BCHM 270: Module 6

NITROGEN METABOLISM

Content Outline

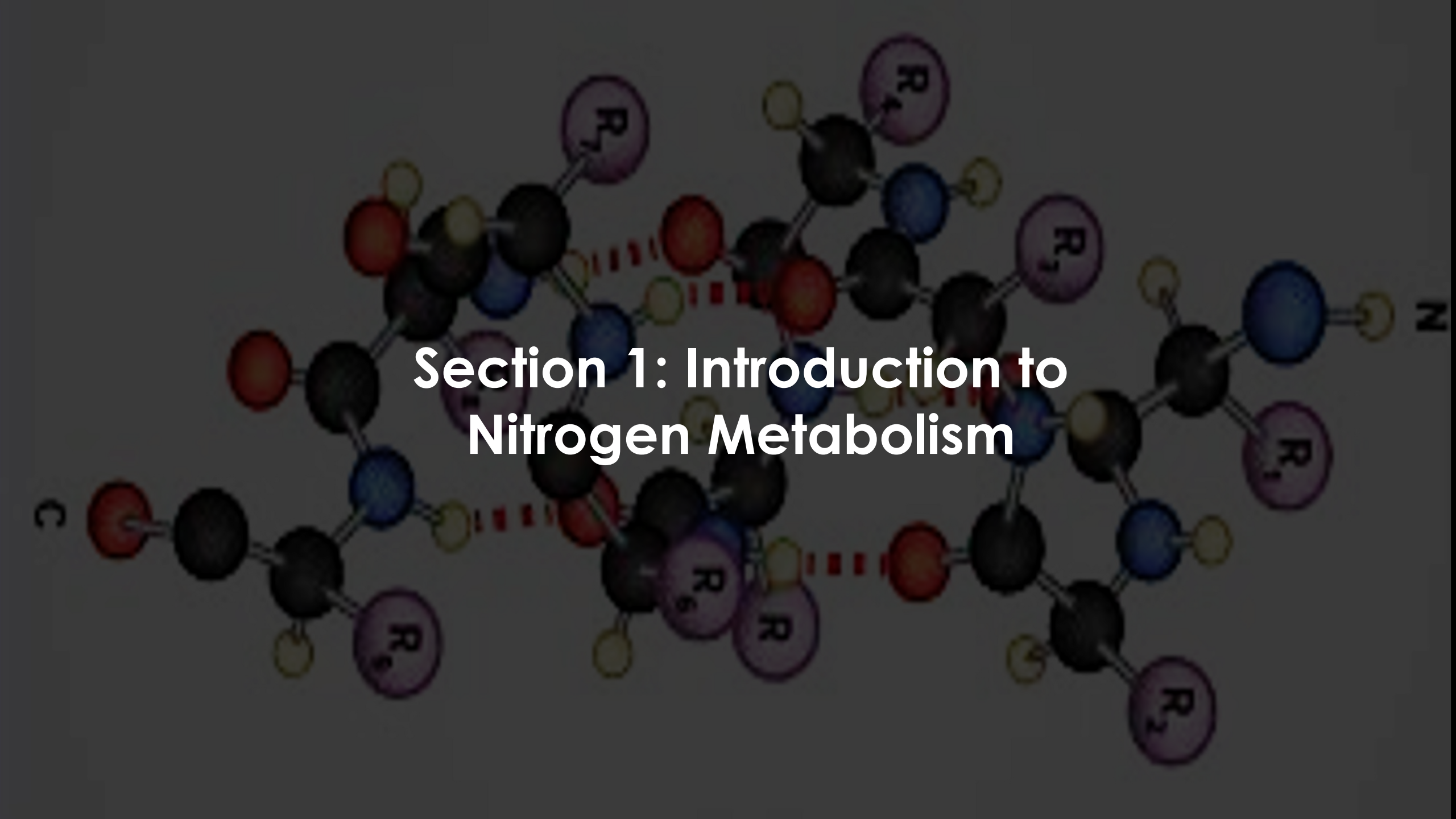
Section 1..... Introduction to Nitrogen Metabolism

Section 2..... Ammonia, the Liver, and the Urea Cycle

Section 3..... Amino Acid Metabolism- Degradation and Synthesis

Section 4..... Specialized Products of Amino Acids

Section 5..... Nucleotide Metabolism



Section 1: Introduction to Nitrogen Metabolism

Concept 1.1: Amino Acid Pool

- Amino acids/proteins do not have a storage in the body.
  - Rather the body has a pool of amino acids (~100 grams)

- Amino acid pool is utilized for many of the body's functions:
  - Protein Synthesis
  - Energy metabolism
  - For the creation of certain nitrogen-containing molecules

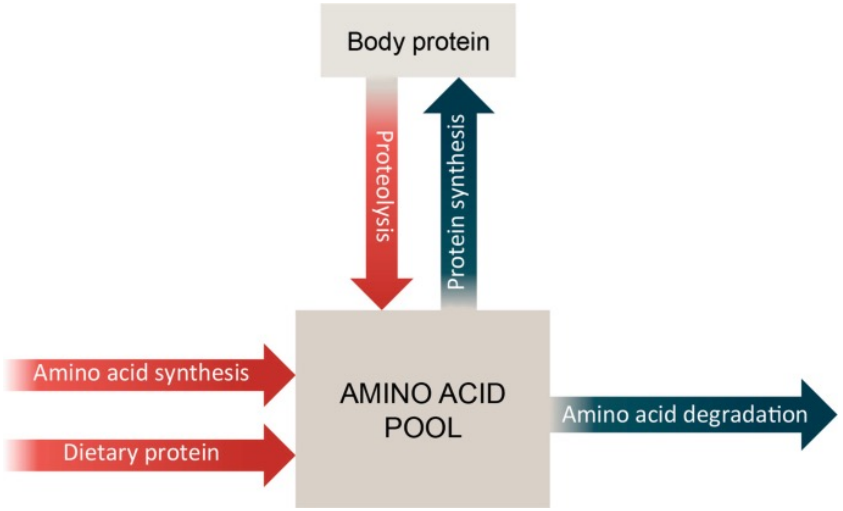


Figure 1: Amino Acid Pool and its Relevant Sources

Concept 1.2: Dietary Protein Digestion

- Proteins from the diet must be broken down to be absorbed into circulation

Components to Protein Digestion in Stomach:

Hydrochloric Acid (HCl) - Responsible for the stomach's acidic environment (~pH 2). Useful for killing bacteria and protein denaturation

Pepsin: The activated form of the zymogen pepsinogen. Activation first consists of the zymogen unfolding from HCl, and then other activated pepsin cleaving the pepsinogen.

Activated pepsin cleaves dietary proteins into small peptides.

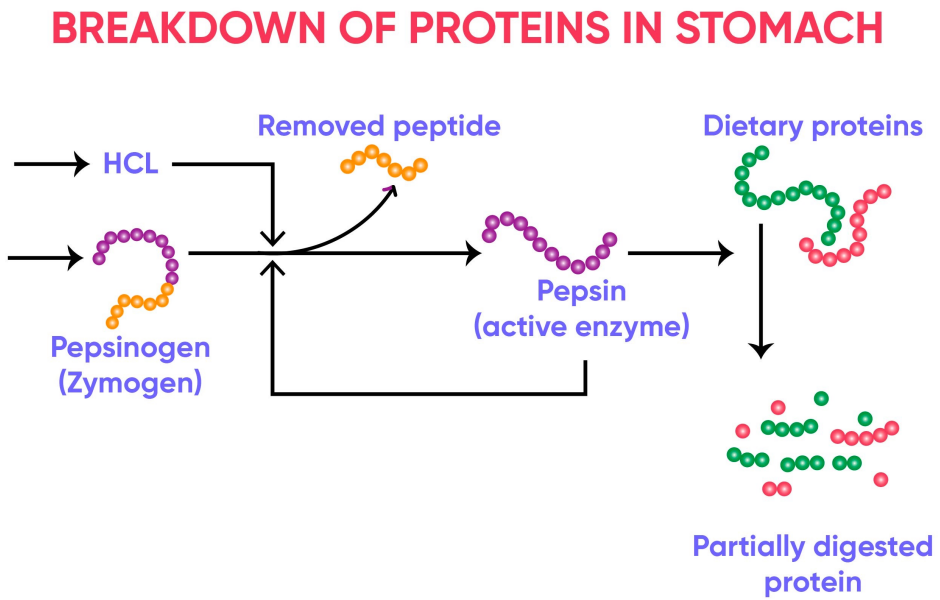


Figure 2: Activation and action of pepsin.

Concept 1.2: Dietary Protein Digestion

Protein Digestion in Small Intestine:

- Pancreatic Enzymes: Trypsin (zymogen: trypsinogen) and chymotrypsin (zymogen chymotrysinogen)
  - Trypsin activation occurs by enteropeptidase; trypsin then activates other enzymes.
  - Breaks proteins into oligopeptides (small amino acid chains)
- Intestinal Epithelium contains peptidases which, breaks peptides into individual amino acids for absorption

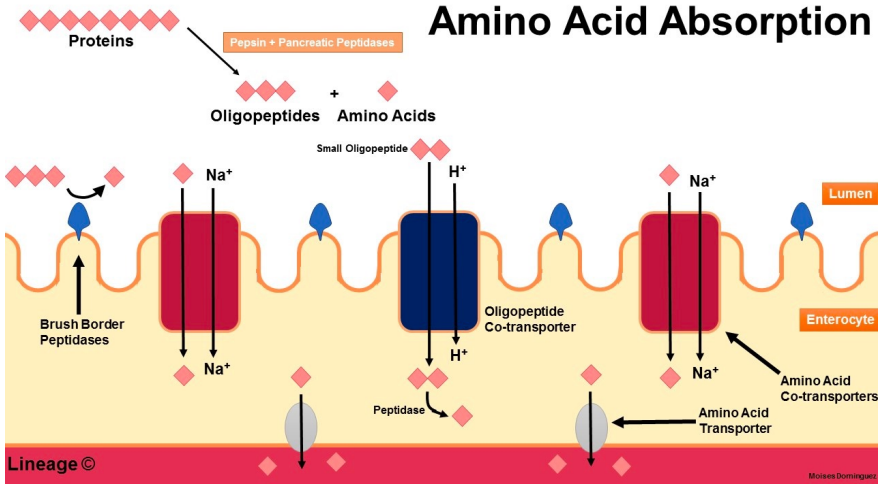


Figure 3: Peptidase in the Intestinal Epithelium and absorption of amino acids.

Amino Acid Absorption in Small Intestine:

- Amino Acid absorption requires active transport from small intestine lumen (requires ATP)
- 7 transporters help with transporting different amino acids

Spotlight on Disease: Acute Pancreatitis

- Caused by pancreatic duct blockage
  - This prevents pancreatic juices from reaching small intestine
- Previously mentioned zymogens (trypsinogen and chymotrypsinogen) become activated inside pancreatic duct
  - Causes enzymes to attack proteins in pancreatic tissue
- Overall, leads to less pancreatic enzymes entering the small intestine
- Proteins and fats are not adequately absorbed and excreted in stool.

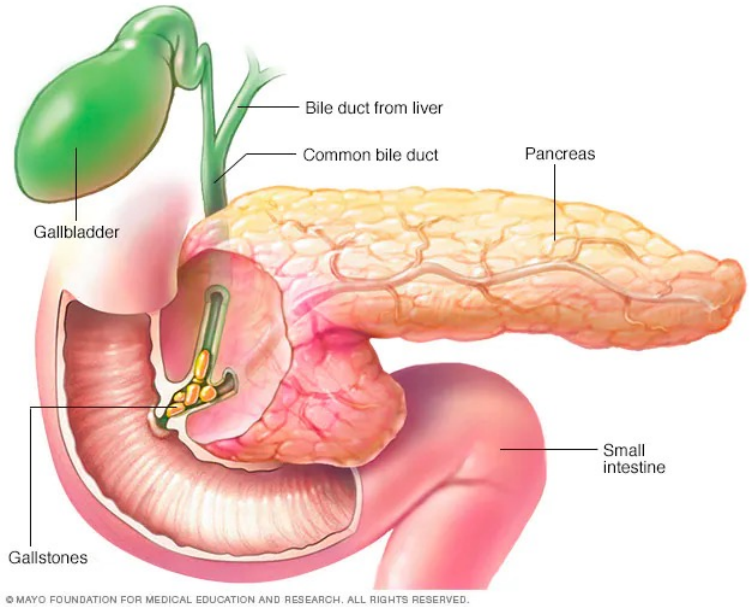


Figure 4: Gallstones causing a blockage in the pancreatic duct causing acute pancreatitis.

Spotlight on Disease: Cystinuria

- Inheritable disease (Autosomal Recessive)
- Results in dysfunctioning amino acid transporter
  - Reduced or complete inability to absorb certain amino acids (arginine, cysteine, ornithine, lysine)
- These amino acids are excreted in urine
  - Kidneys are unable to reabsorb the amino acids
- Can result in kidney, ureter or bladder stones

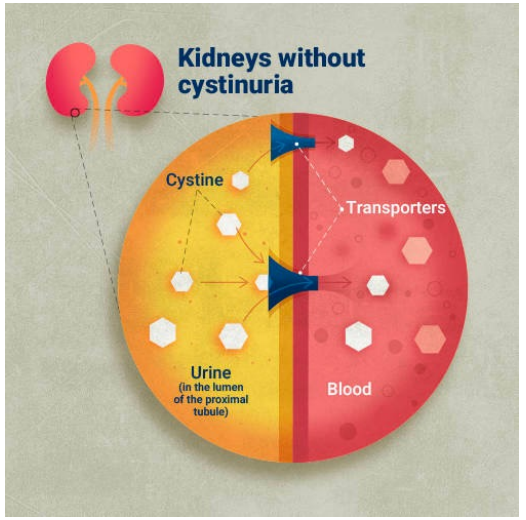


Figure 5: Kidneys without Cystinuria

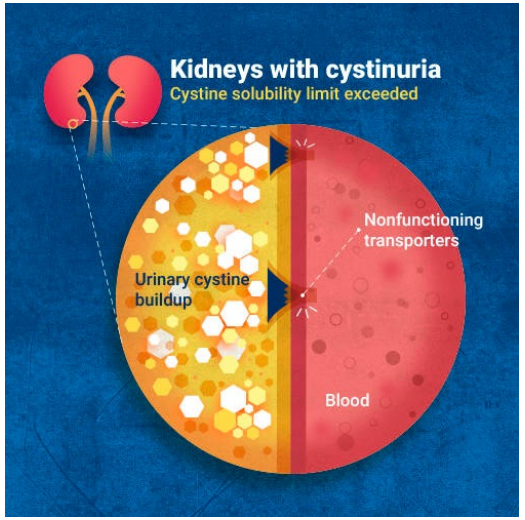
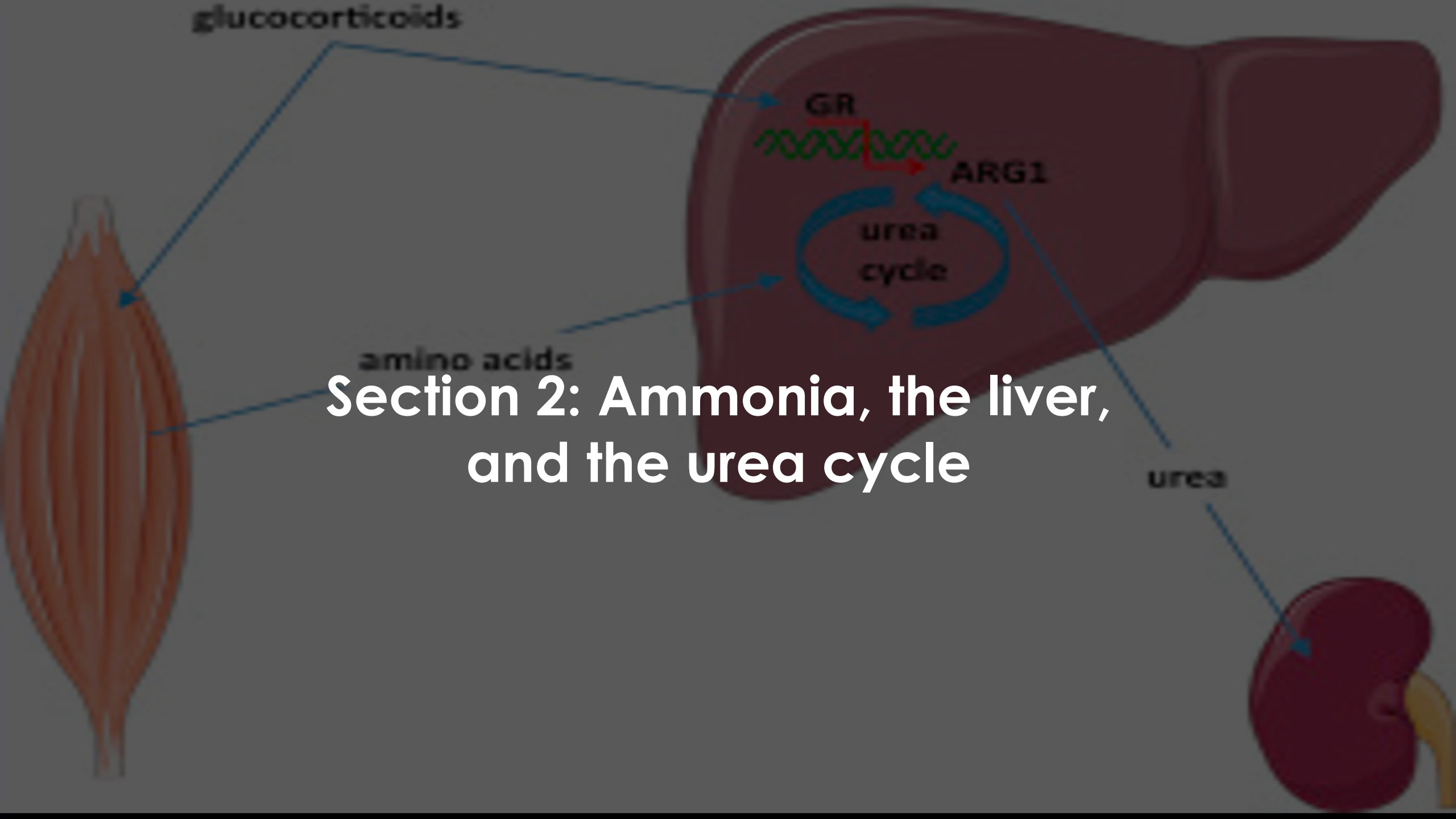


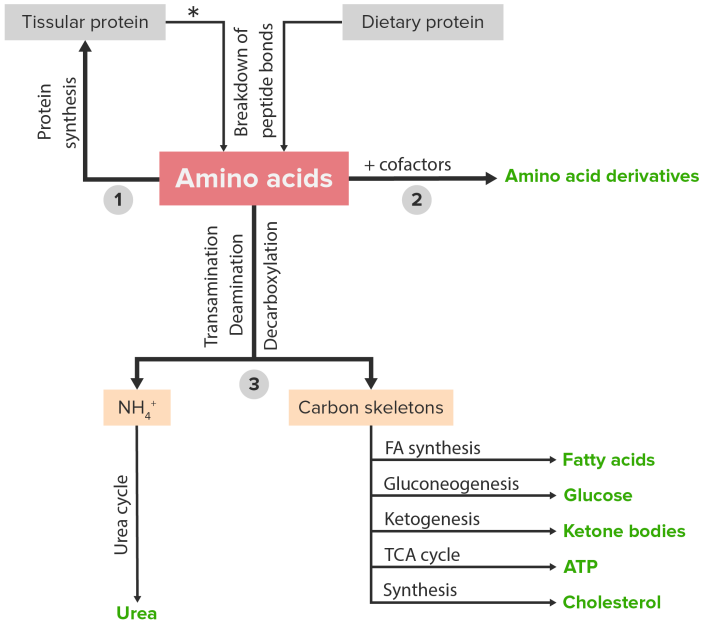
Figure 6: Kidneys with Cystinuria



Section 2: Ammonia, the liver, and the urea cycle

Concept 2.1: Overview of Amino Acid Catabolism

- Removal of a-amino is a committed step in the catabolism of all amino acids
  - Process starts as transamination, then the disposal/synthesis of amino acids, ammonia transport to liver and finally ammonia metabolism
    - Forms ammonia and carbon skeleton
      - Nitrogen later leaves as body as urea, ammonia and other products derived from the urea cycle



* Tissular protein breakdown is increased during periods of fasting/starvation

Figure 1: Amino acid metabolism.

Concept 2.2: Transamination

- Transamination: transfer of a-amino from a-amino acid (NH3+ supplier) to a-keto acid (NH3+ acceptor) to generate a-keto acid and a-amino acid.
- Catalyzed by an aminotransferase
- For example, alanine (a-amino acid)
  loses an NH3+ becoming pyruvate (a-keto acid) while a-ketoglutarate (a-keto acid)
  gains NH3+ becoming glutamate (a-amino acid)

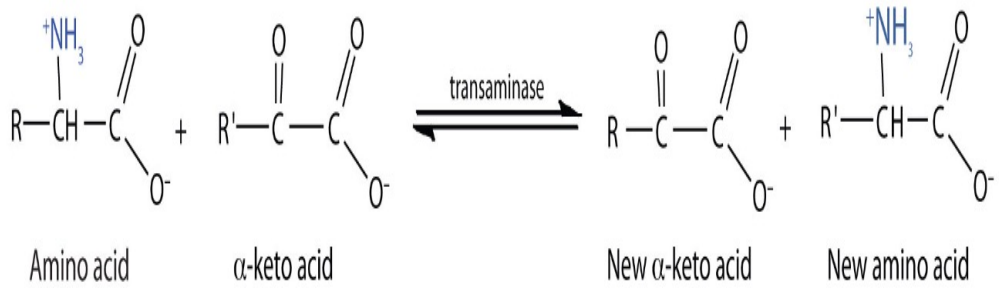


Figure 2: Transamination Reaction

Concept 2.3: Aminotransferases

Aminotransferases
- Enzymes that transfer 1 amino group from 1 carbon skeleton to another
- Use a-ketoglutarate to produce glutamine (most cases), also used in amino acid synthesis
- Each aminotransferase specific for amino group donors
- Require pyridoxal phosphate (from vit B6) as coenzyme

Types:

1. Alanine aminotransferase (ALT)
- Transfers amino group from alanine to a-ketoglutarate, produces pyruvate and glutamate

2. Aspartate aminotransferase (AST)
- ×Transfers amino group from glutamate to oxaloacetate, produces aspartate and a-ketoglutarate
- ×Aspartate is a nitrogen source in urea cycle

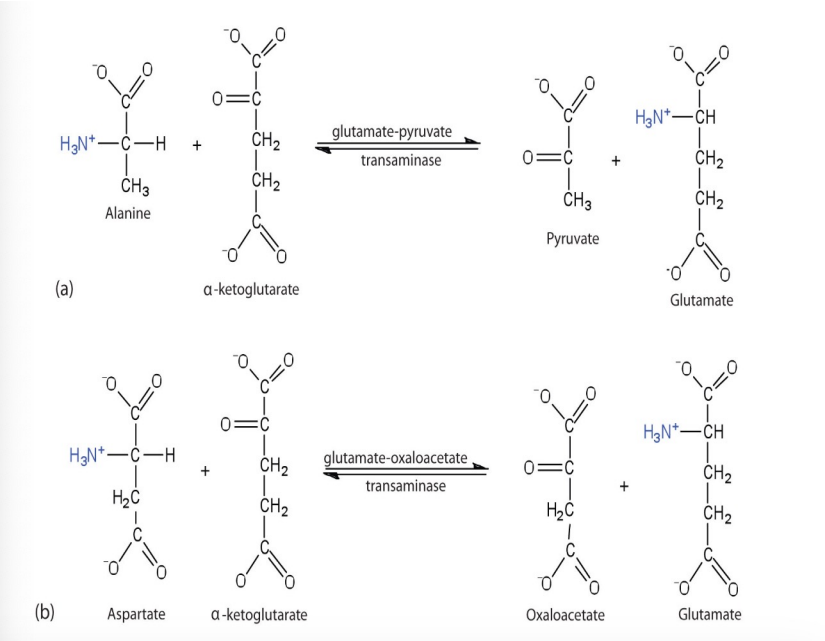


Figure 3: 2 Transamination reactions catalyzed by ALT and AST respectively.

Spotlight on Disease: Diagnostic Value of Plasma Aminotransferases

- Elevated aminotransferases in blood can indicate liver and non-hepatic diseases
- Liver diseases
  - Blood AST and ALT elevated
  - ALT elevations more specific to liver injury
  - AST more sensitive
  - Bilirubin (byproduct of heme degradation) also increases with liver damage but after initial damage
- Non-hepatic diseases (e.g., myocardial infarction, muscle disorders) aminotransferases may be elevated
  - Easily distinguished clinically from liver disease

Panel 1: Typical adult reference ranges for liver function tests

Albumin	35–55 g/L
Total bilirubin	3–20μmol/L
Conjugated bilirubin	0–14μmol/L
Alanine aminotransferase	0–45IU/L
Aspartate aminotransferase	0–50IU/L
Gamma-glutamyl transferase	0–70IU/L ♂ 0–40IU/L ♀
Alkaline phosphatase	90–300IU/L

Figure 4: Reference values for liver function test.

Concept 2.4: Oxidative Deamination and Reductive Amination

- Glutamate dehydrogenase in liver mitochondria removes ammonia from glutamate to generate a-ketoglutarate (reversible)
  - Enzyme can use NADH or NADPH as reducing agents
- Reaction direction depends on concentrations of reactants & products
- Disposal/synthesis of amino acids (liver) use aminotransferase and glutamate dehydrogenase

1. Disposal (oxidative deamination):
   - Glutamate dehydrogenase uses NAD+ as coenzyme
     - Inhibited allosterically by GTP
     - Activated allosterically by ADP (energy is low, catabolism)

2. Synthesis (reductive amination) occurs during starvation or growth
   - Glutamate dehydrogenase uses NADPH as coenzyme
     - Decreased if there is lots of NADH
     - Increased if there is lots of ADP

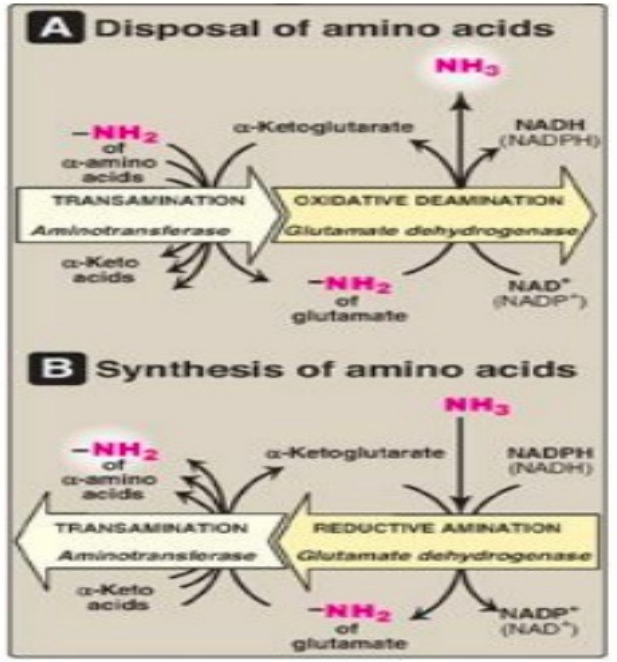


Figure 5: Disposal and Synthesis of Amino Acids.

Concept 2.5: The Urea Cycle

- In liver, ammonia converted to urea via urea cycle (irreversible synthesis)
- Urea diffuses from liver -> blood -> kidney, excreted into urine
- Urea formed from free NH3, N from aspartate, CO2 in form of bicarbonate (glutamate is intermediate precursor of both ammonia and aspartate N)

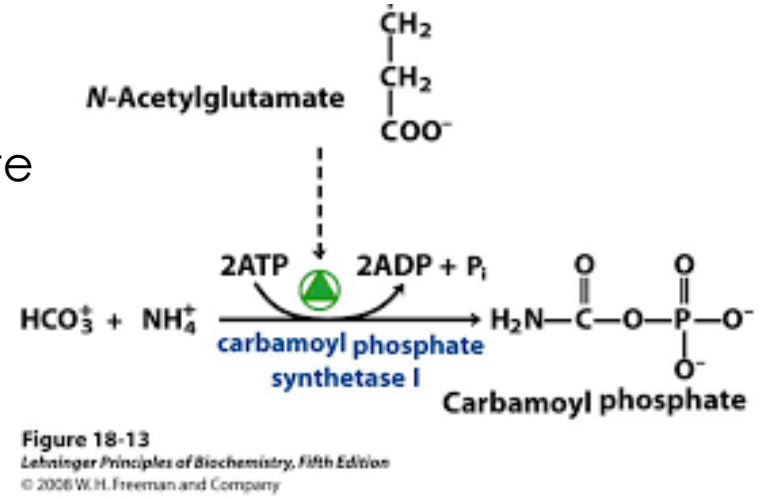


Figure 6: Carbamoyl phosphate formation.

- Step 1 (regulatory):
  - NH3 and CO2 converted to carbamoyl phosphate by carbamoyl phosphate synthase I (CPS I) using 2 ATP (endergonic)
    - CPS I: Activated allosterically by N-acetylglutamate (produced by high arginine when urea production is too low to handle ammonia)

Concept 2.6: Interconnectivity of the Urea and TCA Cycle

- Urea cycle shares several intermediates with TCA cycle, allows several amino acids to be glucogenic when glucose is low

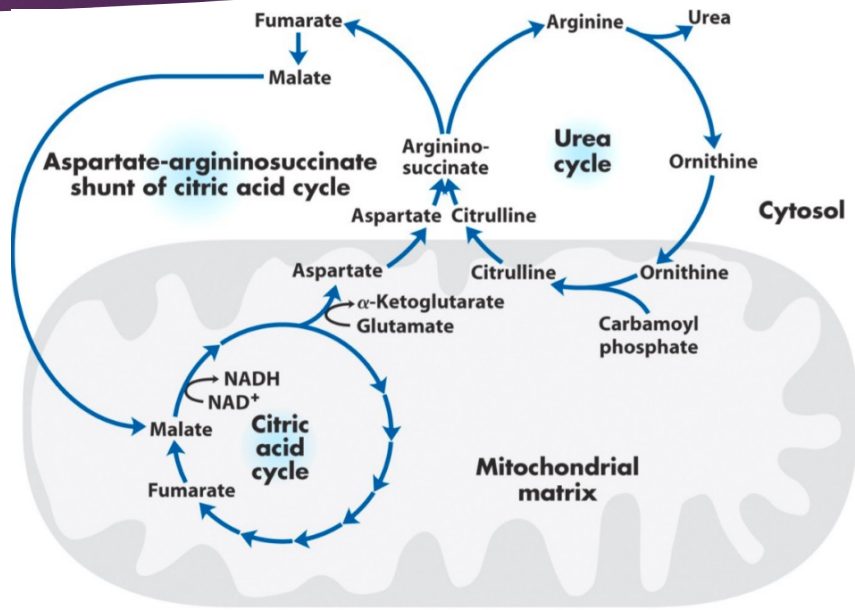


Figure 7: Interconnectivity of Urea Cycle and TCA cycle.

Spotlight on Disease: Hyperammonemia

- Ammonia highly toxic to humans
- Medical emergency, neurotoxic effect on CNS
- Symptoms: tremors, slurring of speech, blurry vision, high concentrations -> coma/death

- Normal situation
  - Capacity of hepatic urea cycle much greater than normal ammonia generation
  - Blood ammonia: 5-50 umol/L
- Diseased situation
  - Genetic defects of urea cycle (CPS I) or liver disease cause capacity of hepatic urea cycle to be much lower than normal ammonia generation
  - Blood ammonia: > 100 umol/L

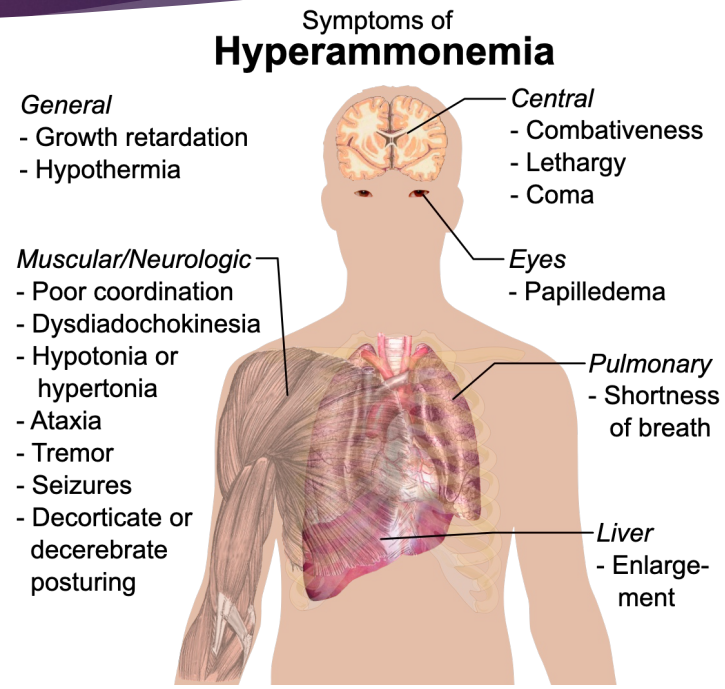


Figure 8: Symptoms of Hyperammoneia

Section 2 Quiz- 1 MC and 1 Short Answer

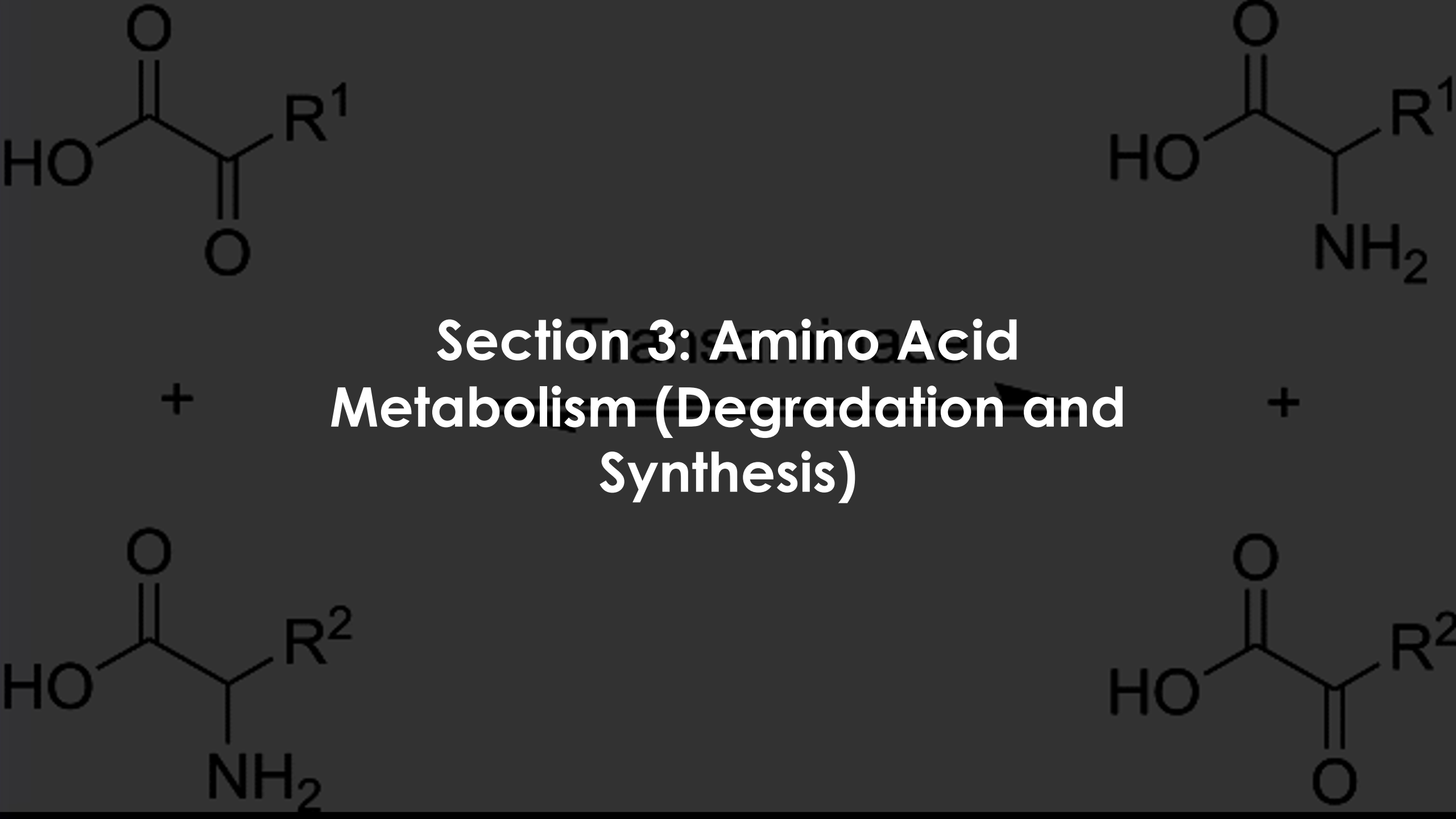
Q1. What are the products of transamination?

A. a-keto acid and sulfuric acid
B. a-keto acid and a-amino acid.
C. a-amino acid and ascorbic acid.
D. a-amino acid and hydrochloric acid

Answer: B

Q2. What is the biochemical basis of disease in hyperammonemia? Explain the affected enzyme, the reaction it is involved in and why the mutation causes the condition.

- Occurs due to liver complications (i.e., CPS I deficiency)
- Catalyzes conversion of NH_3 and CO_2 converted to carbamoyl phosphate
- Regulatory step in urea cycle
- Dysfunction leads to the inability to remove NH_3 leading to ammonia buildup



Section 3: Amino Acid Metabolism (Degradation and Synthesis)

Concept 3.1: Overview of Amino Acid Catabolism

Steps

- a-amino group removal
- Carbon skeleton breakdown
  - Occurs in liver

Carbon skeleton breakdown products

- Can enter glucose or lipid synthesis pathways or be used to produce energy
- Include:
  - Pyruvate
  - Fumarate
  - Oxaloacetate
  - Acetyl-CoA
  - Succinyl-CoA

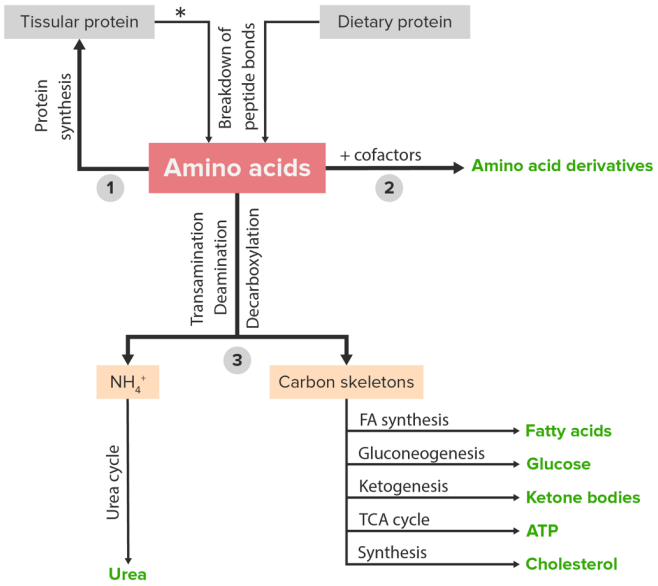


Figure 1. The overall amino acid catabolism pathway.

Concept 3.2: Glucogenic vs. Ketogenic Amino Acids

Ketogenic amino acids

- Produce acetoacetate or one of its precursors to act as substrates for ketone formation
- Involves lysine and leucine (the Ls)

Glucogenic amino acids

- Produce pyruvate or TCA cycle intermediates to act as substrates for gluconeogenesis
- Involves every other amino acid

Ketogenic and glucogenic amino acids

- Can undergo ketone formation AND gluconeogenesis
- *Mnemonic:* FITTT (Phenylalanine, Isoleucine, Tryptophan, Tyrosine, Threonine)

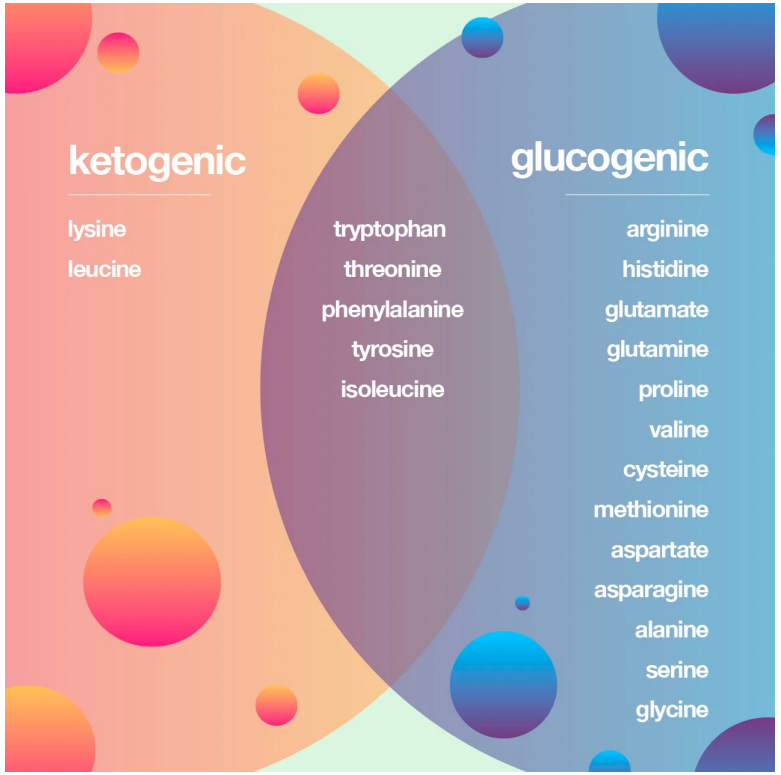


Figure 2. A list of the different amino acid categories.

Spotlight on Disease: Maple-Syrup Urine Disease

Biochemical basis

- Caused by deficiency in *branched chain a-keto acid dehydrogenase*
  - Enzyme involved in breakdown of branched chain amino acids
    - Valine, leucine, isoleucine
- Increases branched chain amino acid and a-keto acid levels in blood and urine

Symptoms

- Causes maple syrup odour in urine
- Can cause death within first few weeks of life

Treatment

- Low protein diet that avoids branched-chain amino acids

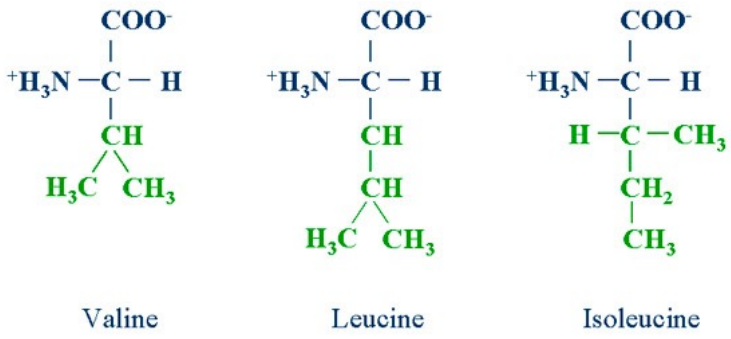


Figure 3. The branched amino acids.

Spotlight on Disease: PKU

Biochemical basis

- Caused by deficiency in *phenylalanine hydroxylase*
  - Converts phenylalanine to tyrosine
- Causes high phenylalanine levels and low tyrosine levels

Symptoms

- High phenylalanine, phenyllactate, phenylacetate, and phenylpyruvate levels in tissues
- Phenylpyruvate excretion in urine
- Low tyrosine levels cause hypopigmentation
- Neurological damage

Treatment

- Low phenylalanine diet

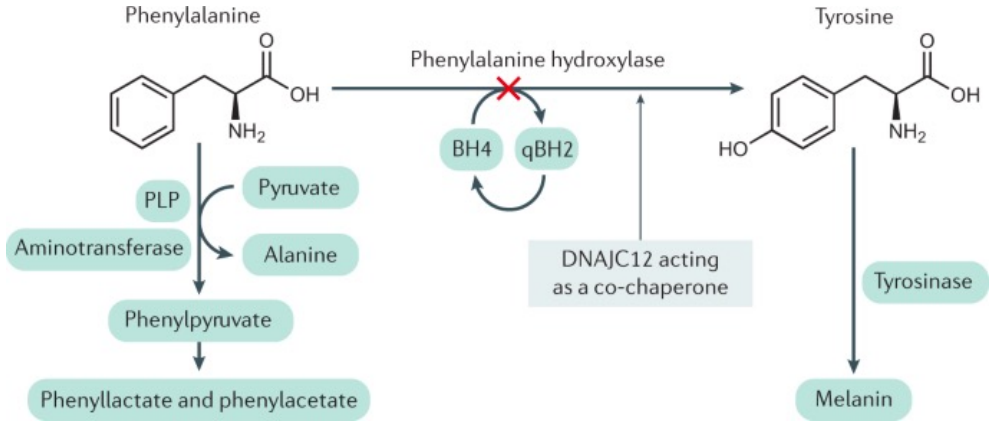


Figure 4. The mechanism behind PKU.

Spotlight on Disease: Albinism

Biochemical basis

- Caused by deficiency of tyrosinase
  - Low tyrosine levels inhibit melanin production

Symptoms

- White skin and hair
- Pink eyes
- Skin and eyes are very sensitive to sunlight
- Nystagmus (near-sightedness or far-sightedness)

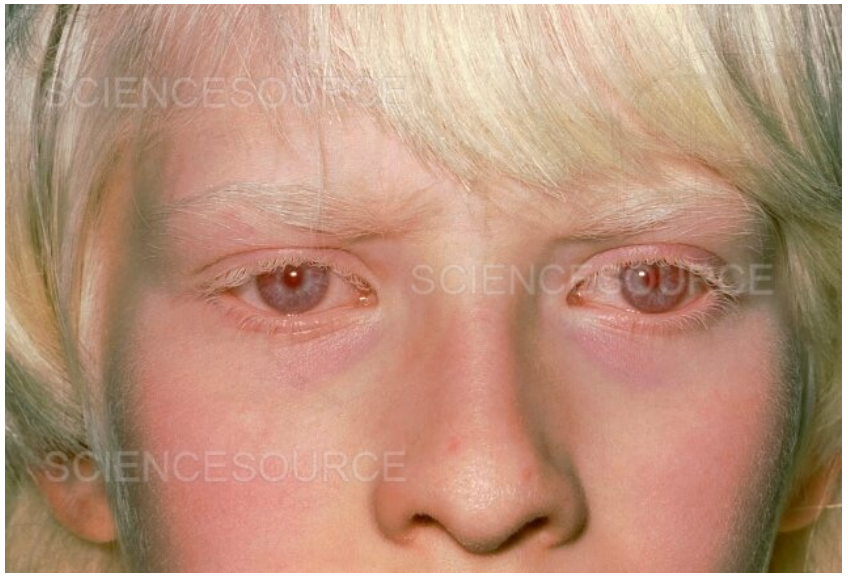


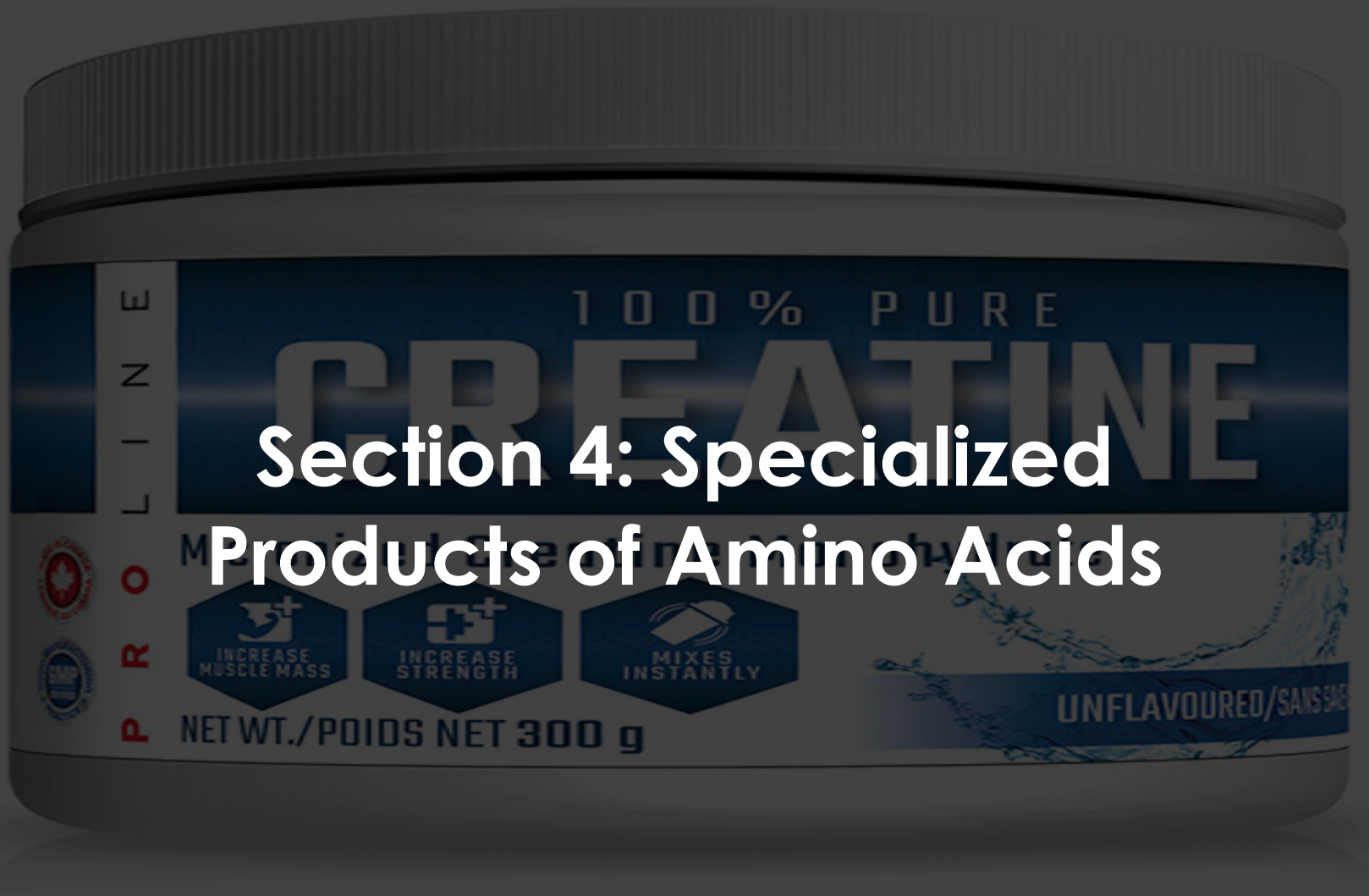
Figure 5. Symptoms of albinism.

Section 3 Quiz: 1 MC

Which of the following is caused by low tyrosine levels in PKU?

a) Neurological damage
b) Hypopigmentation
c) Slurred speech
d) Maple syrup odour in urine

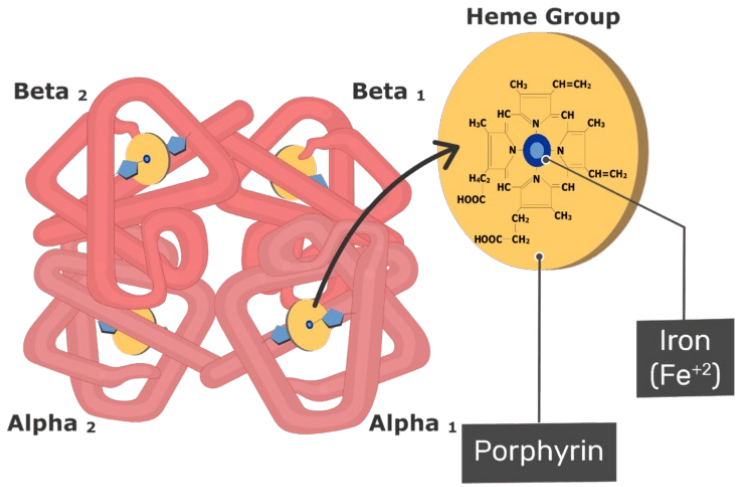
Answer: b



Section 4: Specialized Products of Amino Acids

Concept 4.1: Importance of Heme

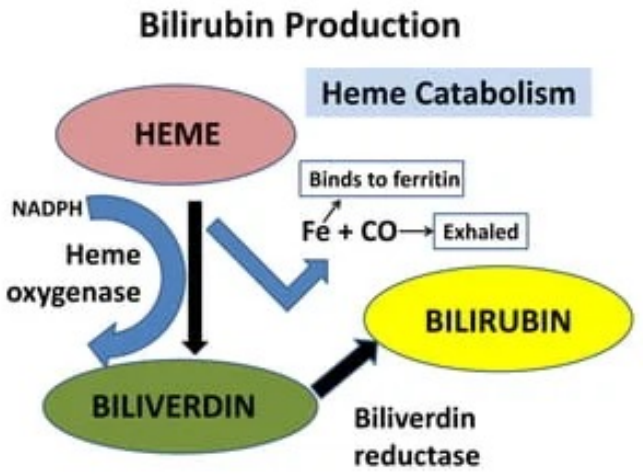
- Porphyrins bind metal ions, commonly Fe2+ or Fe3+.
- Heme is the most abundant porphyrin in humans.
  - Heme is a cofactor of hemoglobin, myoglobin, and cytochromes.
  - Heme is a type of porphyrin known as protoporphyrin IX.
  - Heme is bound to Fe2+.
- The importance of heme lies in its ability to bind and transport oxygen in hemoglobin and myoglobin, and in its role as an electron carrier in cytochromes.



Structure of Hemoglobin subunits

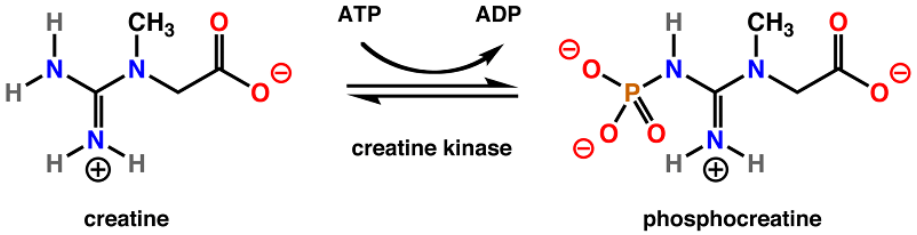
Spotlight on Disease: Jaundice

- Jaundice is yellowing of the skin, nails, and sclera due to bilirubin deposition and caused by:
  - Liver dysfunction
  - Bile duct obstruction
  - Increased red blood cell destruction.
- Hyperbilirubinemia occurs when bilirubin levels in the blood increase.
- Newborns may accumulate bilirubin due to low hepatic bilirubin glucuronyl transferase activity at birth.
  - Hepatic bilirubin glucuronyl transferase activity reaches adult levels around one month after birth.
- Light therapy is used to prevent toxic accumulation of bilirubin in newborns by breaking it down.



Concept 4.2: Creatine and creatine phosphate

- Creatine and creatine phosphate are specialized products of amino acids.
  - Serves as an energy reserve in muscle, alongside glycogen.
- The hydrolysis of creatine phosphate produces more energy than ATP hydrolysis, at -10.3 kcal/mol (vs. -7.3 kcal/mol).
- Creatine kinase can regenerate ADP to ATP during intense muscular contraction.
- Creatine and phosphocreatine are generated from compounds in the urea cycle and can form the cyclic creatinine.
- Creatinine levels in the blood can estimate the amount of muscle in an individual, as a loss in muscle will lead to a decrease in creatinine levels.



Concept 4.3: Creatine Phosphate Converted to Working Muscle

- Creatine kinase generates ATP from ADP when ATP reserves are depleted.
- Muscle cells can use glycolysis and aerobic metabolism to generate more energy after ATP reserves are depleted.
- In resting muscle, ATP is around 4 mM, while ADP is 0.013 mM.
- Creatine phosphate is much more abundant and higher in energy, with levels at 25 mM, and creatine at 13 mM.
- Creatine phosphate is readily catalyzed by creatine kinase to produce more ATP due to its abundance and high energy.
- Creatine phosphate is the major source of phosphoryl groups for ATP regeneration in muscle activities in the long term.

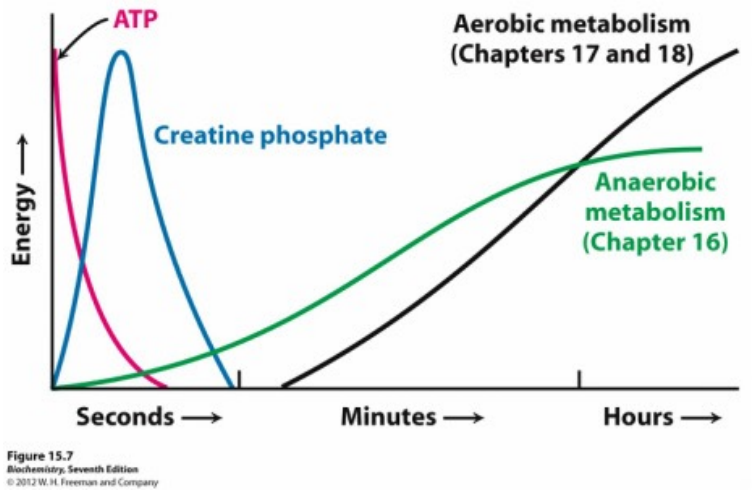


Figure 15.7
Biochemistry, Seventh Edition

Section 4 Quiz: 1 MC

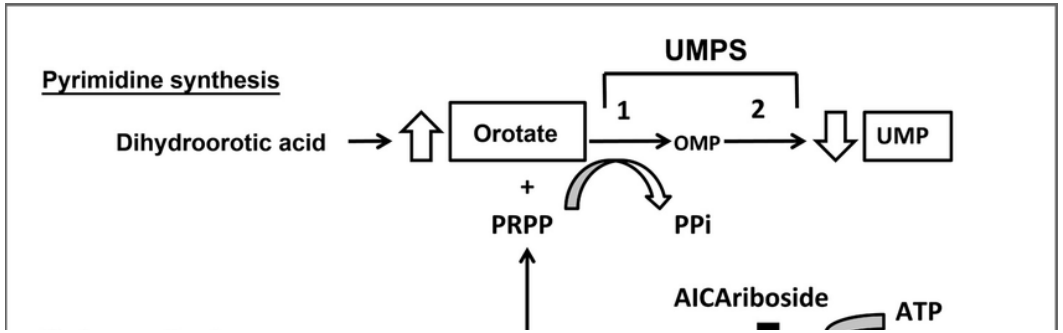
Which of the following is a cause of jaundice?
A) Increased levels of white blood cells
B) Deposition of creatine
C) Hyperbilirubinemia due to liver dysfunction
D) Decreased levels of bilirubin in the blood

Answer: C) Hyperbilirubinemia due to liver dysfunction

Section 5: Nucleotide Metabolism

Concept 5.2: Two steps of purine synthesis

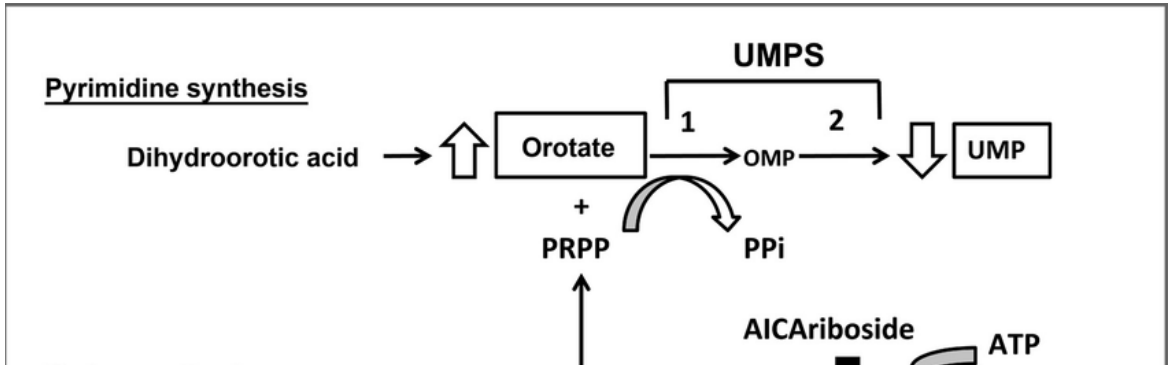
- Step #1: Synthesis of 5-phosphoribsoyl-1-pyrophosphate (PRPP)
  - PRPP synthetase converts ribose-phosphate to PRPP
  - Inhibited by: IMP, AMP, GMP (all purine nucleotides)
  - Activated by: phosphate
- Step #2: Synthesis of 5-phosphoribosyl-1-amine
  - Major regulated step in purine synthesis
  - Glutamine:PRPP amidotransferase converts PRPP to 5-phosphoribosyl-1-amine
  - Activated by: glutamine and PRPP
  - Inhibited by: AMP, GMP, IMP



Pyrimidine synthesis

Concept 5.3: Pyrimidine Synthesis

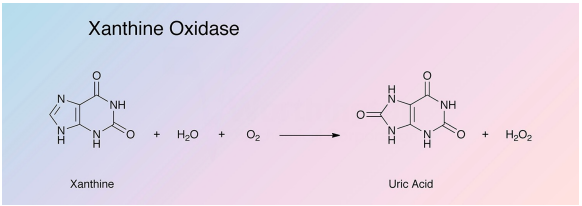
- Synthesis of carbamoyl phosphate
  - Synthesis is a three-step process
  - Carbamoyl phosphate synthetase II (CPS II) Involved in each step
  - Activated by: ATP, PRPP
  - Inhibited by: UTP



Pyrimidine synthesis

Concept 5.4: Degradation of Purines to Uric Acid

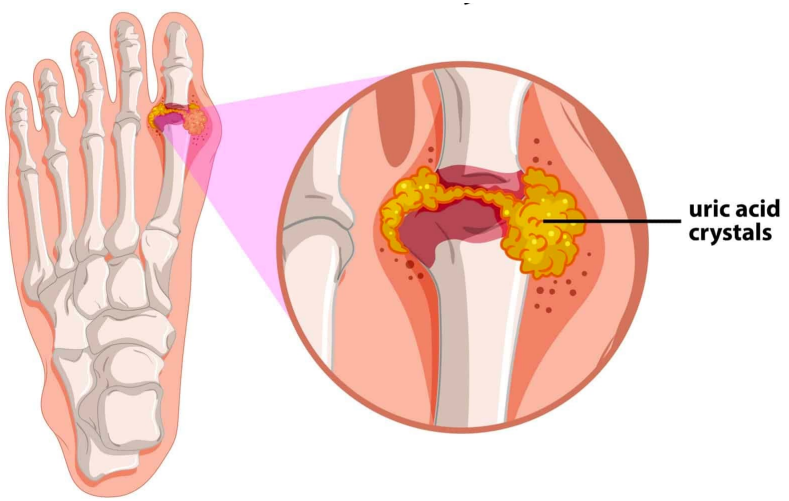
- Adenosine monophosphate and guanine are converted to xanthine
- Xanthine oxidase
  - Converts xanthine to uric acid (less soluble)
  - Uric acid converted to urate
  - Kidney excretes urate in urine



Reaction catalyzed by xanthine oxidase

Spotlight on Disease: Gout

- Caused by uric acid build-up
- Build-up due to hyperuricemia (caused by inborn metabolism error or elevated dietary purine intake)
- Build-up leads to uric acid crystal deposits in joints (arthritis) and kidneys
- Treatment
  - Allopurinol --> inhibits xanthine oxidase --> prevents formation of uric acid



Arthritis, a key symptom of gout