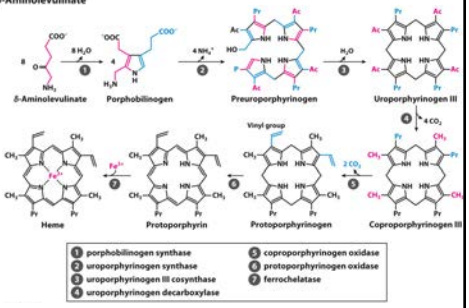
Porphyrin Biosynthesis

Glycine is the Precursor to Porphyrins; synthesis of δ-Aminolevulinate



- Porphyrin makes up the heme of hemoglobin, cytochromes, myoglobin.
- In higher animals, porphyrin arises from reaction of glycine with succinyl-CoA.
  - In plants and bacteria, glutamate is the precursor.
- The pathway generates two molecules of the important intermediate *δ-aminolevulinate.*
- *Porphobilinogen* is another important intermediate.

Synthesis of Heme from δ-Aminolevulinate



1. Two molecules of δ-aminolevulinate condense to form porphobilinogen.
2. Four molecules of porphobilinogen combine to form protoporphyrin.
3. Fe ion is inserted into protoporphyrin with the enzyme *ferrochelatase.*

Biosynthesis Amino Acids & Nucleotides

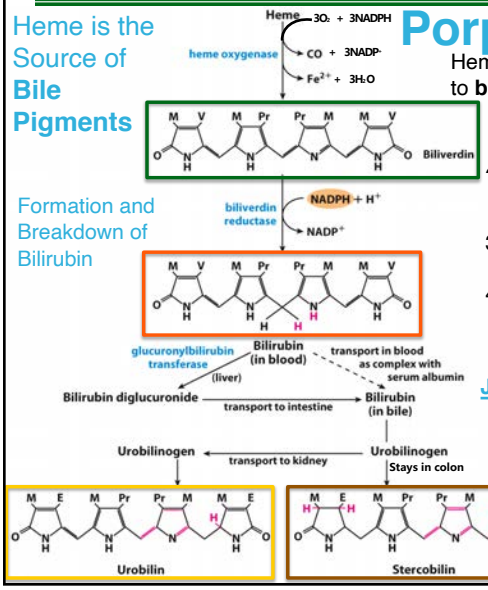
Defects in Heme Biosynthesis

- Most animals synthesize their own heme.
- Mutations or mis-regulaton of enzymes in the heme biosynthesis pathway lead to **porphyrias** (pour-fear-ia).
  - Precursors accumulate in red blood cells, body fluids, and liver.
- Accumulation of precursor uroporphyrinogen I
  - Urine becomes discolored (pink to dark purplish depending on light, heat exposure).
  - Teeth may show red fluorescence under UV light.
  - Skin is sensitive to UV light.
  - There is a craving for heme.
- Explored as possible biochemical basis for vampire myths

Biosynthesis Amino Acids & Nucleotides

Porphyrin Degradation

Heme is the Source of **Bile Pigments**



Formation and Breakdown of Bilirubin

Heme, from degradation of erythrocytes, is converted to **bilirubin** in two steps:

1. *Heme oxygenase* linearizes heme to create **biliverdin**, a green compound (bruises).
2. *Biliverdin reductase* converts biliverdin to **bilirubin,** a red-orange compound that travels bound to serum albumin in the bloodstream.
3. Bilirubin is oxidized in the kidney to **urobilin**, the major pigment of urine.
4. Urobiligen not taken up by the kidney stays in the colon and is reduced by intestinal microbiota to **stercobilin.**

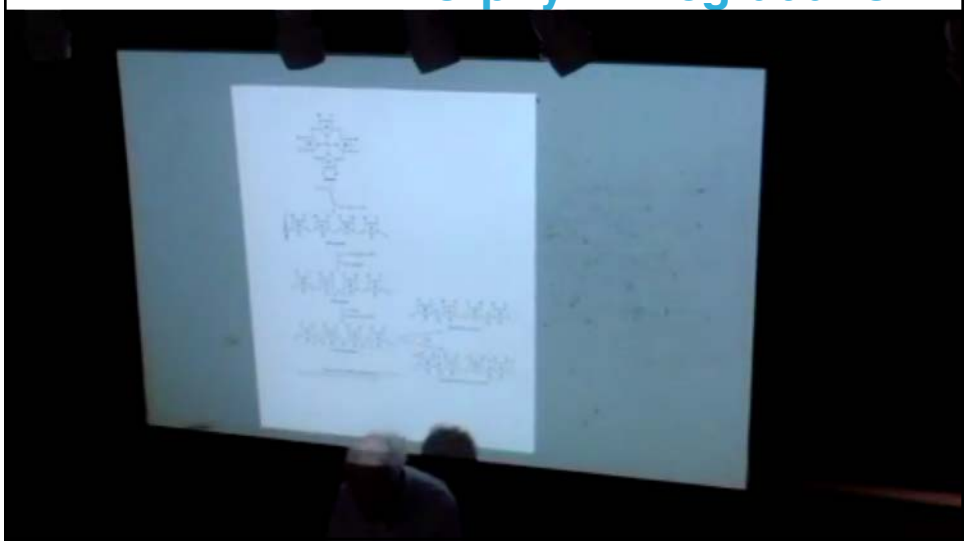
Jaundice is Caused by Bilirubin Accumulation

Jaundice (yellowish pigmentation of skin, whites of eyes, etc.) can result from:

- impaired liver (in liver cancer, hepatitis)
- blocked bile secretion (due to gallstones, pancreatic cancer)
- insufficient *glucouronyl bilirubin transferase* to process bilirubin (occurs in infants)
  - treated with UV to cause photochemical breakdown of bilirubin

Biosynthesis Amino Acids & Nucleotides

Porphyrin Degradation



ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

Summary

What we learned:

- Methods for fixation of molecular nitrogen to nitrates, nitrates, and **ammonia**
- Gln serves as the primary entry point of assimilation of ammonia via *Gln Synthetase* in animals; but made useful by *Glu Synthase* to make net Glu.
- The 20 common amino acids are synthesized from α-ketoglutarate, 3-phosphoglycerate, oxaloacetate, pyruvate, phosphoenolpyruvate, erythrose 4-phosphate, and ribose-5-phosphate (through phosphoribosyl pyrophosphate (PRPP).
- About half are non-essential in humans and are made much like they are degraded
- About half are essential and are made through extensive and inter-related paths
- Nucleotides can be synthesized either *de novo* from simple precursors, or reassembled from the salvage pathway using PRPP.
- *De novo* purines are synthesized on the ribose starting with PRPP., while pyrimidine rings are assembled prior to attachment to ribose using PRPP.
- Ribonucleotides(NDP) are converted to deoxyribonucleotides(dNTP) by *ribonucleotide reductase*, which is regulated in ways to ensure equal amounts of A:T & G:C.
- Regulation of amino-acid biosynthesis, as well as nucleotide synthesis, is by various types of feedback inhibition; in particular, the cumulative type at Gln synthetase
- Porphyrin biosynthesis and degradation is one example of 2° product of amino acids

Farewell to Biochemistry II (BI/CH 422 & BI/CH 622)

This course is Dedicated to the memory of Sir Hans Kornberg

January 14, 1928 ~ December 16, 2019

I want to thank Dr. Kornberg for all his help this semester and the inspiration!