"Amino Acids: Nitrogen Disposal"

I. Overview

- Unlike fats and carbohydrates, amino acids are not stored by the body.
    - No protein exists whose sole function is to maintain a supply of amino acids for future use.
- Therefore, amino acids must be:
    - Obtained from the diet
    - Synthesized de novo
    - Produced from the degradation of body protein
- Any amino acids in excess of the biosynthetic needs of the cell are rapidly degraded.
- The first phase of catabolism involves:
    - Removal of the α-amino groups
        - Usually by:
            - Transamination
            - Subsequent oxidative deamination
    - Formation of:
        - Ammonia
        - Corresponding α-keto acids (the carbon skeletons of amino acids)

- Fate of free ammonia:
    - A portion is excreted in the urine
    - Most is used in the synthesis of urea
        - Urea: Quantitatively the most important route for disposing of nitrogen from the body

- The second phase of amino acid catabolism:
    - Involves the conversion of the carbon skeletons of α-keto acids to common intermediates of energy-producing metabolic pathways

- These compounds can be metabolized to:
    - Carbon dioxide (CO_2) and water (H_2O)
    - Glucose
    - Fatty acids
    - Ketone bodies

II. Overall Nitrogen Metabolism

- Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules.

- Nitrogen enters the body in a variety of compounds present in food:
    - The most important being amino acids contained in dietary protein
- Nitrogen leaves the body as:
    - Urea
    - Ammonia
    - Other products derived from amino acid metabolism
        - e.g., Creatinine
- The role of body proteins in these transformations involves two important concepts:
    - Amino acid pool
    - Protein turnover

A. Amino Acid Pool

- Free amino acids are present throughout the body:
    - In cells
    - In blood
    - In extracellular fluids

- For the purpose of discussion:
    - Envision all of these amino acids as belonging to a single entity called the amino acid pool

Sources that Supply the Amino Acid Pool:

1. Degradation of endogenous (body) proteins
    - Most of which are reutilized

2. Amino acids derived from exogenous (dietary) protein

3. Nonessential amino acids
    - Synthesized from simple intermediates of metabolism

Routes of Depletion of the Amino Acid Pool:

1. Synthesis of body protein

2. Consumption of amino acids as precursors
    - For essential nitrogen-containing small molecules

3. Conversion of amino acids to:

    - Glucose
    - Glycogen
    - Fatty acids
    - Ketone bodies
    - Or oxidation to $CO_2 + H_2O$

- Size of the amino acid pool:

    - Small: 90 to 100 g of amino acids
    - Compared with total body protein: 12 kg in a 70-kg man

- Conceptual importance:

    - The amino acid pool is conceptually at the center of whole-body nitrogen metabolism

Nitrogen Balance in Healthy, Well-Fed Individuals:

- Input to the amino acid pool = Output

- The amount of amino acids in the pool remains constant

- The amino acid pool is said to be in a steady state

- The individual is said to be in nitrogen balance

B. Protein Turnover

- Most proteins in the body:
    - Are constantly being synthesized and then degraded (turned over)
- Purpose of turnover:
    - Permits removal of abnormal or unneeded proteins

Regulation of Protein Concentration:

- For many proteins:
    - Regulation of synthesis determines concentration in the cell
    - Protein degradation assumes a minor role

- For other proteins:
    - Rate of synthesis is constitutive
        - i.e., essentially constant
    - Cellular levels of the protein are controlled by selective degradation

1. Rate of Protein Turnover

- In healthy adults:
    - The total amount of protein in the body remains constant
        - Because the rate of protein synthesis is just sufficient to replace degraded protein

- This process is called protein turnover
    - Leads to the hydrolysis and resynthesis of 300 to 400 g of body protein each day

Variation in Turnover Rates:

- Short-lived proteins:
    - e.g., Many regulatory proteins and misfolded proteins
    - Are rapidly degraded
    - Have half-lives measured in minutes or hours
- Long-lived proteins:
    - Have half-lives of days to weeks
    - Constitute the majority of proteins in the cell
- Structural proteins (e.g., collagen):
    - Are metabolically stable
    - Have half-lives measured in months or years

2. Protein Degradation

- Two major enzyme systems degrade proteins:

Enzyme System	Location	Energy Dependency	Characteristics
ATP-dependent ubiquitin-proteasome system	Cytosol	Requires ATP	Selectively degrades damaged or short-lived proteins
ATP-independent lysosomal enzyme system	Lysosomes	Does not require ATP	Nonselectively degrades: - Intracellular proteins (autophagy) - Extracellular proteins (heterophagy, e.g., plasma proteins via endocytosis) - Uses acid hydrolases

a. Ubiquitin-Proteasome System

- Target proteins are first modified by covalent attachment of ubiquitin (Ub)
    - Ub: A small, globular, nonenzymic protein that is highly conserved across eukaryotes
- Ubiquitination process:
    - Involves isopeptide linkage:
        - Between the α-carboxyl group of C-terminal glycine of Ub
        - And the ε-amino group of a lysine in the protein substrate
    - Occurs via a three-step, enzyme-catalyzed, ATP-dependent process:

Enzyme	Role
E1 (activating enzyme)	Activates Ub
E2 (conjugating enzyme)	Receives Ub from E1
E3 (ligase)	Identifies the target protein and interacts with E2-Ub - There are many more E3s than E1 or E2

- Polyubiquitination:
    - Four or more Ub molecules are added to the target protein
    - Forms a polyubiquitin chain
- Recognition and degradation:
    - Polyubiquitinated proteins are recognized by a proteasome
        - A large, barrel-shaped, macromolecular proteolytic complex
    - Proteasome actions:
        - Unfolds the protein
        - Deubiquitinates it
        - Cuts it into fragments
    - Fragments are further degraded by cytosolic proteases into amino acids
        - These enter the amino acid pool
    - Ub is recycled
- ATP hydrolysis is required for this process
    - Unlike simple hydrolysis by proteolytic enzymes, Ub-proteasome degradation is ATP-dependent

b. Degradation Signals

- Protein degradation is not random
    - It is influenced by structural aspects of the protein that act as degradation signals
    - These signals are recognized and bound by an E3 ligase

Factors Determining Half-Life:

Factor	Effect
N-terminal residue (N-end rule)	Influences half-life; ranges from minutes to hours
Destabilizing N-terminal amino acids	e.g., Arginine, acetylated alanine
Stabilizing N-terminal amino acid	e.g., Serine

- PEST sequences:
    - Sequences rich in:
        - Proline (P)
        - Glutamate (E)
        - Serine (S)
        - Threonine (T)
    - Rapidly ubiquitinated and degraded
    - Result in short half-lives

III. Dietary Protein Digestion

- Most of the nitrogen in the diet is consumed in the form of protein
    - Typically 70 to 100 g/day in the American diet
- Proteins are generally too large to be absorbed by the intestine
    - Exception: Newborns can take up maternal antibodies in breast milk

- Therefore, proteins must be hydrolyzed to yield:
    - Di-peptides
    - Tri-peptides
    - Individual amino acids
        - These can be absorbed
- Proteolytic enzymes responsible for degrading proteins are produced by three different organs:
    - Stomach
    - Pancreas
    - Small intestine

A. Digestion by Gastric Secretion

- Protein digestion begins in the stomach
- The stomach secretes gastric juice
    - A unique solution containing:
        - Hydrochloric acid (HCl)
        - Proenzyme pepsinogen

1. Hydrochloric Acid (HCl)

- Stomach HCl is too dilute (pH 2 to 3) to hydrolyze proteins
- Secreted by: Parietal cells of the stomach
- Functions of HCl:
    - Kills some bacteria
    - Denatures proteins
        - Makes proteins more susceptible to subsequent hydrolysis by proteases

2. Pepsin

- Pepsin is an acid-stable endopeptidase
- Secreted by: Chief cells of the stomach
- Secreted in inactive form: Pepsinogen (a zymogen or proenzyme)

- Note:
    - Zymogens contain extra amino acids in their sequences
    - These extra amino acids prevent catalytic activity
    - Removal of these amino acids permits proper folding needed for an active enzyme

- In the presence of HCl:
    - Pepsinogen undergoes a conformational change
    - Allows it to cleave itself (autocatalysis) into the active form: Pepsin

- Pepsin function:
    - Releases polypeptides
    - Releases a few free amino acids from dietary proteins

B. Digestion by Pancreatic Enzymes

- Upon entering the small intestine, the polypeptides produced in the stomach by pepsin are:
    - Further cleaved into oligopeptides and amino acids
    - By a group of pancreatic proteases

- These pancreatic proteases include:
    - Endopeptidases: cleave within peptide chains
    - Exopeptidases: cleave at an end of peptide chains
- Note:
    - Bicarbonate (HCO_3^-) is secreted by the pancreas
        - In response to the intestinal hormone secretin
        - Raises the intestinal pH

1. Specificity

- Each pancreatic protease has different specificity for the amino acid R-groups adjacent to the susceptible peptide bond
- Example:
    - Trypsin cleaves only when the carbonyl group of the peptide bond is contributed by:
        - Arginine
        - Lysine

- These enzymes, like pepsin, are:
    - Synthesized and secreted as inactive zymogens

2. Zymogen Release

- Release and activation of the pancreatic zymogens is mediated by:
    - Cholecystokinin
        - A polypeptide hormone of the small intestine

3. Zymogen Activation

- Enteropeptidase (also called enterokinase):
    - A serine protease
    - Synthesized by and present on the luminal (apical) surface of intestinal mucosal cells (enterocytes) of the brush border

- Function of enteropeptidase:
    - Converts the pancreatic zymogen trypsinogen to trypsin
        - By removal of a hexapeptide from the N-terminus of trypsinogen

- Trypsin then:
    - Converts other trypsinogen molecules to trypsin
    - By cleaving a limited number of specific peptide bonds in the zymogen

- Thus, enteropeptidase unleashes a cascade of proteolytic activity
    - Because trypsin is the common activator of all the pancreatic zymogens

4. Digestion Abnormalities

- In individuals with deficiency in pancreatic secretion (e.g., due to):
    - Chronic pancreatitis
    - Cystic fibrosis
    - Surgical removal of the pancreas

- Result:
    - Incomplete digestion and absorption of fat and protein

- Leads to:
    - Abnormal appearance of lipids in feces
        - A condition called steatorrhea (see p. 196)
    - Presence of undigested protein in feces

C. Digestion of Oligopeptides by Small Intestine Enzymes

- The luminal surface of enterocytes contains:
    - Aminopeptidase
        - An exopeptidase
        - Repeatedly cleaves the N-terminal residue from oligopeptides
        - Produces:
            - Smaller peptides
            - Free amino acids

D. Amino Acid and Small Peptide Intestinal Absorption

1. Free Amino Acids

- Most free amino acids are taken into enterocytes via:
    - Sodium-dependent secondary active transport
    - Transported by:
        - Solute carrier (SLC) proteins of the apical membrane
- There are:
    - At least seven different transport systems
    - With overlapping amino acid specificities

2. Di- and Tripeptides

- Taken up by:
    - Proton-linked peptide transporter (PepT1)
- After uptake:
    - Peptides are hydrolyzed to free amino acids

3. Exit into Portal Circulation

- Regardless of their source, free amino acids are released from enterocytes into the portal system via:
    - Sodium-independent transporters of the basolateral membrane
- Therefore:
    - Only free amino acids are found in the portal vein after a protein-containing meal

4. Fate of Absorbed Amino Acids

- These amino acids are:
    - Either metabolized by the liver
    - Or released into the general circulation
- Note:
    - Branched-chain amino acids (BCAAs):
        - Are not metabolized by the liver
        - Instead, they are sent from the liver to muscle via the blood

E. Absorption Abnormalities

- The small intestine and the proximal tubules of the kidneys share:
    - Common transport systems for amino acid uptake
- Consequence:
    - A defect in any one of these systems results in:
        - Inability to absorb particular amino acids into the intestine
        - And into the kidney tubules

Example: Cystinuria

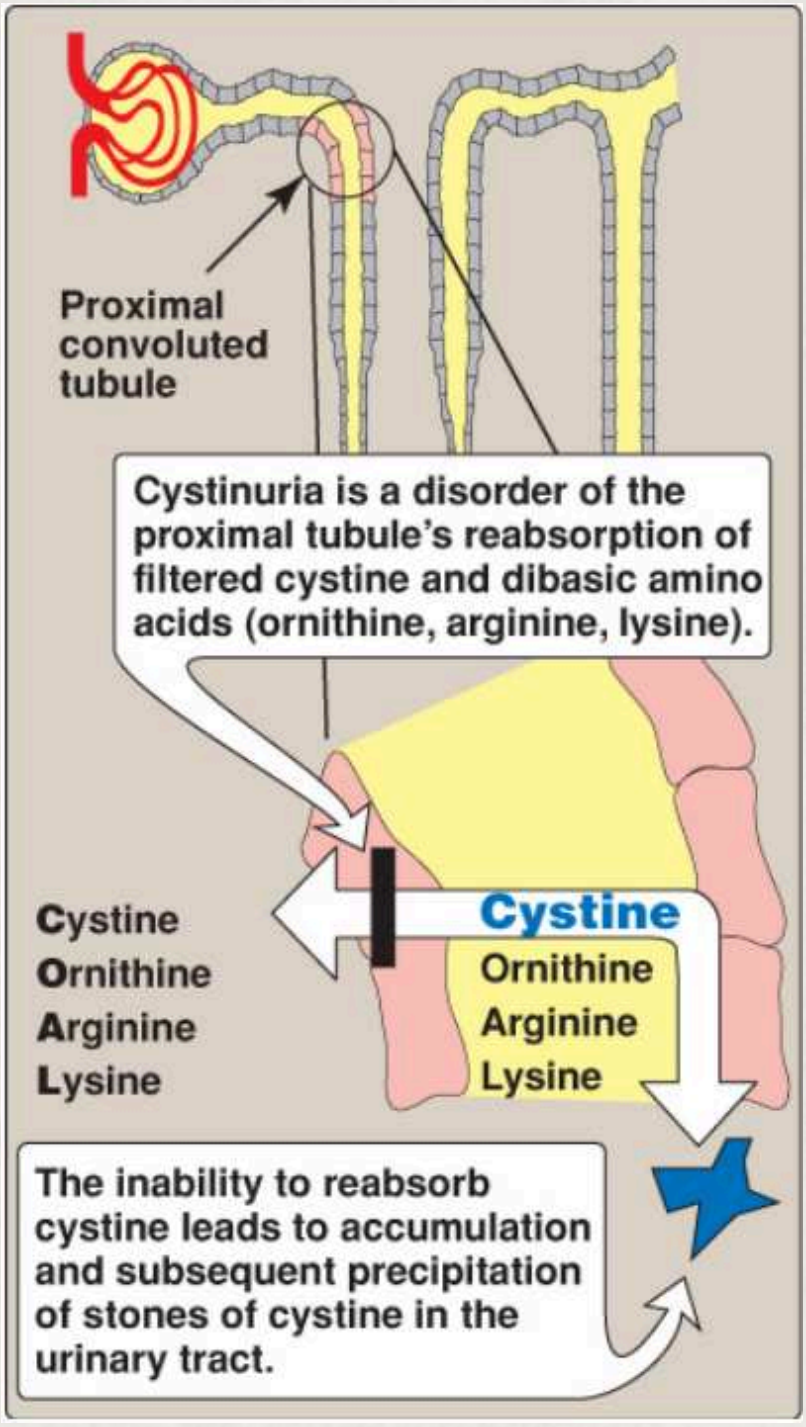
- One specific transport system is responsible for uptake of:
    - Cystine
    - Ornithine
    - Arginine
    - Lysine
        - Represented as: COAL

- In the inherited disorder cystinuria:
    - This carrier system is defective
    - All four amino acids appear in the urine
- Epidemiology:
    - Occurs with a frequency of 1 in 7,000 individuals
    - One of the most common inherited diseases
    - The most common genetic error of amino acid transport
- Clinical expression:
    - Cystine precipitates to form kidney stones (calculi)
    - Stones may block the urinary tract
- Treatment:
    - Oral hydration is an important part of management

Note: Hartnup Disorder

- Defects in the uptake of tryptophan:
    - Due to mutation in a neutral amino acid transporter
- Can result in:
    - Hartnup disorder
    - Pellagra-like dermatologic and neurologic symptoms

Genetic Defect Seen in Cystinuria



(Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and widespread tissue damage.)

IV. Nitrogen Removal From Amino Acids

- The α-amino group keeps amino acids safely locked away from oxidative breakdown
- Removal of the α-amino group is:
    - Essential for producing energy from any amino acid
    - An obligatory step in the catabolism of all amino acids
- Once removed, the nitrogen can be:
    - Incorporated into other compounds
    - Or excreted as urea
- The carbon skeletons of amino acids are then metabolized
- This section describes:
    - Transamination
    - Oxidative deamination
        - These reactions ultimately provide ammonia and aspartate
            - The two sources of urea nitrogen

A. Transamination: Funneling Amino Groups to Form Glutamate

- The first step in the catabolism of most amino acids is:
    - Transfer of their α-amino group to α-ketoglutarate
- This reaction produces:
    - An α-keto acid (derived from the original amino acid)
    - Glutamate (derived from α-ketoglutarate)
- α-Ketoglutarate:
    - A citric acid cycle ketoacid intermediate
    - Plays a pivotal role in amino acid metabolism
    - Accepts amino groups from most amino acids
    - Becomes its structurally related amino acid: glutamate

- Glutamate produced by transamination can be:
    - Oxidatively deaminated (see section B below)
    - Or used as an amino group donor in the synthesis of nonessential amino acids
- This transfer of amino groups from one carbon skeleton to another is catalyzed by:
    - A family of readily reversible enzymes called:
        - Aminotransferases (also called transaminases)
- Location of aminotransferases:
    - Found in the cytosol and mitochondria of cells throughout the body
- All amino acids, except the following participate in transamination at some point in their catabolism:
    - Lysine
    - Threonine
- Note:
    - Lysine and Threonine lose their α-amino groups by deamination

1. Substrate Specificity of Aminotransferases

- Each aminotransferase is specific for:
    - One or, at most, a few amino group donors
- Naming:
    - Aminotransferases are named after the specific amino group donor
    - Because the acceptor of the amino group is almost always α-ketoglutarate
- Two important aminotransferase reactions:

a. Alanine aminotransferase (ALT)
b. Aspartate aminotransferase (AST)

- Coenzyme requirement:
    - All aminotransferases require pyridoxal phosphate
        - A derivative of vitamin B_6
    - Pyridoxal phosphate is:
        - Covalently linked to the ε-amino group of a specific lysine residue
        - At the active site of the enzyme

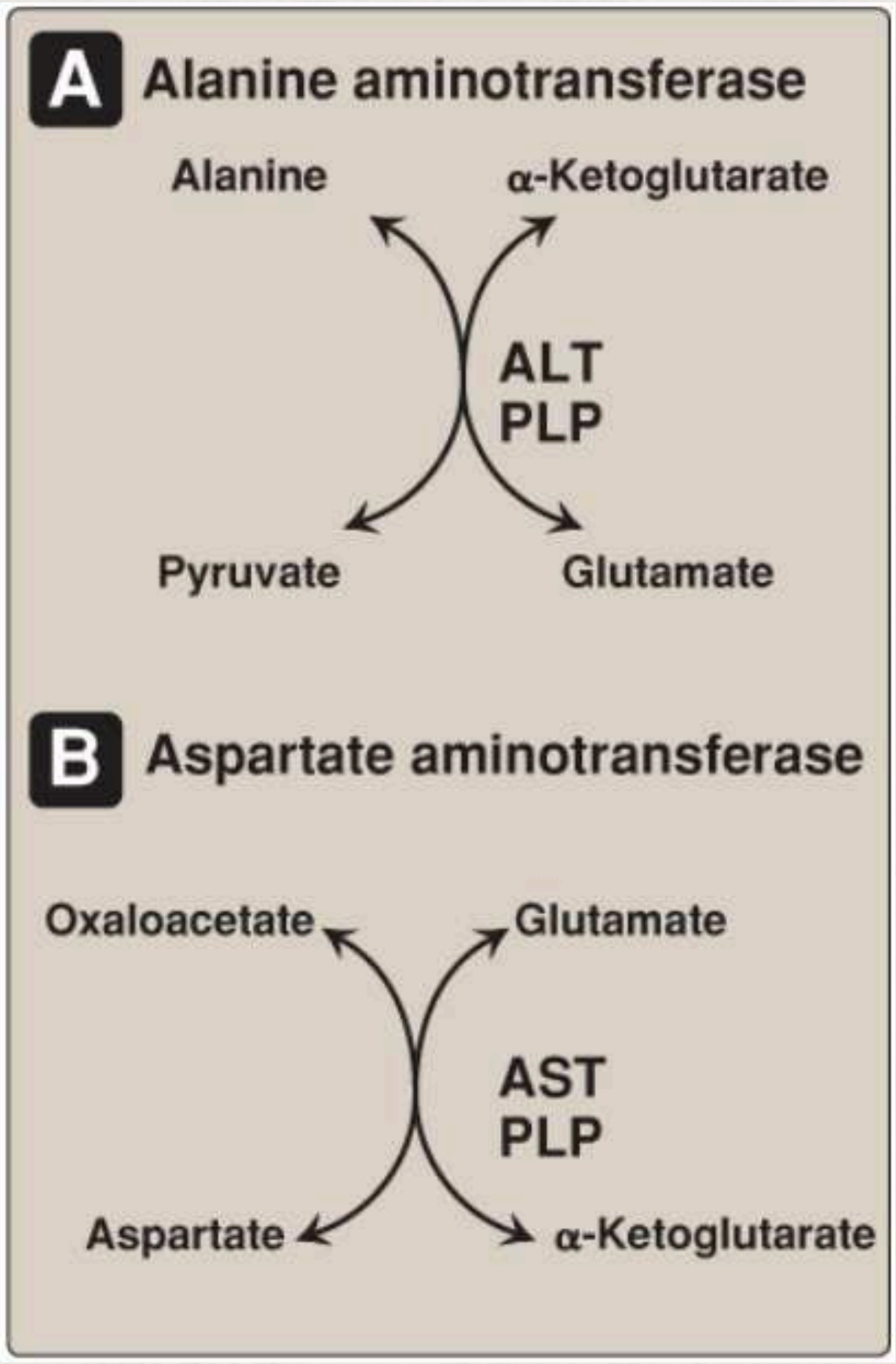
a. Alanine Aminotransferase (ALT)

- Present in: Many tissues
- Catalyzed reaction:
    - Transfers the amino group of alanine to α-ketoglutarate
    - Forms:
        - Pyruvate
        - Glutamate
- The reaction is readily reversible
- During amino acid catabolism:
    - ALT primarily functions in the direction of glutamate synthesis
- Note:
    - In effect, glutamate acts as a collector of nitrogen from most amino acids

b. Aspartate Aminotransferase (AST)

- AST is an exception to the general rule:
    - Most aminotransferases funnel amino groups to form glutamate
- During amino acid catabolism:
    - AST primarily transfers amino groups from glutamate to oxaloacetate
    - Forms:
        - α-Ketoglutarate
        - Aspartate
- Aspartate:
    - Used as a source of nitrogen in the urea cycle
- The AST reaction is reversible, like other transamination reactions

Reactions Catalyzed During Amino Acid Catabolism



2. Mechanism of Aminotransferase Reactions

- Aminotransferases act by:
    - Transferring the amino group of an amino acid substrate (glutamate) to the pyridoxal part of the coenzyme
- This generates:
    - Pyridoxamine phosphate
- At the same time:
    - The amino acid substrate glutamate is converted to an α-keto acid product (α-ketoglutarate)
- The pyridoxamine form of the coenzyme then reacts with:
    - An α-keto acid substrate (oxaloacetate) to form:
        - An amino acid product (aspartate)
        - Regenerates the original aldehyde form of the coenzyme

3. Equilibrium of Transamination Reactions

- For most transamination reactions, the equilibrium constant is near 1
- This allows the reaction to function in both directions:
    a. Amino acid degradation via removal of α-amino groups
        - Example: After consumption of a protein-rich meal
    a. Biosynthesis of nonessential amino acids via addition of amino groups to α-keto acid carbon skeletons
        - Example: When dietary amino acid supply is inadequate to meet cellular synthetic needs

4. Diagnostic Value of Aminotransferases

- Aminotransferases are normally intracellular enzymes
- Low levels in plasma reflect:
    - Release of cellular contents during normal cell turnover

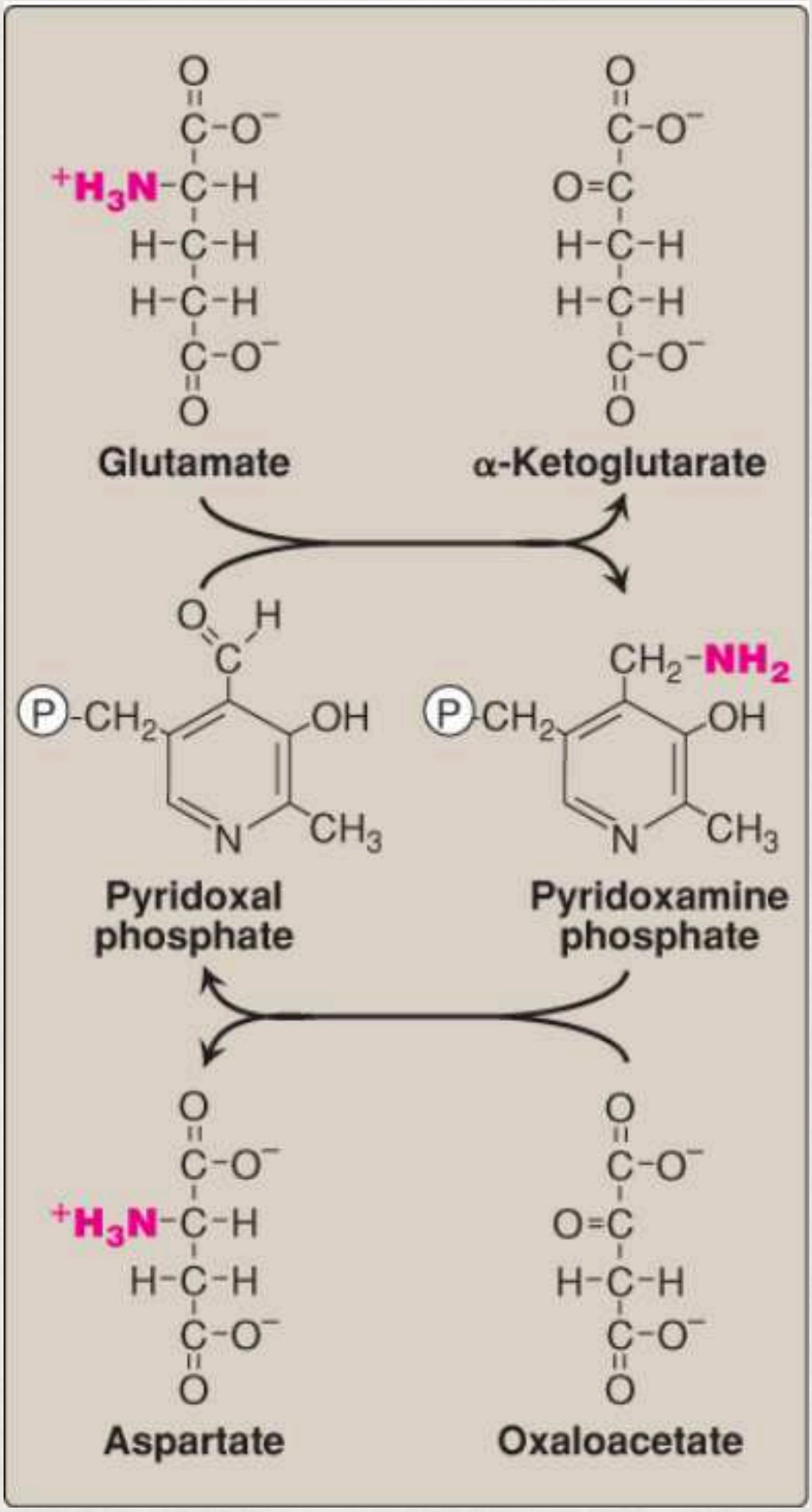
- Elevated plasma levels of aminotransferases indicate:
    - Damage to cells rich in these enzymes
- Causes of elevation:
    - Physical trauma
    - Disease processes causing cell lysis
        - Leads to release of intracellular enzymes into the blood
- Two aminotransferases of particular diagnostic value when found in plasma:
    - AST
    - ALT

a. Hepatic Disease

- Plasma AST and ALT are elevated in nearly all hepatic diseases

- Particularly high in conditions with extensive cell necrosis, such as:
    - Severe viral hepatitis
    - Toxic injury
    - Prolonged circulatory collapse
- ALT is:
    - More specific for liver disease
- AST is:
    - More sensitive for liver damage
    - Because the liver contains larger amounts of AST
- Serial measurements of AST and ALT:
    - Often used as liver function tests
    - Help in determining the course of liver damage
- Note:
    - Elevation in bilirubin results from:
        - Hepatocellular damage
        - Which decreases hepatic conjugation and excretion of bilirubin

Cyclic Interconversion of Pyridoxal Phosphate and Pyridoxamine Phosphate during the Aspartate Aminotransferase Reaction



b. Nonhepatic Disease: Diagnostic Considerations for Aminotransferase Elevation

- Aminotransferases may also be elevated in nonhepatic diseases such as:
    - Conditions that cause damage to cardiac or skeletal muscle
- However:
    - These disorders can usually be distinguished clinically from liver disease
    - Using additional lab tests

When muscle damage is suspected:

- Plasma levels of the following may be increased:
    - Creatine kinase
    - Lactate dehydrogenase (LDH)
    - Myoglobin
    - Along with AST and ALT

- Other lab values would be in the normal range:
    - Blood urea nitrogen (BUN)
    - Bilirubin
    - γ-Glutamyl transferase (GGT)
    - Alkaline phosphatase (ALP)

When bone disease is suspected:

- ALP levels will be disproportionately higher than:
    - AST
    - ALT
    - GGT

B. Oxidative Deamination: Amino Group Removal

- In contrast to transamination, which transfers amino groups:

- Oxidative deamination:
    - Results in liberation of the amino group as free ammonia

- These reactions occur primarily in:
    - The liver
    - The kidney
- Products of oxidative deamination:
    - α-Keto acids
        - Can enter central pathways of energy metabolism
    - Ammonia
        - A source of nitrogen in hepatic urea synthesis
- Note:
    - Ammonia (NH_3) exists primarily as ammonium (NH_4^+) in aqueous solution
    - But it is the unionized form (NH_3) that crosses membranes

1. Glutamate Dehydrogenase (GDH)

- As previously described:
    - Amino groups of most amino acids are ultimately funneled to glutamate
        - By transamination with α-ketoglutarate

- Glutamate is unique because:
    - It is the only amino acid that undergoes rapid oxidative deamination
- The reaction is catalyzed by:
    - Glutamate dehydrogenase (GDH)

Sequential Action of Transamination and Oxidative Deamination

- Transamination transfers amino groups from most amino acids to α-ketoglutarate, producing glutamate
- Oxidative deamination of glutamate then:
    - Regenerates α-ketoglutarate
    - Releases amino groups as ammonia
- Together, this sequential action:
    - Provides a pathway for the amino groups of most amino acids to be released as ammonia

a. Coenzymes of Glutamate Dehydrogenase (GDH)

- GDH is a mitochondrial enzyme
- GDH is unusual because:
    - It can use either:
        - Nicotinamide adenine dinucleotide (NAD^+)
        - Or its phosphorylated reduced form (NADPH) as a coenzyme
- NAD^+ is primarily used in:
    - Oxidative deamination
        - Simultaneous loss of ammonia + oxidation of carbon skeleton
- NADPH is used in:
    - Reductive amination
        - Simultaneous gain of ammonia + reduction of carbon skeleton

b. Direction of the GDH Reaction

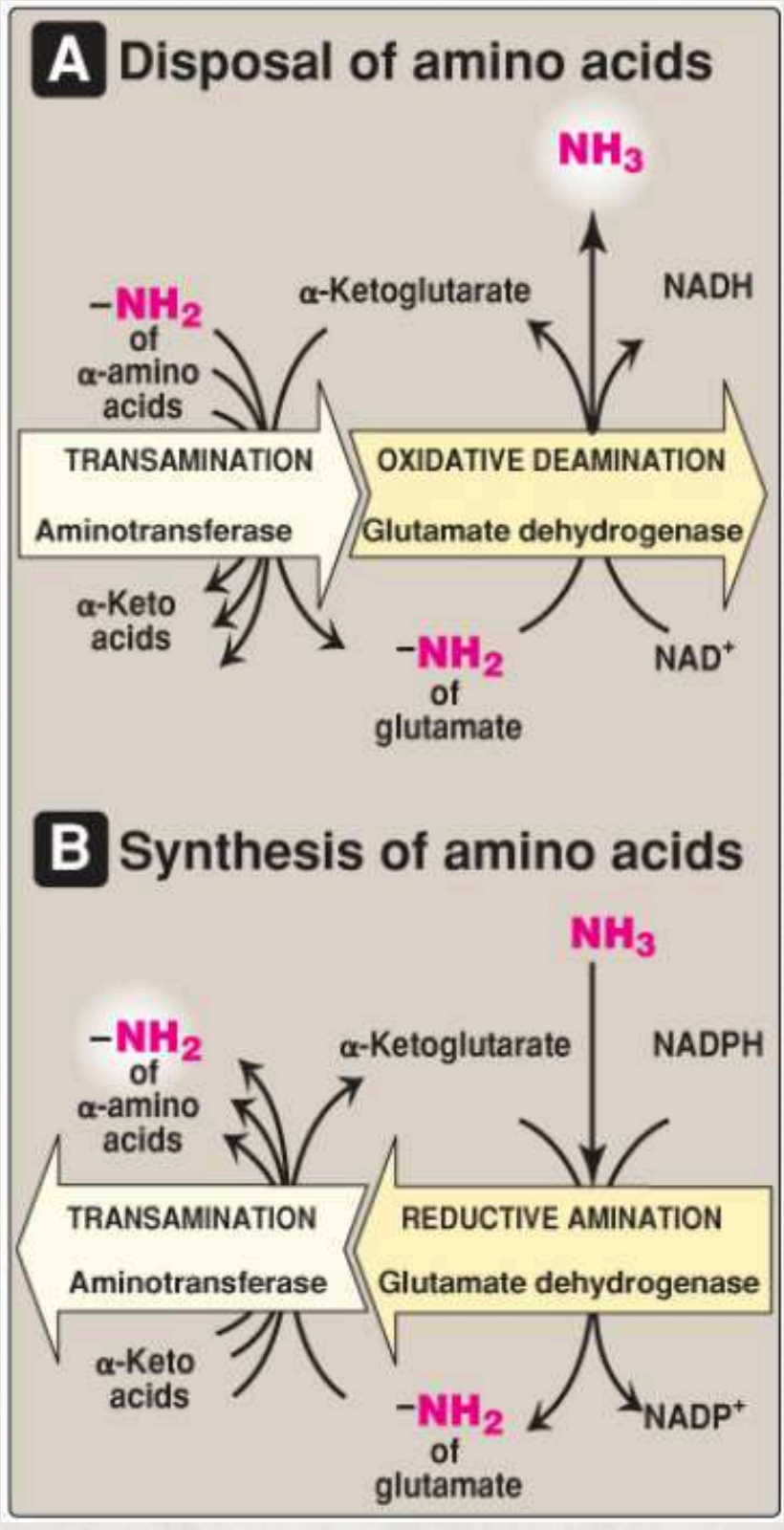
- Depends on:
    - Relative concentrations of:
        - Glutamate
        - α-Ketoglutarate
        - Ammonia
    - Ratio of:
        - Oxidized to reduced coenzymes
- Example:
    - After ingestion of a protein-containing meal:
        - Glutamate levels in the liver are elevated
        - Reaction proceeds in the direction of:
            - Amino acid degradation
            - Formation of ammonia
- High ammonia levels are required to:
    - Drive the reaction toward glutamate synthesis

c. Allosteric Regulators of GDH

- Guanosine triphosphate (GTP):
    - Allosteric inhibitor of GDH
- Adenosine diphosphate (ADP):
    - Allosteric activator of GDH
- Therefore:
    - When cellular energy levels are low:
        - Amino acid degradation by GDH is high
        - This facilitates energy production from:
            - The carbon skeletons derived from amino acids

A, B: Combined actions of aminotransferase and glutamate dehydrogenase reactions

(Note: Reductive amination occurs only when ammonia [NH3] level is high.)



2. D-Amino Acid Oxidase (DAO)

- D-Amino acids:
    - Supplied by the diet
    - Not used in the synthesis of mammalian proteins
    - Efficiently metabolized in liver and kidney peroxisomes
- DAO enzyme:
    - Flavin adenine dinucleotide (FAD)-dependent
    - Catalyzes metabolism of D-amino acids to:
        - α-Keto acids
        - Ammonia (NH_3)
        - Hydrogen peroxide (H_2O_2)
- Fate of α-keto acids:
    - Can enter general amino acid metabolic pathways
    - May be:
        - Reaminated to L-isomers
        - Or catabolized for energy

- Important Notes:
    - DAO degrades D-serine, an isomer of serine that:
        - Modulates NMDA-type glutamate receptors
        - Increased DAO activity is linked to:
            - Increased susceptibility to schizophrenia
    - DAO also converts glycine to glyoxylate

- L-Amino acid oxidases:
    - Found in snake venom

C. Ammonia Transport to the Liver

- Purpose: Conversion of toxic ammonia to urea

- Two mechanisms for transporting ammonia from peripheral tissues to the liver:

1. Glutamine Pathway

- Enzyme: Glutamine synthetase
    - Combines:
        - Ammonia + Glutamate → Glutamine

- Glutamine:
    - A nontoxic transport form of ammonia
    - Transported in blood to liver
- In the liver:
    - Glutamine is cleaved by glutaminase →
        - Glutamate + Ammonia
    - Glutamate undergoes:
        - Oxidative deamination by GDH →
            - Ammonia + α-Ketoglutarate
    - Ammonia is then:
        - Converted to urea

2. Alanine Pathway (Glucose-Alanine Cycle)

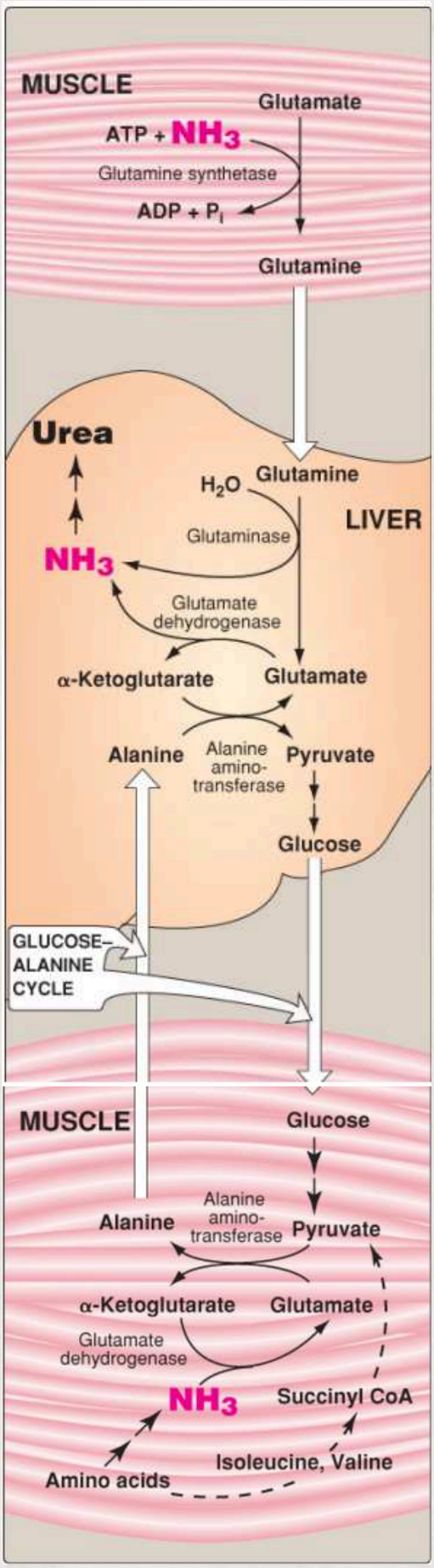
- Alanine formation:
    - Formed by transamination of pyruvate
- Sources of pyruvate:
    - Aerobic glycolysis
    - Succinyl-CoA metabolism from:
        - Branched-chain amino acids (BCAA): Isoleucine and Valine

- Transport:
    - Alanine is transported in blood to liver
- In the liver:
    - Alanine is transaminated by ALT →
        - Pyruvate + Glutamate
    - Fate of pyruvate:
        - Used to synthesize glucose
        - Glucose enters blood and is used by muscle
    - This process is called:
        - Glucose-alanine cycle
    - Fate of glutamate:
        - Deaminated by GDH →
            - Ammonia (to urea)

Summary

- Both glutamine and alanine:
    - Carry ammonia from peripheral tissues to the liver
    - Where it is ultimately converted to urea

Transport of Ammonia (NH3) from Muscle to the Liver



V. Urea Cycle

- Urea is the major disposal form of amino groups derived from amino acids
- Accounts for ~90% of nitrogen-containing components of urine
- Nitrogen sources in urea:
    - One nitrogen: free ammonia
    - Other nitrogen: aspartate
        - Note: Glutamate is the immediate precursor of:
            - Ammonia (via oxidative deamination by GDH)
            - Aspartate nitrogen (via transamination of oxaloacetate by AST)
- Carbon and oxygen of urea:
    - Derived from CO_2 (as HCO_3^-)
- Site of production:
    - Urea is produced by the liver

- Transport:
    - Urea is carried in the blood as blood urea nitrogen
    - Transported to kidneys for urinary excretion

A. Reactions of the Urea Cycle

- First two reactions:
    - Occur in the mitochondrial matrix
- Remaining enzymes:
    - Located in the cytosol
- (Note: Other pathways using both mitochondrial matrix and cytosol include:
    - Gluconeogenesis
    - Heme synthesis

1. Carbamoyl Phosphate Formation

- Enzyme: Carbamoyl phosphate synthetase I (CPS I)

- Reaction is:
    - Driven by cleavage of 2 ATP molecules
- Ammonia source:
    - Provided primarily by oxidative deamination of glutamate by mitochondrial GDH
- Final fate of nitrogen from ammonia:
    - Becomes one of the nitrogens in urea
- Activator of CPS I:
    - N-acetylglutamate (NAG) — positive allosteric activator
- (Note:
    - Carbamoyl phosphate synthetase II:
        - Participates in pyrimidine biosynthesis
        - Does not require NAG
        - Uses glutamine as nitrogen source
        - Occurs in cytosol)

2. Citrulline Formation

- Enzyme: Ornithine transcarbamylase (OTC)
- Reaction:
    - Carbamoyl group of carbamoyl phosphate transferred to ornithine
    - Phosphate released as inorganic phosphate
    - Citrulline formed → transported to cytosol
- Transport:
    - Ornithine and citrulline cross inner mitochondrial membrane via antiporter
- Note:
    - Ornithine and citrulline are not incorporated into proteins due to absence of codons
    - Ornithine is regenerated in each cycle
        - Similar to oxaloacetate regeneration in TCA cycle

3. Argininosuccinate Formation

- Enzyme: Argininosuccinate synthetase

- Reaction:
    - Citrulline + Aspartate → Argininosuccinate
- Nitrogen source:
    - α-Amino group of aspartate provides the second nitrogen in urea
- ATP usage:
    - Reaction requires cleavage of ATP → AMP + pyrophosphate
    - This is the third ATP consumed in urea formation

4. Argininosuccinate Cleavage

- Enzyme: Argininosuccinate lyase
- Products:
    - Arginine (immediate precursor of urea)
    - Fumarate

- Fate of fumarate:
    - Hydrated to malate
        - Links urea cycle to multiple metabolic pathways
- Malate metabolism options:
    - Oxidized by malate dehydrogenase to oxaloacetate
        - Can be transaminated to aspartate
        - Aspartate reenters urea cycle
    - Transported to mitochondria via malate-aspartate shuttle
        - Reenters TCA cycle
        - Can be oxidized to oxaloacetate for:
            - Gluconeogenesis
- Note:
    - Malate oxidation generates NADH
        - Supports oxidative phosphorylation
        - Reduces the energy cost of urea cycle

5. Arginine Cleavage to Ornithine and Urea

- Enzyme: Arginase-I
    - Function: Hydrolyzes arginine → ornithine + urea
- Tissue specificity:
    - Arginase-I is virtually exclusive to the liver
    - Therefore, only the liver can cleave arginine and synthesize urea
- Other tissues:
    - Tissues like the kidney can synthesize arginine from citrulline
- Note:
    - Arginase-II (present in kidneys)
        - Controls arginine availability for nitric oxide synthesis

6. Fate of Urea

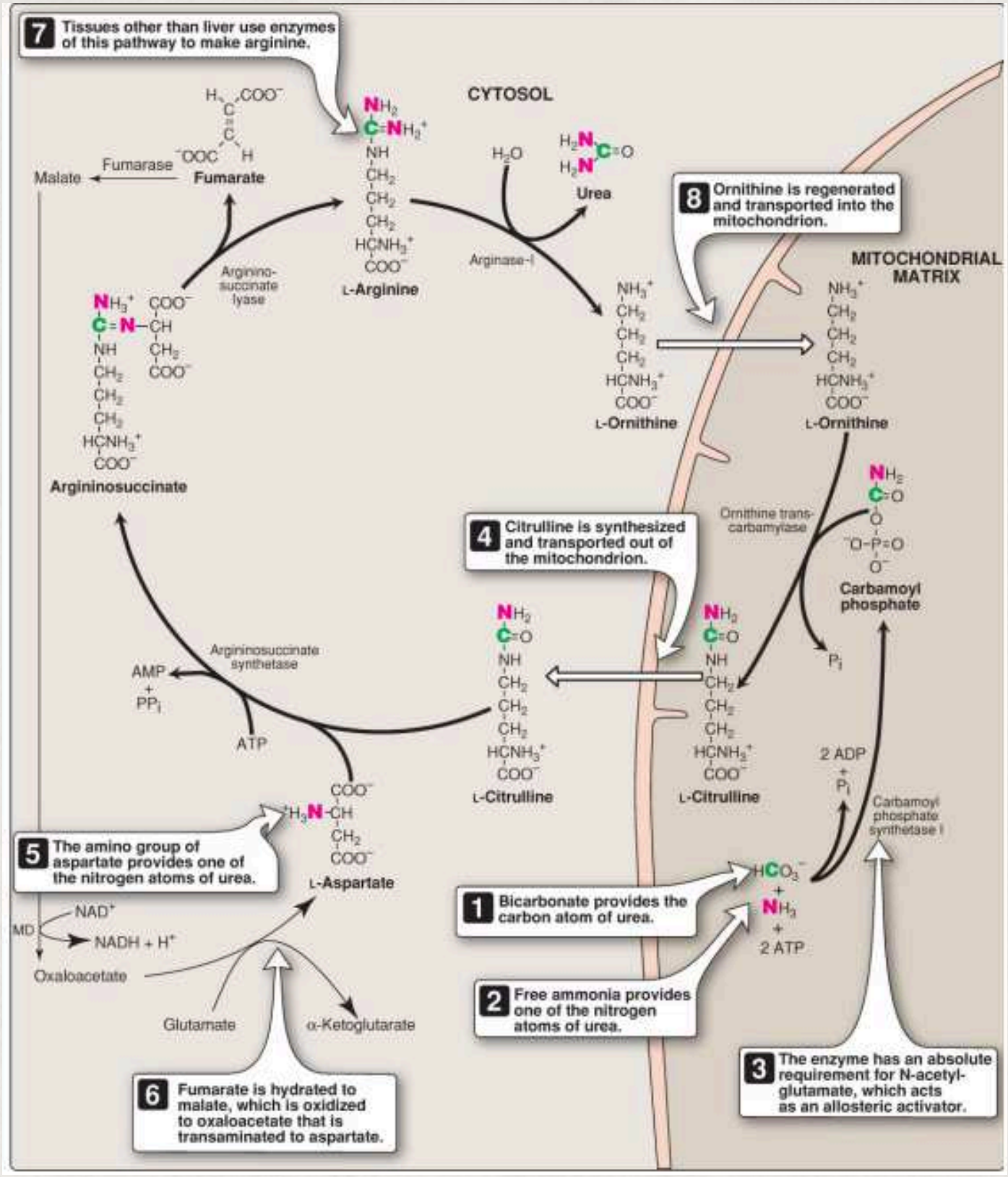
- Transport:
  - Urea diffuses from the liver
  - Transported in the blood to the kidneys
- Renal handling:
  - Urea is filtered and excreted in the urine
- Gastrointestinal interaction:
  - A portion of urea diffuses from blood into the intestine
  - Cleaved to CO_2 and ammonia by bacterial urease
- Fate of ammonia:
  - Partly lost in feces
  - Partly reabsorbed into the blood

- Kidney failure:
    - Plasma urea levels are elevated
    - Leads to greater transfer of urea from blood into gut
    - Bacterial urease activity in gut becomes a clinically important source of ammonia
    - This contributes to hyperammonemia in these patients

- Treatment:
    - Oral antibiotics reduce the number of intestinal bacteria
    - Lowers ammonia production from urea

Reactions of the Urea Cycle

(Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.)



B. Overall Stoichiometry

- Overall reaction:

$$\text{Aspartate} + NH_3 + HCO_3^- + 3ATP + H_2O \rightarrow \text{Urea} + \text{Fumarate} + 2ADP + AMP + 2Pi + PPi$$

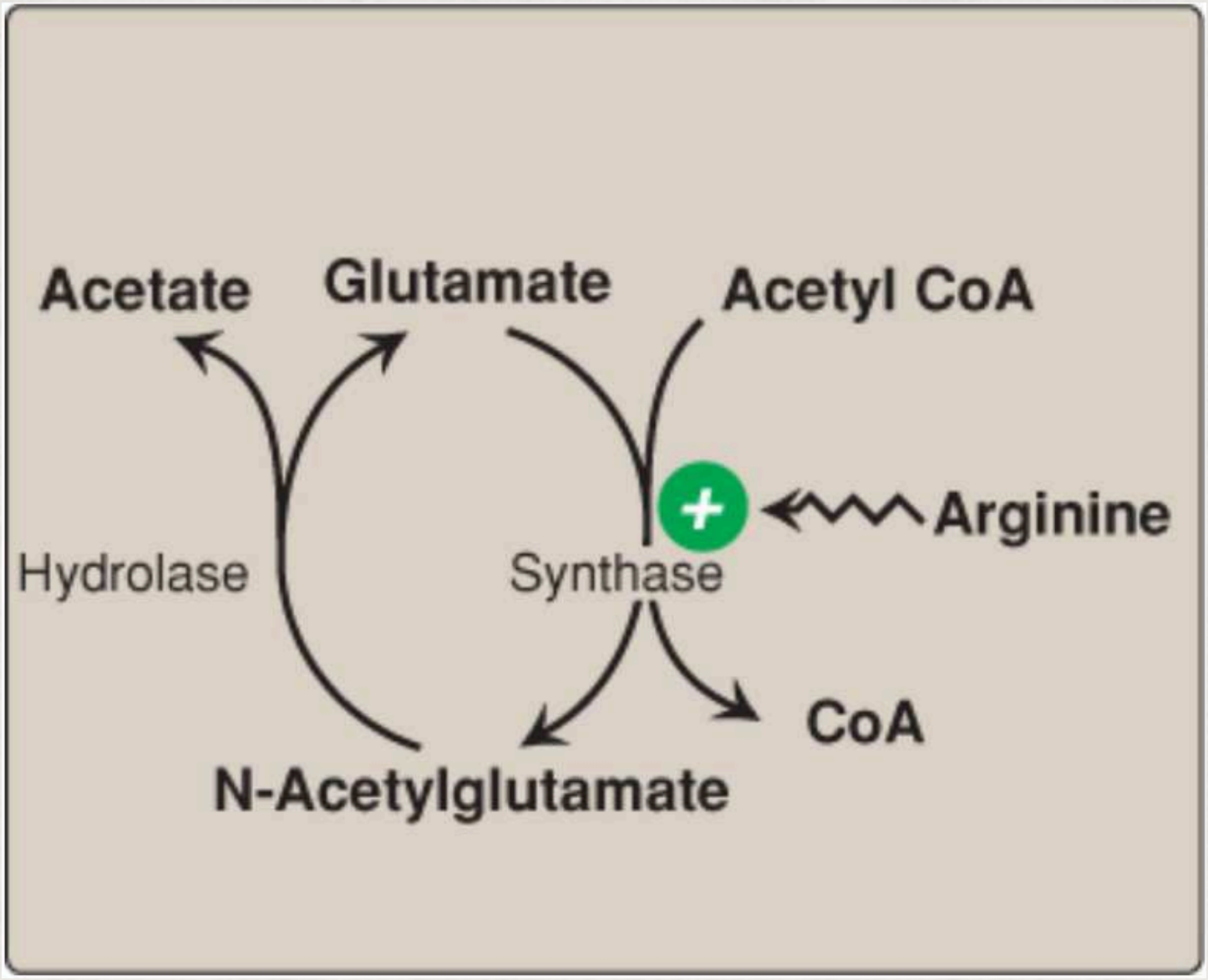
- Energy requirement:
    - 4 high-energy phosphate bonds are consumed per molecule of urea synthesized
    - Therefore, urea synthesis is irreversible
    - Associated with a large, negative ΔG
- Nitrogen sources in urea:
    - One nitrogen is from free ammonia
    - One nitrogen is from aspartate
- Role of glutamate:
    - Immediate precursor of both:
        - Ammonia → via oxidative deamination by GDH
        - Aspartate nitrogen → via transamination of oxaloacetate by AST

- Thus, both nitrogen atoms of urea arise from glutamate
- Glutamate gathers nitrogen from other amino acids

C. Regulation

- N-Acetylglutamate (NAG):
  - Essential activator for CPS I, the rate-limiting step in the urea cycle
  - Function: Increases CPS I affinity for ATP
- NAG synthesis:
  - Enzyme: N-Acetylglutamate Synthase (NAGS)
  - Substrates: Acetyl CoA + Glutamate
  - Arginine is an activator of this reaction
- Additional regulatory mechanisms:
  - Substrate availability
  - → Short-term regulation
  - Enzyme induction
  - → Long-term regulation

Formation and Degradation of N-acetylglutamate



VI. Ammonia Metabolism

- Ammonia production:
    - Occurs in all tissues during metabolism of various compounds
    - Disposed of primarily by urea formation in the liver

- Toxicity of ammonia:
    - Blood ammonia must remain very low
    - Even slightly elevated levels (hyperammonemia) are toxic to the CNS
- Requirement:
    - A mechanism is needed to transport nitrogen from peripheral tissues to the liver for disposal as urea
    - Simultaneously, circulating free ammonia must be kept low

A. Sources

1. Amino Acids (Main Source)

- Quantitatively the most important source of ammonia
- Reason: High protein intake in most Western diets → excess amino acids

- Amino acids travel to the liver and undergo:
    - Transdeamination: a combination of aminotransferase and glutamate dehydrogenase (GDH) reactions → produces ammonia
- The liver primarily catabolizes straight-chain amino acids

2. Glutamine

- Major plasma glutamine source: catabolism of BCAAs in skeletal muscle
- Target tissues for glutamine uptake:
    - Intestine
    - Liver
    - Kidneys
- Ammonia generation:
    - Enzymes involved:
        - Glutaminase
        - GDH

- In kidneys:
  - Ammonia → excreted into urine as NH_4^+
  - Role: Maintains acid–base balance by excreting protons

- In liver:
  - Ammonia → detoxified to urea and excreted

- α-Ketoglutarate (second product of GDH):
  - Is a glucogenic precursor in liver and kidneys

- Ammonia is also generated by:
  - Intestinal glutaminase
  - Enterocyte glutamine sources:
    - From blood
    - From dietary protein digestion

- Additional products of intestinal glutamine metabolism:
  - Alanine → used by liver for gluconeogenesis
  - Citrulline → used by kidneys to synthesize arginine

3. Intestinal Bacteria

- Urea is converted to ammonia by bacterial urease in intestinal lumen

- Ammonia is:

    - Absorbed via portal vein
    - Almost completely removed by the liver via conversion to urea

4. Amines

- Sources:

    - Amines from diet
    - Monoamines (hormones or neurotransmitters)

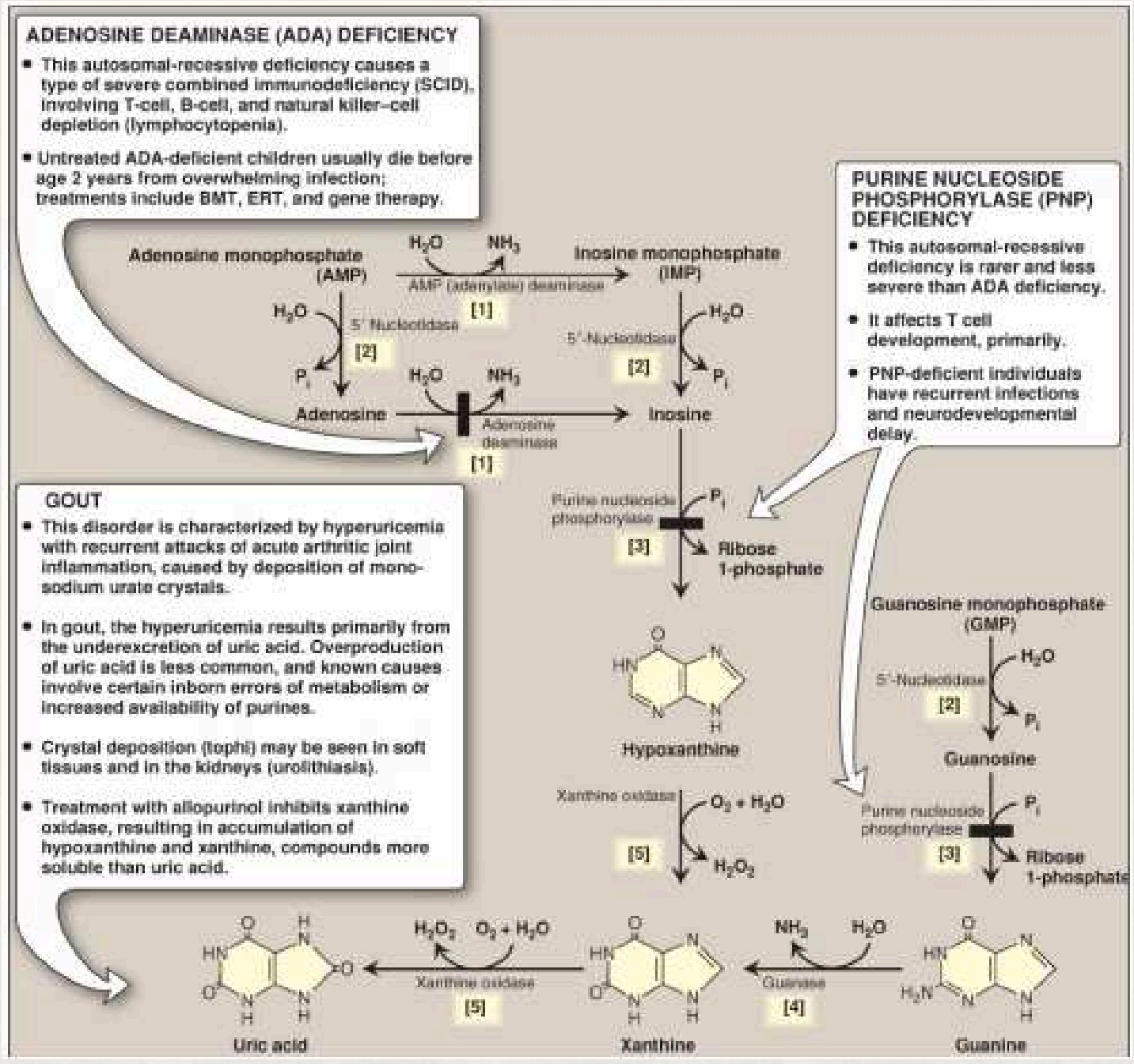
- Enzyme involved: Monoamine oxidase

- → Converts amines to ammonia

5. Purines and Pyrimidines

- During catabolism:
  - Amino groups attached to ring atoms are released as ammonia

For Concept:



B. Transport in the Circulation

- Ammonia production:
    - Constantly produced in tissues
    - Present at very low levels in blood due to:
        - Rapid hepatic removal
        - Peripheral tissues (especially muscle) releasing nitrogen as glutamine and alanine, not as free ammonia

1. Urea

- Primary disposal route for ammonia
- Formed in the liver
- Transport:
    - Urea travels via blood from liver → kidneys
    - Excreted into the glomerular filtrate

2. Glutamine

- Definition: Amide of glutamate

- Function:
    - Provides a nontoxic transport and storage form of ammonia
- Synthesis:
    - Enzyme: Glutamine synthetase
    - Requires ATP
    - Reaction: Glutamate + NH_3 → Glutamine
    - Occurs primarily in:
        - Skeletal muscle
        - Liver
        - Also important in CNS
- CNS importance:
    - Major mechanism for removal of ammonia in the brain
- Plasma levels:
    - Glutamine is found at higher concentrations than other amino acids
    - Reflects its key role in nitrogen transport

3. Liver Zonation for Ammonia Clearance

- Periportal hepatocytes (close to inflow of blood):
  - Use glutaminase, GDH, and urea cycle for ammonia detoxification

- Perivenous hepatocytes:
  - Use glutamine synthetase as an ammonia scavenger

- This dual-zonal system keeps blood ammonia levels low

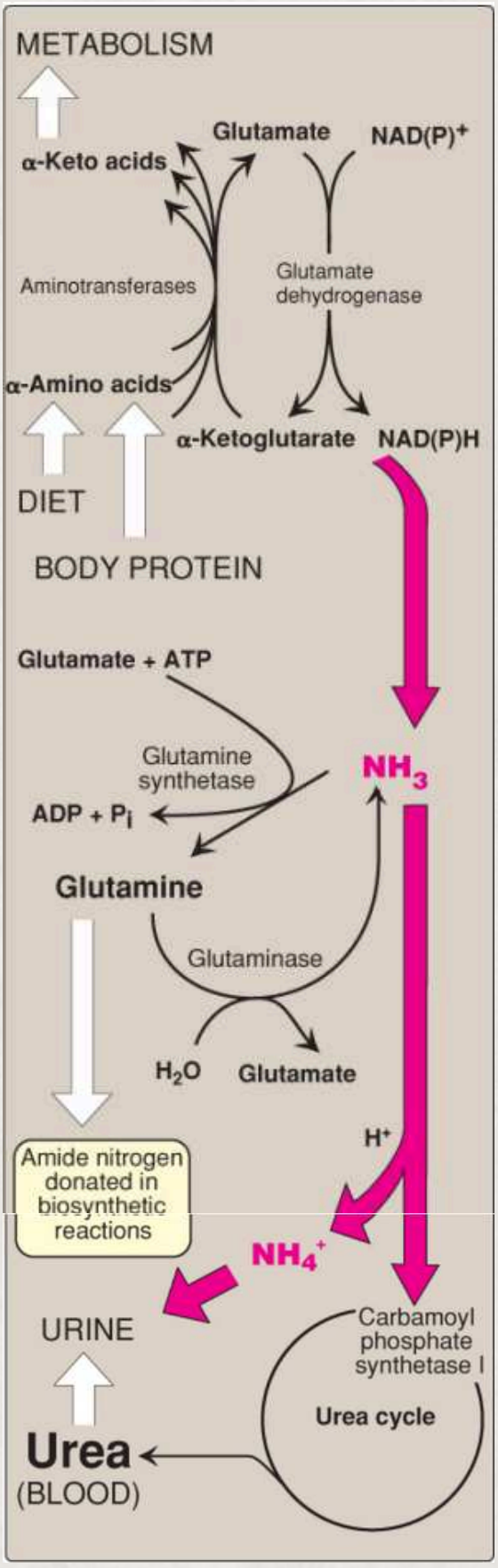
Note:

- Urea content in the urine is reported as urinary urea nitrogen, or UUN.

- Urea in blood is reported as BUN (blood urea nitrogen).

- The enzymes glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I fix NH_3 into organic molecules.

Ammonia (NH3) Metabolism.



C. Hyperammonemia

- Normal blood ammonia level:
    - Ranges between 5–35 µmol/L
    - Due to the high capacity of the hepatic urea cycle to remove ammonia

Causes of Hyperammonemia

- Occurs when liver function is compromised, such as:
    - Genetic defects in urea cycle enzymes
    - Liver diseases (e.g., cirrhosis, hepatitis, liver failure)

- In these conditions, blood ammonia levels can exceed 1,000 µmol/L

Clinical Significance

- Hyperammonemia is a medical emergency

- Ammonia is neurotoxic, especially to the central nervous system (CNS)

Symptoms of Ammonia Intoxication

- Neurological signs:
    - Tremors
    - Slurred speech
    - Somnolence (excessive drowsiness)
    - Vomiting
    - Cerebral edema
    - Blurred vision
- Severe effects (at high ammonia levels):
    - Coma
    - Death

I. Causes of Hyperammonemia

A. Acquired Hyperammonemia

- Most common in adults, due to liver disease:
    - Viral hepatitis
    - Hepatotoxins, e.g., alcohol

- Cirrhosis of the liver:
    - Leads to formation of collateral circulation
    - Portal blood bypasses the liver and enters systemic circulation directly
    - Result: Ammonia is not converted to urea
    - Leads to elevated blood ammonia levels

B. Congenital Hyperammonemia

- Due to inherited deficiencies of the urea cycle enzymes or N-acetylglutamate synthase (NAGS)

- Overall incidence: ~1 in 25,000 live births

i. Enzymes affected:

- All five urea cycle enzymes can be deficient

- NAGS deficiency is also documented

ii. Inheritance pattern:

- Ornithine transcarbamylase (OTC) deficiency:
    - X-linked
    - Affects males predominantly
    - Female carriers may also become symptomatic
- All other urea cycle enzyme deficiencies:
    - Autosomal recessive inheritance

iii. Onset:

- Symptoms appear within the first weeks after birth
- Due to failure to synthesize urea, leading to accumulation of ammonia

iv. Symptoms (similar to general hyperammonemia):

- Tremors
- Slurred speech
- Somnolence (drowsiness)
- Vomiting
- Cerebral edema
- Blurred vision

- Intellectual and developmental disability
- In severe cases: Coma and death

v. Diagnosis:

- Based on:
    - Clinical symptoms
    - Laboratory findings (e.g., plasma ammonia, urea cycle intermediates)
    - Genetic testing

vi. Prognosis:

- Historically associated with high morbidity (especially neurological damage) and high mortality

a. Ornithine Transcarbamylase (OTC) Deficiency

- OTC deficiency is the most common urea cycle disorder

.

- Laboratory findings:
    - Decrease in:
        - Reaction rate of the OTC-catalyzed step
        - Downstream products: citrulline and arginine
    - Increase in:
        - Detectable serum and urinary orotic acid levels
- Pathophysiology:
    - Carbamoyl phosphate, a substrate of OTC, accumulates and instead:
        - Becomes a substrate for pyrimidine biosynthesis
        - Enters the pathway downstream of the regulatory reaction
    - This results in overproduction of orotic acid, a pyrimidine biosynthesis intermediate
- Differential Diagnosis Note:
    - Elevated orotic acid is also seen in hereditary orotic aciduria
        - Caused by deficiency of UMP synthase (UMPS)

OTC vs. UMPS Deficiency

Feature	OTC Deficiency	UMPS Deficiency
Orotic acid	Elevated	Elevated
Hyperammonemia	Present	Absent
Megaloblastic anemia	Absent	May be present
Genetic Testing	Used for confirmation	Used for confirmation

b. Argininosuccinate Synthetase Deficiency

(Also known as Citrullinemia Type I)

- Characterized by accumulation of the substrate citrulline in:
    - Blood
    - Urine

- Clinical Forms:
    - Neonatal acute (classic) form
    - Milder late-onset form
    - Form beginning during or after pregnancy
    - Asymptomatic form

- Neonatal Screening:
    - Citrulline can be detected in newborn screening
    - Early detection is critical to prevent:
        - Hyperammonemia
        - Brain damage

c. Argininosuccinate Lyase Deficiency

- Results in accumulation of the substrate argininosuccinate in urine

- Leads to argininosuccinic aciduria
    - This finding is diagnostic
    - Included in newborn screening

- Clinical Features (more severe and late-onset forms):
    - Neurologic abnormalities
    - Developmental delays
    - Cognitive impairment

d. Arginase-I Deficiency

- In arginase-I deficiency:
    - Accumulation of substrate arginine occurs in:
        - Blood
        - Urine
    - Referred to as:
        - Argininemia
        - Hyperargininemia

- Hyperammonemia:
    - Often less severe
    - Reason: Arginine contains two waste nitrogens
        - Can be excreted in urine

- Clinical Course:
    - Patients may:
        - Appear healthy at birth
        - Have normal development during the first 1 to 3 years
    - First symptoms may appear later and include:
        - Developmental delays
        - Loss of developmental milestones
        - Intellectual disability

- Nature of Hyperammonemia:
    - Episodic
    - Triggered by:
        - High-protein meals
        - Stress, such as:
            - Illness
            - Fasting

e. N-Acetylglutamate Synthase (NAGS) Deficiency

- Like arginase-I deficiency, NAGS deficiency can lead to:
    - Developmental delays
    - Intellectual disability

- Less severe forms:
    - May be episodic later in life
    - Triggered by:
        - High-protein meals
        - Stress
        - Fasting
- Treatment:
    - Carglumic acid (FDA-approved therapy)
        - Synthetic form of NAG (N-acetylglutamate)
        - Functions as a positive allosteric activator of:
            - Carbamoyl phosphate synthetase I

f. Treatment for Hyperammonemia

General Treatment Principles:

- Goal: Manage urea cycle enzyme deficiencies by:
    - Limiting protein intake while ensuring:
        - Sufficient calories to prevent protein catabolism
    - Removing excess ammonia from blood

- Individualized treatment varies with:
    - Type of enzyme deficiency
    - Severity of defect

Dietary Management:

- Low-protein diet:
    - With minimum protein needed for health
    - Requirements depend on:
        - Age
        - Weight
- Special formulas/medical foods:
    - Protein content tailored to individual needs

Nitrogen-Scavenging Medications

Medication	Mechanism	Final Product	Excretion
Benzoate	Combines with glycine	Hippurate	Urine
Phenylbutyrate	Converted to phenylacetate → combines with glutamine	Phenylacetylglutamine	Urine

- Effect:
    - Excretes glycine and glutamine
    - Stimulates their resynthesis
    - Lowers ammonia levels
    - Reduces potential for hyperammonemia

Severe Hyperammonemia Management:

- May require:
    - Dialysis
    - Intravenous fluids
    - Other emergency treatments
- Purpose: Rapidly reduce blood ammonia levels
- Prevents: Permanent brain damage

Treatment of Patients with Urea Cycle Defects by Administration of Phenylbutyrate to Aid in Excretion of Ammonia (NH3)