

AMINO ACID AND PROTEIN METABOLISM

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Amino Acids: Disposal of Nitrogen

- Unlike fats and carbohydrates, amino acids are not stored by the body
- Therefore, amino acids must be obtained from the diet, synthesized de novo or produced from normal protein degradation
- Any amino acid in excess of the biosynthetic needs of the cell are rapidly degraded

- The first phase of catabolism involves the removal of α -amino groups (usually by transamination and subsequent oxidative deamination) forming ammonia and the corresponding α -keto acid
- In the second phase of amino acid catabolism, the carbon skeletons of the α -keto acids are converted to common intermediates of energy producing, metabolic pathways

- These compounds can be metabolized to CO_2 and water, glucose, fatty acids or ketone bodies by the central pathways of metabolism

OVERALL NITROGEN METABOLISM

- Nitrogen enters the body in a variety of compounds present in foods, the most important being amino acids contained in dietary proteins
- Nitrogen leaves the body as urea, ammonia and other products derived from amino acid metabolism
- The role of body protein in these transformations involves two important concepts: the **amino acid pool** and **protein turnover**

Amino acid pool

- Amino acids released by hydrolysis of dietary or tissue protein, or synthesized de novo, mix with other free amino acids distributed throughout the body
- Collectively, they constitute amino acid pool
- Amino acid pool, containing about 100g of amino acids is small in comparison with the amount of protein in the body (about 12 kg in 70 kg man)

- Only about 75% of amino acids obtained through hydrolysis of body protein are recaptured through the biosynthesis of new tissue protein
- The remainder are metabolized or serve as precursors of a variety of compounds
- The primary role of amino acids is to serve as building blocks in biosynthetic reactions, particularly synthesis of tissue protein
- Amino acids are used secondarily as fuel

Consequences of high and low intake of dietary protein

- Diets low in protein – there will be a deficiency in essential amino acids. This will result in net breakdown of tissue protein, leading to clinical symptoms of protein deficiency as described for **Kwashiorkor**
- Diets high in protein – excess amino acids are metabolized, their carbon skeletons converted to glucose or to fat

Protein turnover

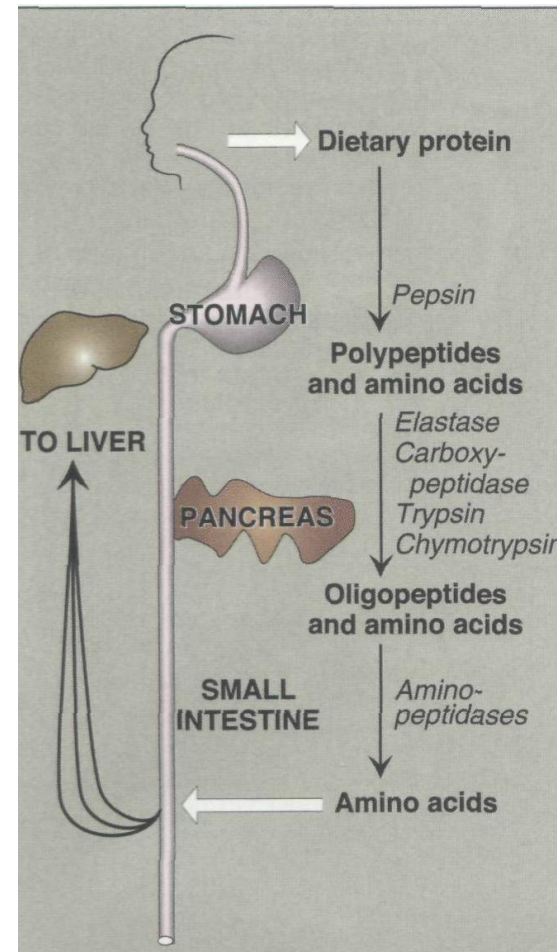
- Most proteins in the body are constantly being synthesized and then degraded, permitting the removal of abnormal or unneeded protein
- **Rate of turnover:**
- In healthy adults, the total amount of protein in the body remains constant, because the rate of protein synthesis is just sufficient to replace the protein that is degraded

- This process, called **protein turnover**, leads to the hydrolysis and re-synthesis of 300 to 400 g of body protein each day
- Short-lived proteins (e.g. regulatory proteins) are rapidly degraded, having half-lives measured in minutes or hours
- Long-lived proteins, with half lives of days to weeks, constitute the majority of proteins in the cell

- Structural proteins, such as collagen, are metabolically stable, and have half-lives measured in months or years

DIGESTION OF PROTEINS

- Digestion of dietary proteins by the proteolytic enzymes of the gastro-intestinal tract



- Proteins are generally too large to be absorbed by the intestine
- *an exception to this rule is that newborns can take up maternal antibodies in breast milk
- They must therefore be hydrolyzed to yield their constituent amino acids, which can be absorbed

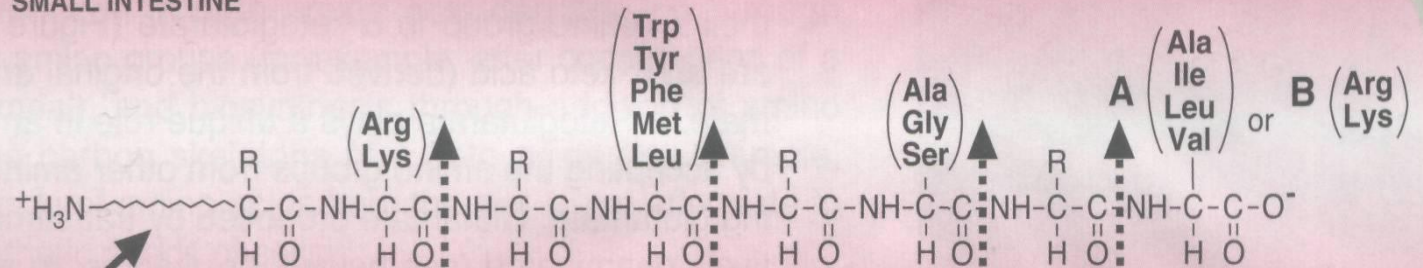
- Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas and the small intestine
- **A. Digestion of proteins by gastric secretion:**
- Digestion of proteins of proteins begin in the **stomach**, which secretes gastric juice - containing **HCl** and proenzyme, **pepsinogen**

- **Hydrochloric acid** – is too dilute (pH 2 to 3) to hydrolyze proteins
- This acid functions instead to kill some bacteria and to denature proteins
- **Pepsinogen** - is acid-stable endopeptidase secreted by the serous cells of the stomach as an inactive zymogen (or proenzyme)

- Pepsinogen is activated to pepsin, either by HCl, or autocatalytically by other pepsin molecules that have already been activated
- Pepsin releases peptides and a few free amino acids from dietary proteins

- **B. Digestion of proteins by pancreatic enzymes**
- On entering the small intestine, large polypeptides are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases
- Each has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond

SMALL INTESTINE



Dietary protein

Trypsin

Chymotrypsin

Elastase

Carboxypeptidase A
Carboxypeptidase B

Enteropeptidase

Trypsinogen

Chymotrypsinogen

Proelastase

Procarboxypeptidase A
Procarboxypeptidase B

- These enzymes, like pepsin, are synthesized and secreted as inactive zymogens
- The release and activation of the pancreatic zymogens is mediated by the secretion of **cholecystokinin** and **secretin**
- **Enteropeptidase** – an enzyme synthesized by and present on luminal surface of intestinal mucosal cells of brush border membrane – converts the pancreatic zymogen **trypsinogen** to **trypsin**

- Trypsin subsequently converts other trypsinogen molecules to trypsin by cleaving a limited number of specific peptide bonds in the zymogen
- Enteropeptidase thus unleashes a cascade of proteolytic activity

- **Abnormalities in protein digestion:**
- In individuals with deficiency in pancreatic secretion (e.g due to chronic pancreatitis, cystic fibrosis or surgical removal of the pancreas), the digestion and absorption of fat and protein is incomplete
- This results in the abnormal appearance of lipids (called steatorrhea) and undigested protein in the feaces

- **C. Digestion of oligopeptides by enzymes of the small intestine:**
- The luminal surface of the intestine contains aminopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce free amino acids and smaller peptides

- **D. Absorption of amino acids and dipeptides**
- Free amino acids and dipeptides are taken up by the intestinal epithelial cells
- There, the dipeptides are hydrolyzed in the cytosol to amino acids before being released into the portal system
- These amino acids are either metabolized by the liver or released into the general circulation

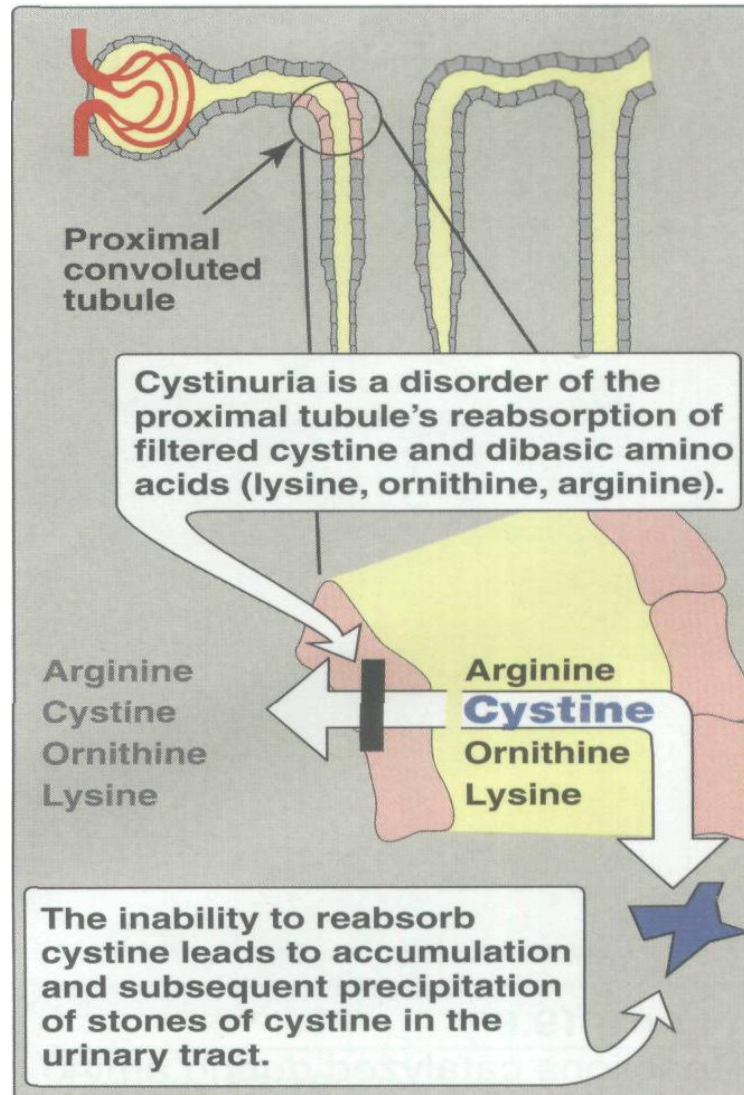
Transport of amino acids into the cell

- Concentration of amino acids in extracellular fluids is significantly lower than that within the cells. This concentration gradient is maintained by active transport systems driven by the hydrolysis of ATP
- At least seven different transport systems are known that have overlapping specificities for different amino acids

- One transport system is responsible for re-absorption of the amino acids cysteine, ornithine, arginine and lysine.
- In inherited disorder **cystinuria**, this carrier system is defective
- Cystinuria is one of the most common inherited diseases and the most common genetic error of amino acid transport

- The disease expresses itself clinically by the precipitation of cystine to form kidney stones (**calculi**) which can block the urinary tract
- Oral hydration is an important part of treatment for this disorder

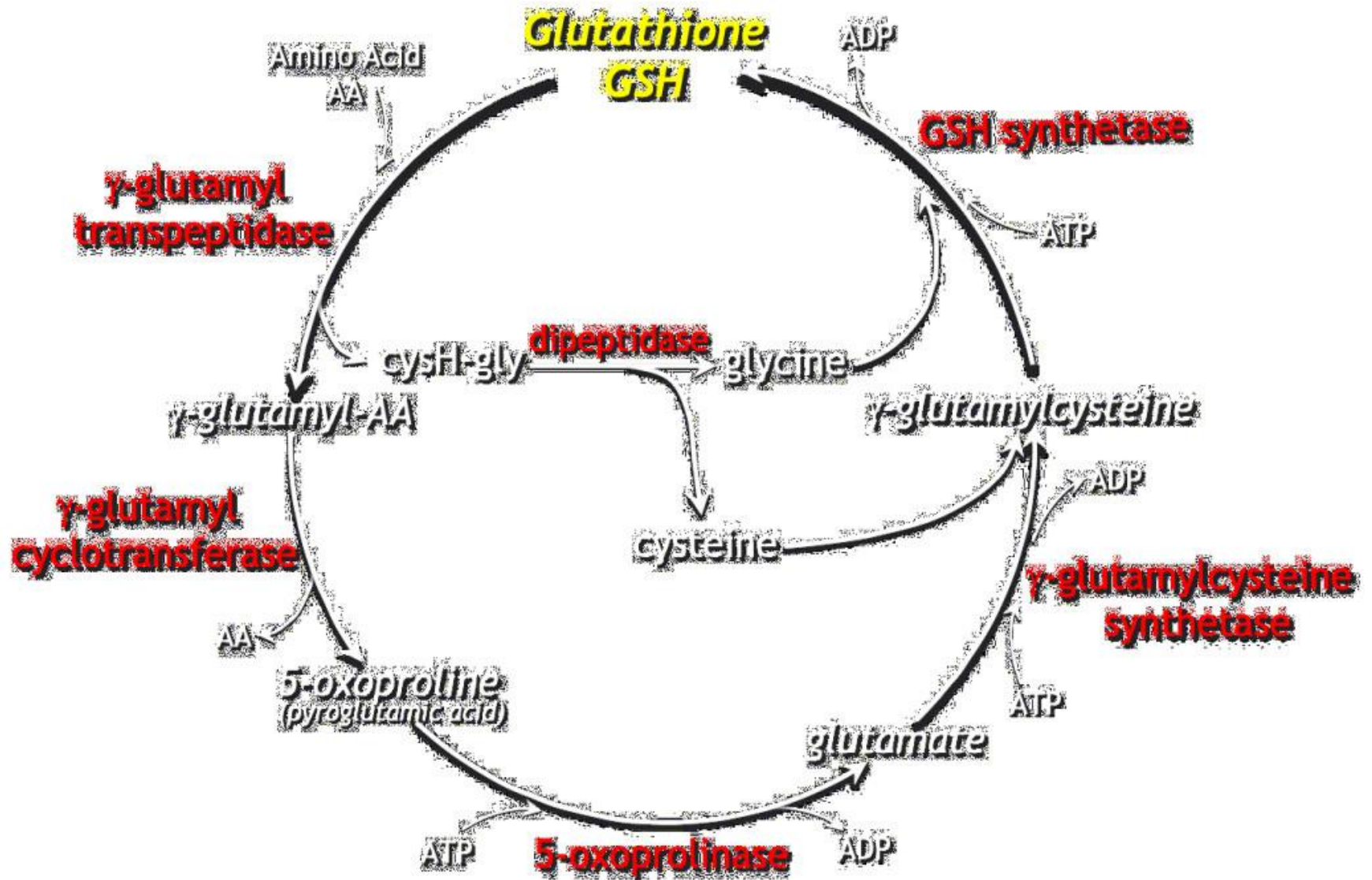
Genetic defect seen in cystinuria



General amino acid transport system: the γ -glutamyl cycle

- Occurs in brain, intestine and kidney
- Most active in the transport of neutral amino acids, such as methionine and glutamine
- The cycle serves two functions:
- In γ -glutamyl cycle, transport of amino acids across membrane is driven by hydrolysis of three ATP for each amino acid that enters the cell

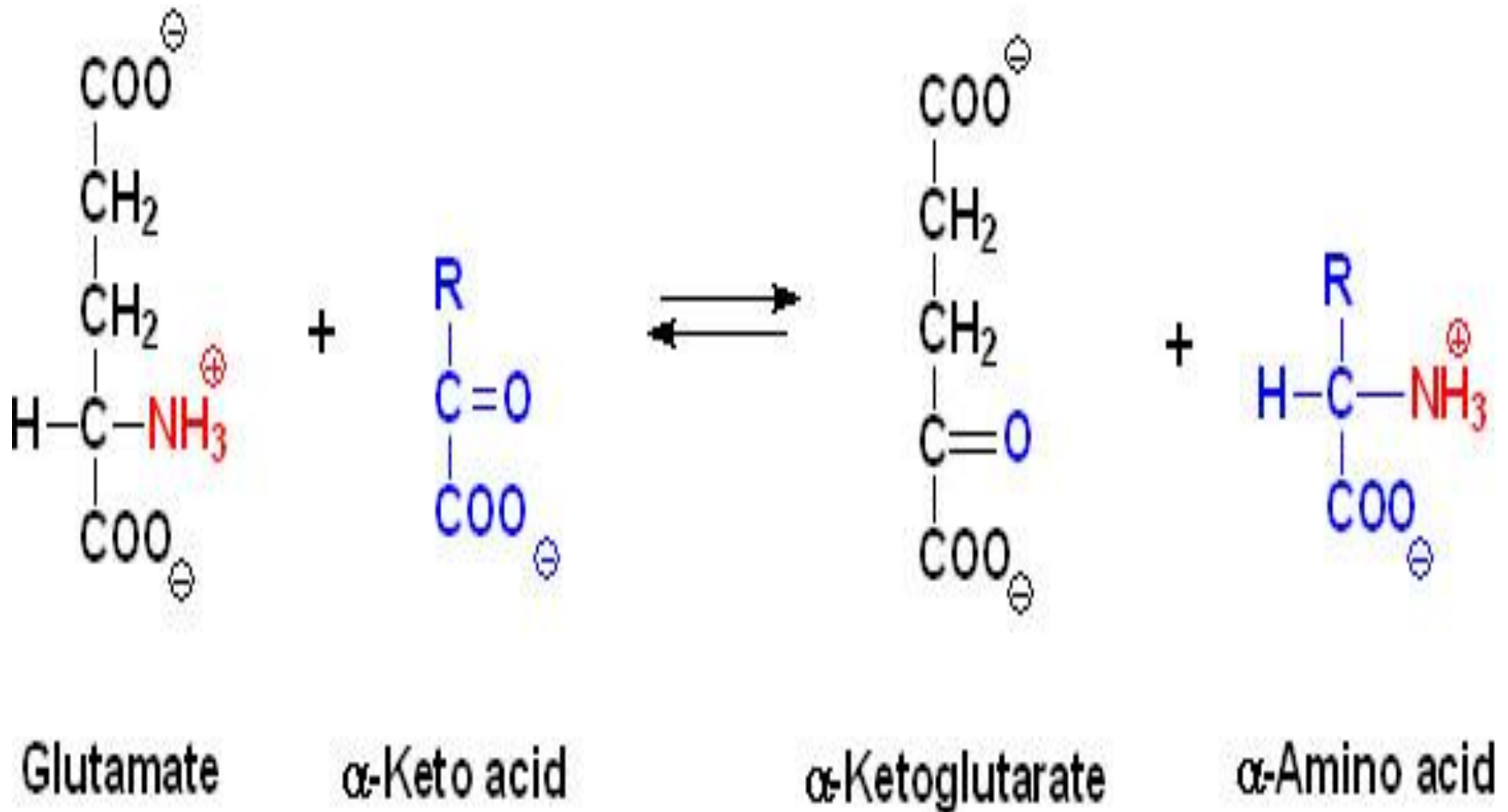
- **Oxoprolinuria** – inherited disease in which elevated levels of 5-oxoproline occur in blood and urine, resulting in acidosis and neurologic damage
- The disease is due to a deficiency in the enzyme **glutathione synthetase**



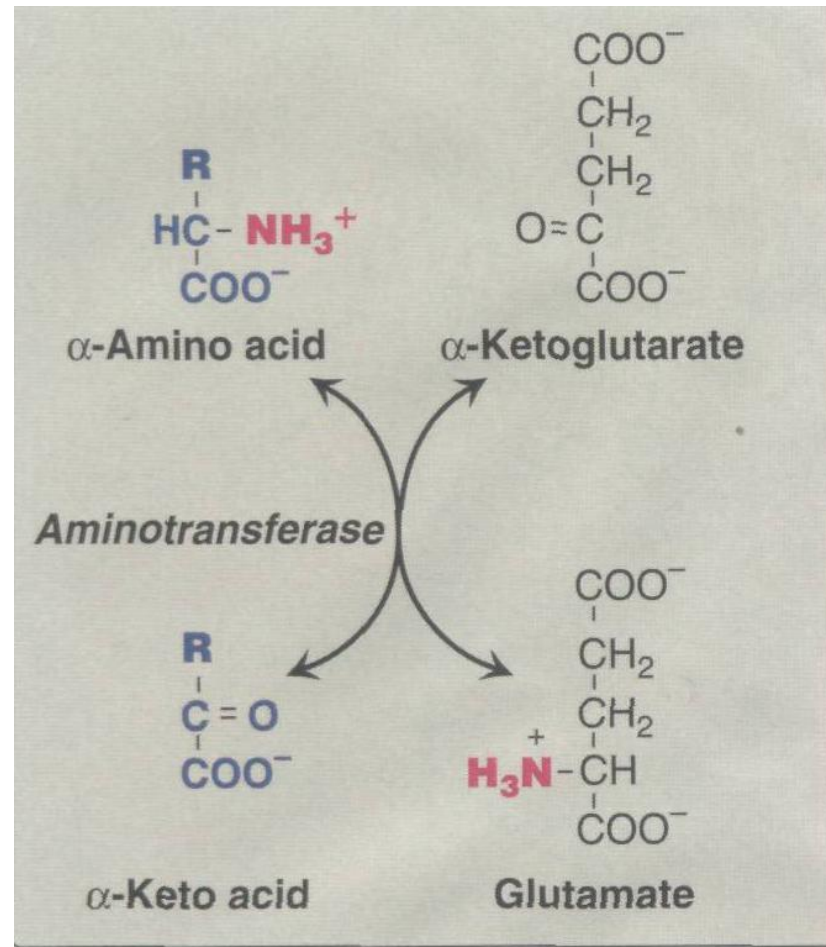
Removal of nitrogen from amino acids

- Accomplished by **transamination** and **oxidative deamination** – reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen
- The first step in the catabolism of most amino acids is the transfer of their α -amino group to α -KG, thus becoming glutamate
- This transfer of amino groups is catalyzed by a family of enzymes called **aminotransferases** (formerly called transaminases)

Aminotransferase catalyzed reaction



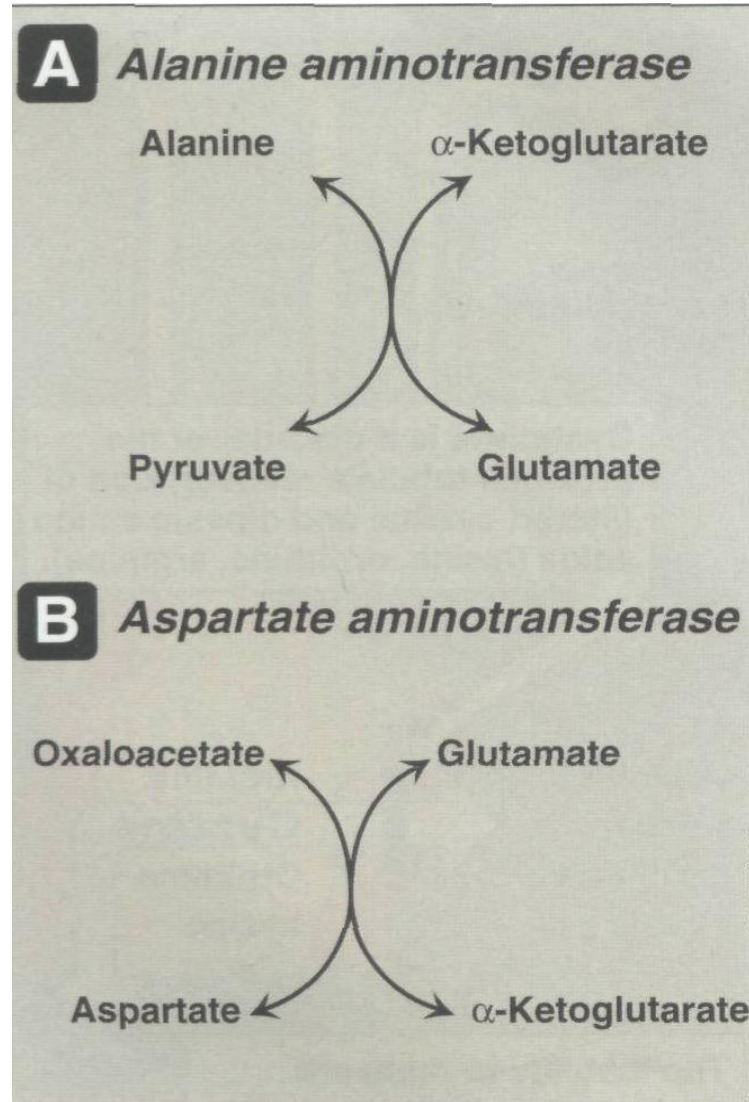
Aminotransferase reaction



- All amino acids, with exception of lysine and threonine, participate in transamination at some point in their catabolism
- These two amino acids lose their α -amino groups by deamination
- **Substrate specificity of aminotransferases:**
- Each aminotransferase is specific for one or at most a few amino acid nitrogen donor

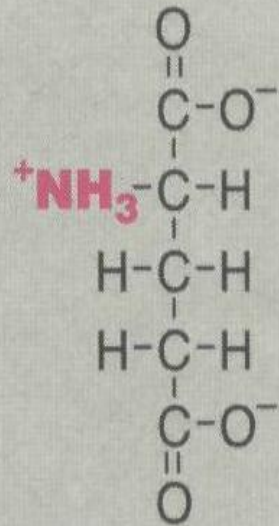
- Aminotransferases are named after the specific amino acid nitrogen donor
- The two most important aminotransferase reactions are:
 - i) **Alanine aminotransferase - ALT (GPT)**
 - Alanine + α -KG \leftrightarrow pyruvate + glutamate
 - ii) **Aspartate aminotransferase – AST (GOT)**
 - Glutamate + OAA \leftrightarrow α -KG + Aspartate
 - Aspartate is used as a source of nitrogen in the urea cycle

- **A. Alanine aminotransferase reaction**
- **B. Aspartate aminotransferase reaction**

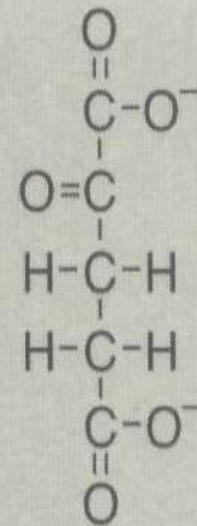


Mechanism of action of aminotransferases

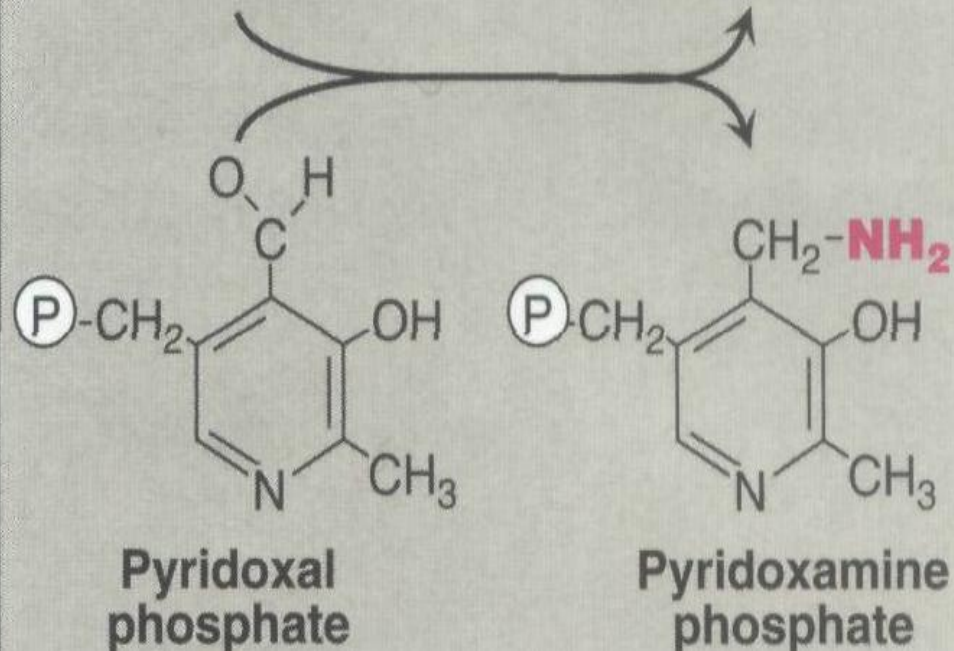
- All aminotransferases require cofactor pyridoxal phosphate

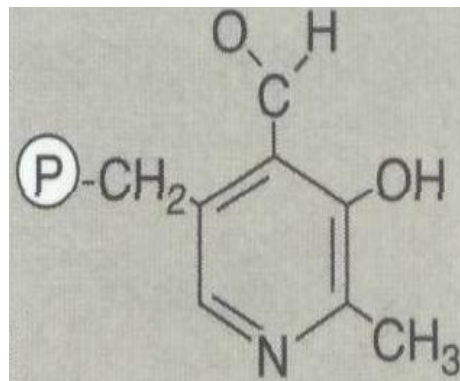


Glutamate

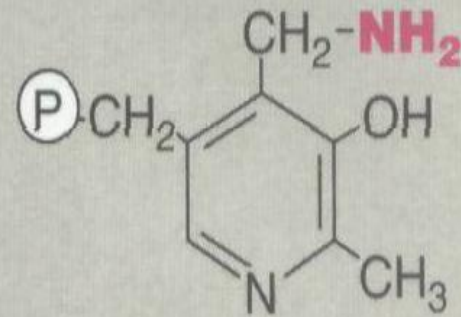


α -Ketoglutarate

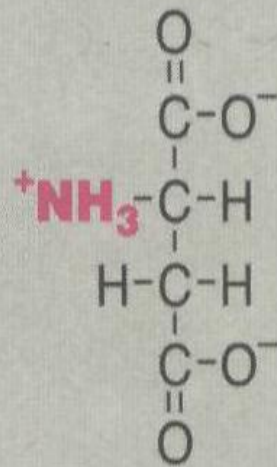




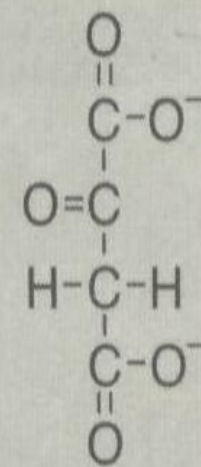
**Pyridoxal
phosphate**



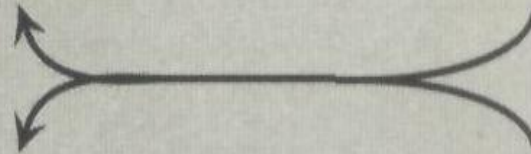
**Pyridoxamine
phosphate**



Aspartate



Oxaloacetate



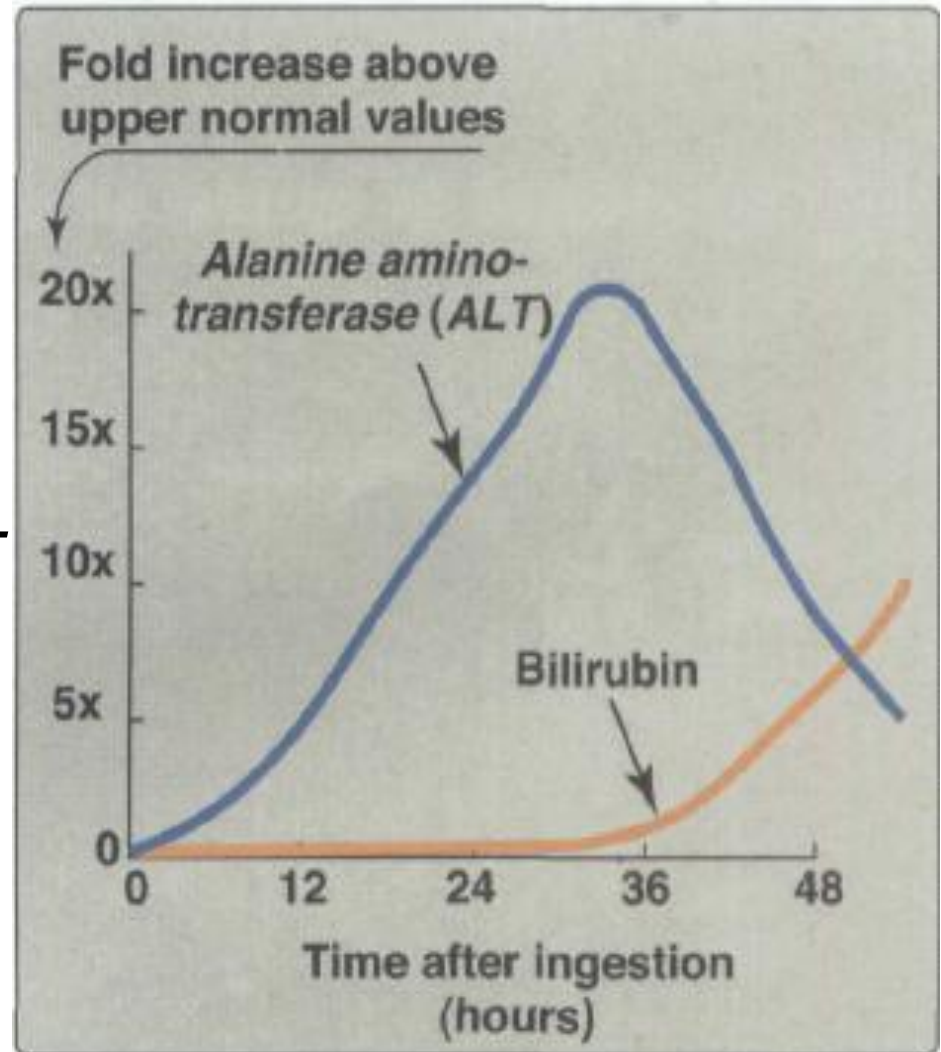
Diagnostic value of plasma aminotransferases

- Are normally intracellular enzymes, with low levels found in the plasma representing the release of cellular plasma contents during normal cell turnover
- Presence of elevated plasma levels of aminotransferases indicates damage to cells rich in these enzymes

Liver disease

- Plasma AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury and prolonged circulatory collapse
- ALT is more specific for liver disease than AST, but the latter is more sensitive because the liver contains larger amounts of AST

- The figure shows the early release of *ALT* into the serum, following ingestion of a liver toxin
- Pattern of serum *ALT* and bilirubin in the plasma following poisoning with toxic mushroom *Amanita phalloides*



Nonhepatic disease

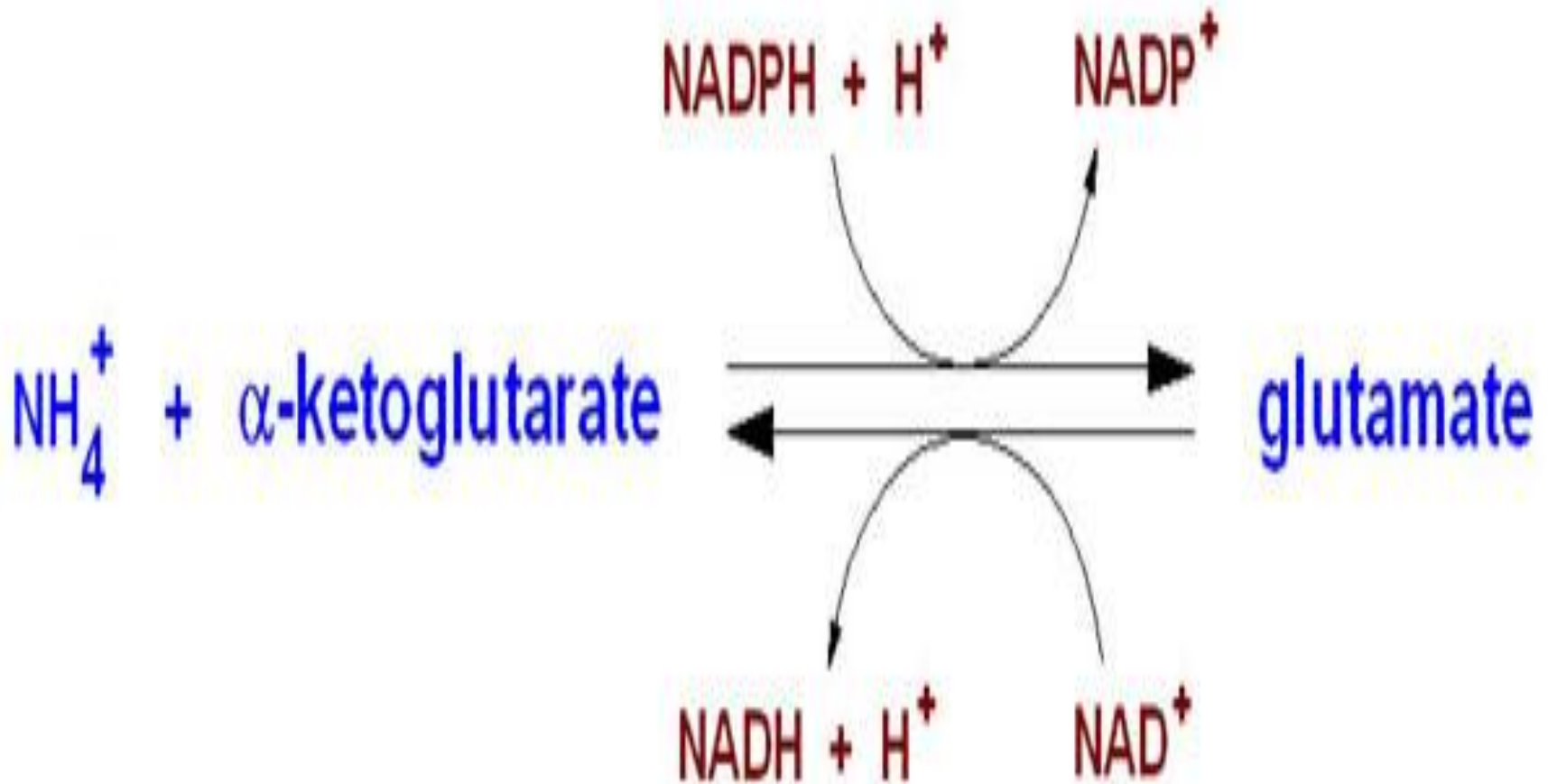
- Aminotransferases may be elevated in nonhepatic disease, such as myocardial infarction and muscle disorders, however, these disorders can usually be distinguished clinically from liver disease

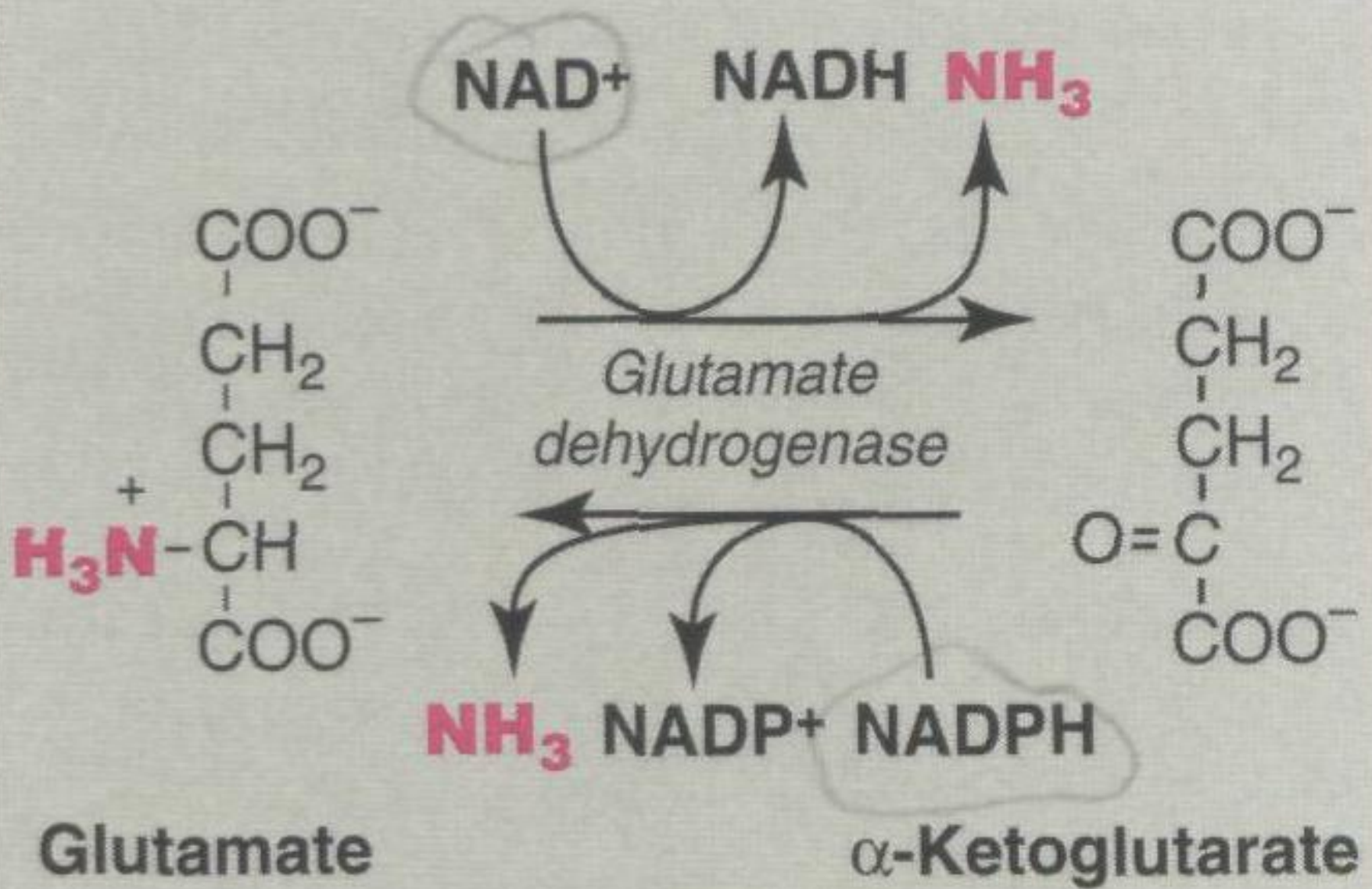
Oxidative deamination

- These reactions occur primarily in the liver and kidney
- Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, catalyzed by **glutamate dehydrogenase**
- The sequential action of transamination and the subsequent oxidative deamination provides a pathway whereby amino groups of most amino acids can be released as free ammonia

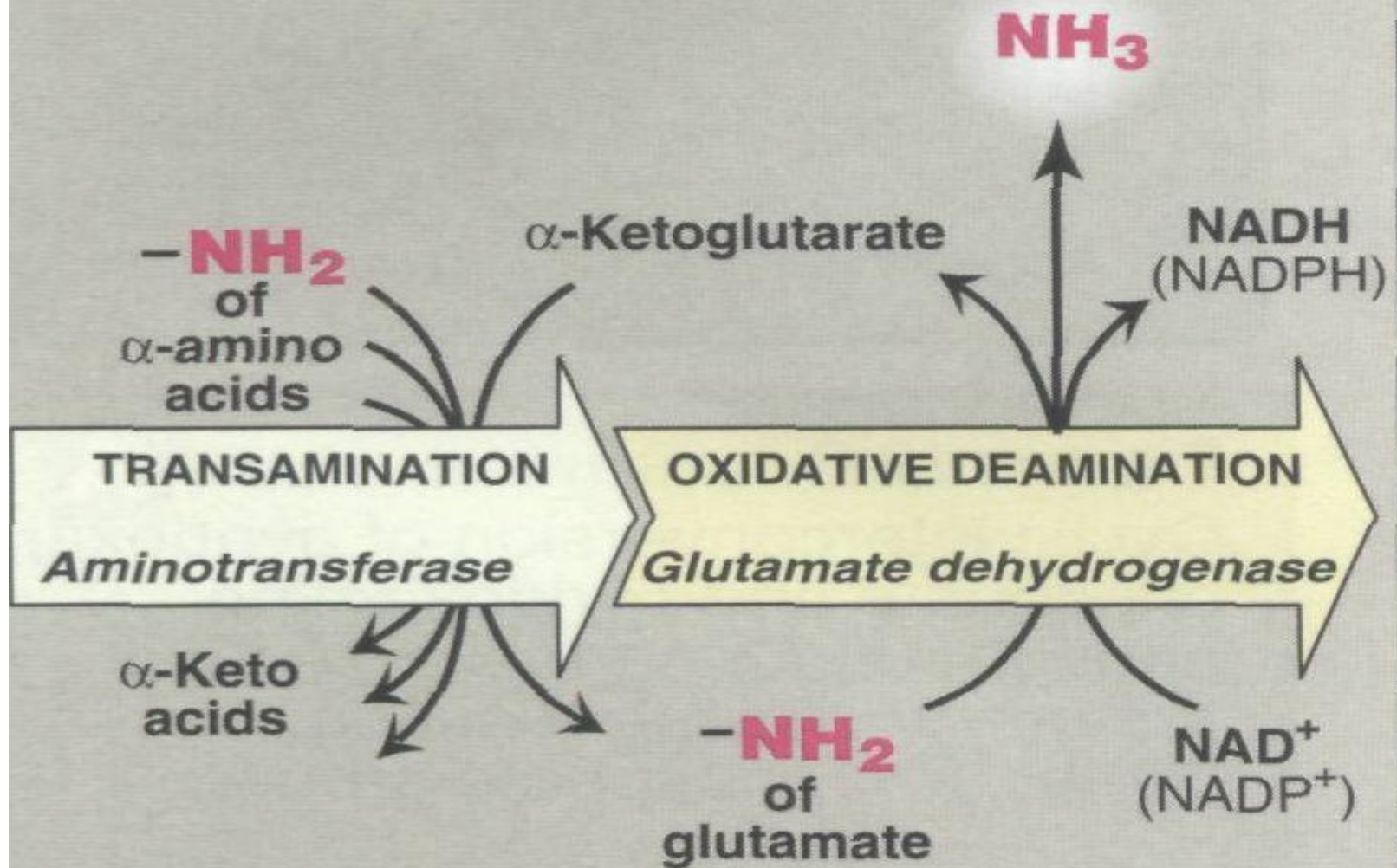
- Glutamate dehydrogenase can use either NAD^+ or NADP^+ as a cofactor.
- NAD^+ is used primarily in oxidative deamination and NADPH is used in reductive amination
- **Allosteric regulators:**
- ATP and GTP are allosteric inhibitors of glutamate dehydrogenase whereas ADP and GDP are activators of the enzyme

The Glutamate Dehydrogenase Reaction

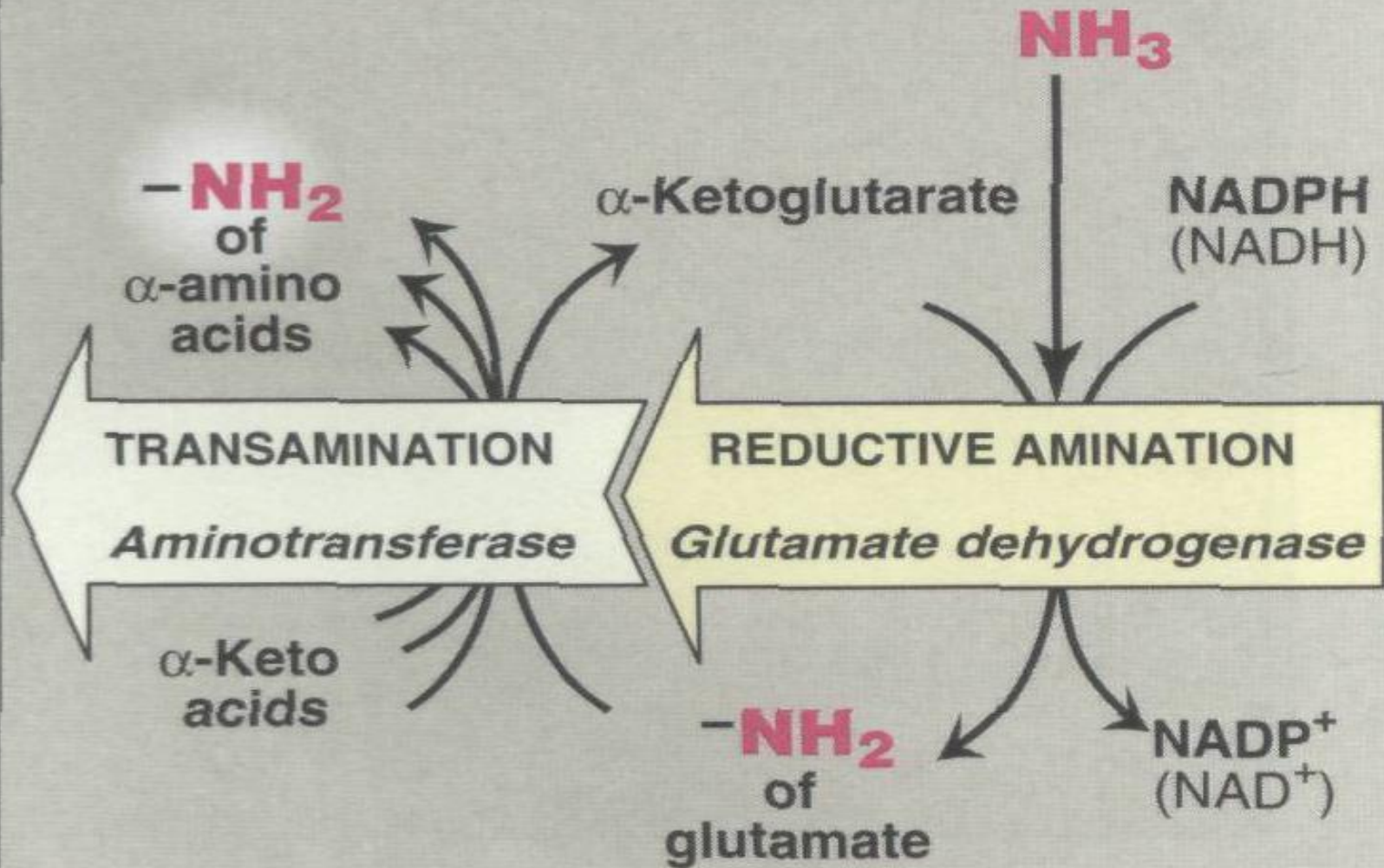




A Disposal of amino acids



B Synthesis of amino acids



D-amino acid oxidase

- D-amino acids are found in plants and cell walls of microorganisms but are not used in the synthesis of mammalian proteins
- They are, however, present in the diet and are efficiently metabolized by the liver
- D-amino acid oxidase is an FAD dependent enzyme that catalyses oxidative deamination of these amino acid isomers

- Resulting α -keto acids can enter the general pathways of amino acid metabolism, and be reaminated to L-isomers or catabolized for energy

Non-oxidative deamination

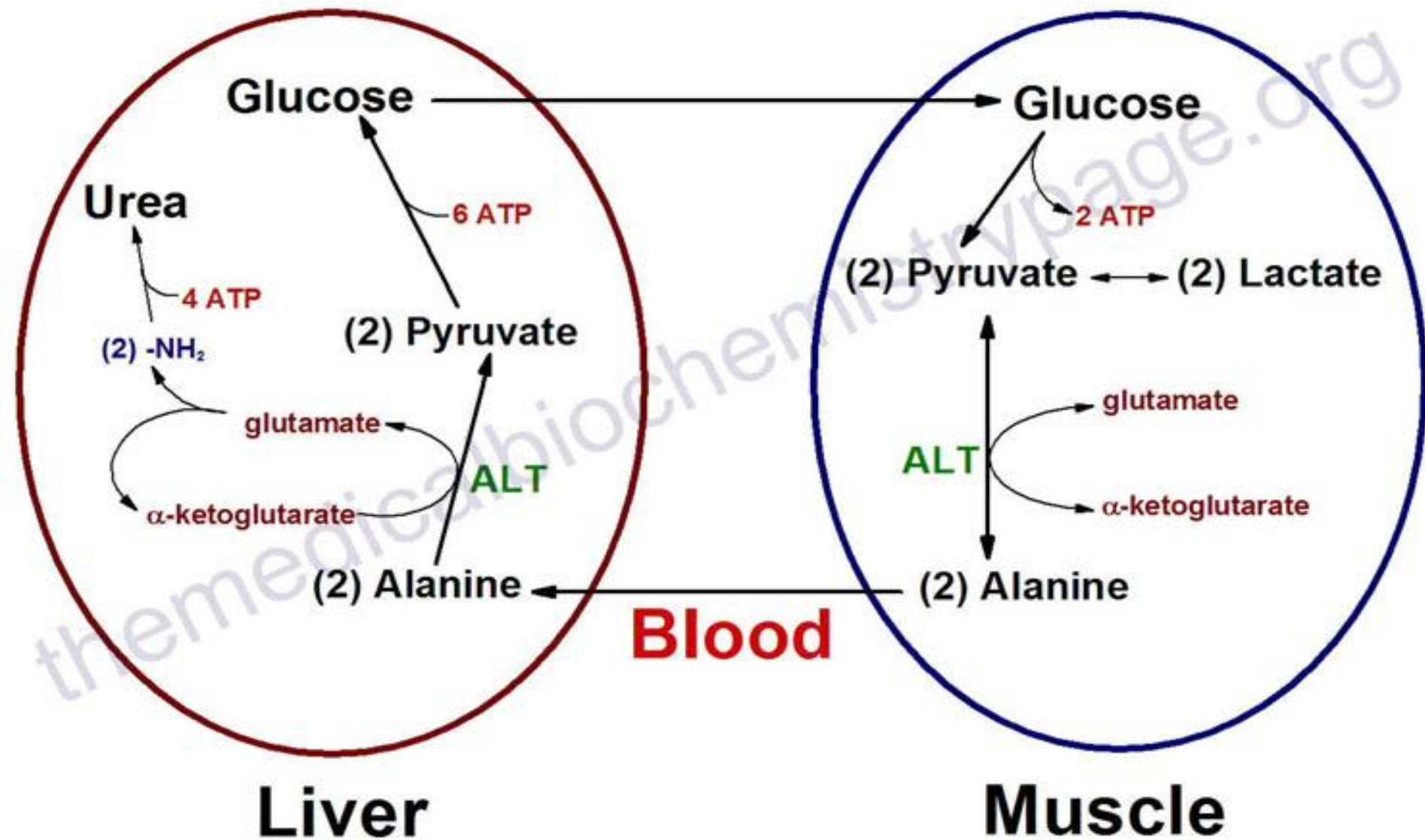
- Certain amino acids e.g serine, threonine and cysteine are deaminated by specific lyases that require pyridoxal phosphate
- These enzymes release ammonia into the medium
- **Examples:**
- Serine \rightarrow pyruvate + NH_3 + H_2O
- Histidine \rightarrow urocanate + NH_3

Transport of ammonia to the liver

- Two mechanisms are available in humans
- The first, found in most tissues, uses **glutamine synthetase** to combine ammonia with glutamate to form glutamine - a non-toxic transport form of ammonia
- Glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia

- The second mechanism used primarily by the muscle, involves transamination of pyruvate to form alanine.
- Alanine is transported by the blood to the liver where it is converted to pyruvate, again by transamination
- In the liver gluconeogenesis can use the pyruvate to synthesize glucose, which can enter the blood and be used by muscle
- This pathway is called **glucose-alanine cycle**

Glucose-Alanine Cycle



UREA CYCLE

- Urea is the major disposal form of amino groups from amino acids and accounts for 90% of nitrogen-containing components of urine
- One nitrogen of the urea molecule is supplied by free ammonia, and the other nitrogen by aspartate
- The carbon and oxygen of urea are derived from CO_2

- Urea is produced by the **liver**, and then is transported in the blood to the kidney for ultimate excretion in the urine
- **Reactions of the cycle:**
- The first two reactions occur in the mitochondria
- The remaining cycle enzymes are located in the cytosol

- **1. Formation of carbamoyl phosphate (CP):**
- Formation of CP by **carbamoyl phosphate synthetase I** is driven by the cleavage of two molecules of ATP
- Ammonia incorporated into CP is provided primarily by oxidative deamination of glutamate by mitochondrial glutamate dehydrogenase

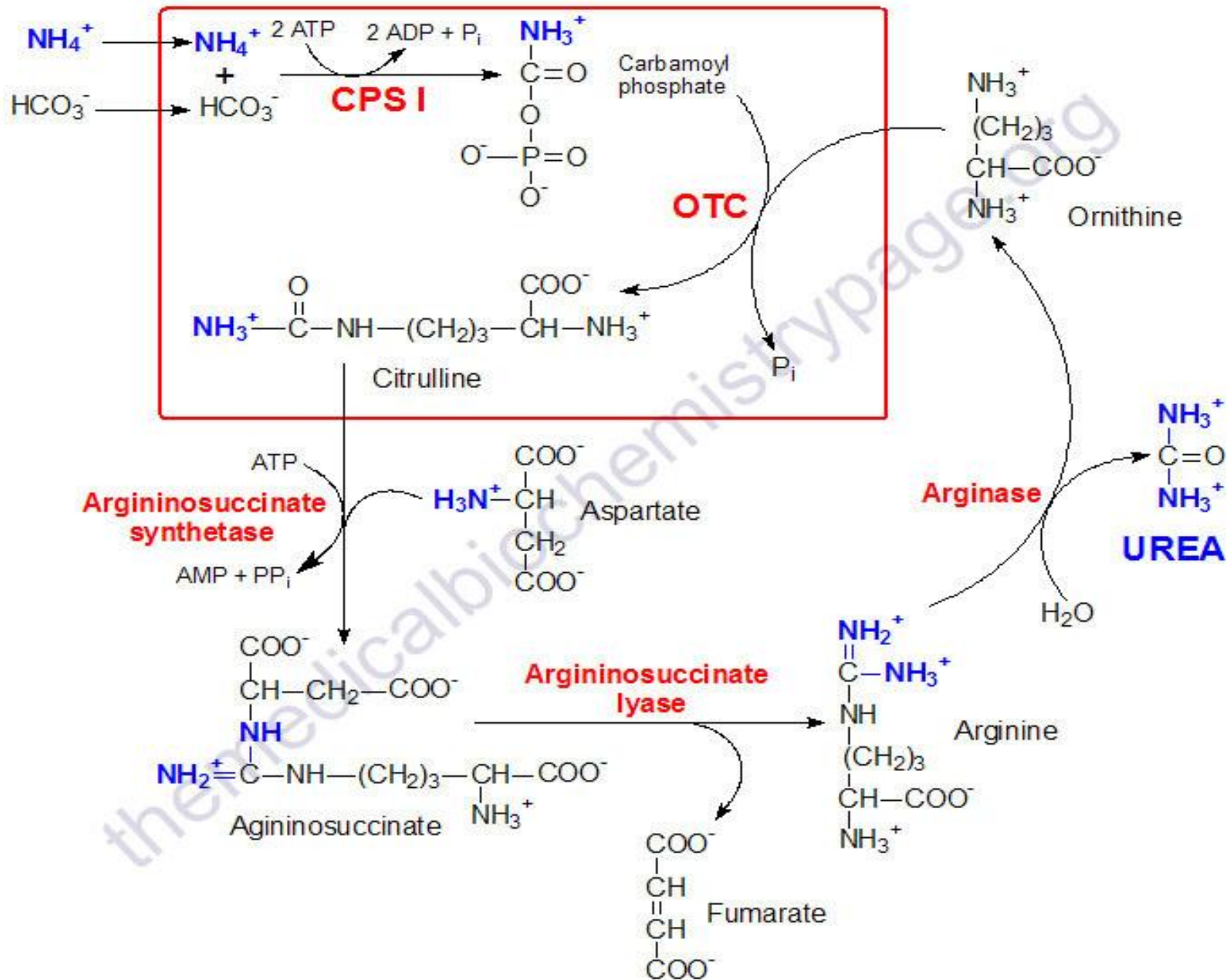
- Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of the urea molecule
- CP synthetase requires N-acetylglutamate as a positive allosteric activator
- **2. Formation of citrulline:**
- Ornithine and citrulline are basic amino acids that participate in the urea cycle but are not incorporated into cellular proteins

- Ornithine is regenerated with each turn of the urea cycle
- The release of the high-energy phosphate of CP as P_i drives the reaction in the forward direction
- The reaction is catalyzed by **ornithine transcarbamoylase**
- The reaction product, citrulline, is transported to the cytosol

- **3. Synthesis of argininosuccinate (AS):**
- citrulline condenses with aspartate to form AS, catalyzed by **argininosuccinate synthase**
- The α -amino group of aspartate provides the second nitrogen that is ultimately incorporated in to the urea
- The formation of AS is driven by the cleavage of ATP to AMP and PPi

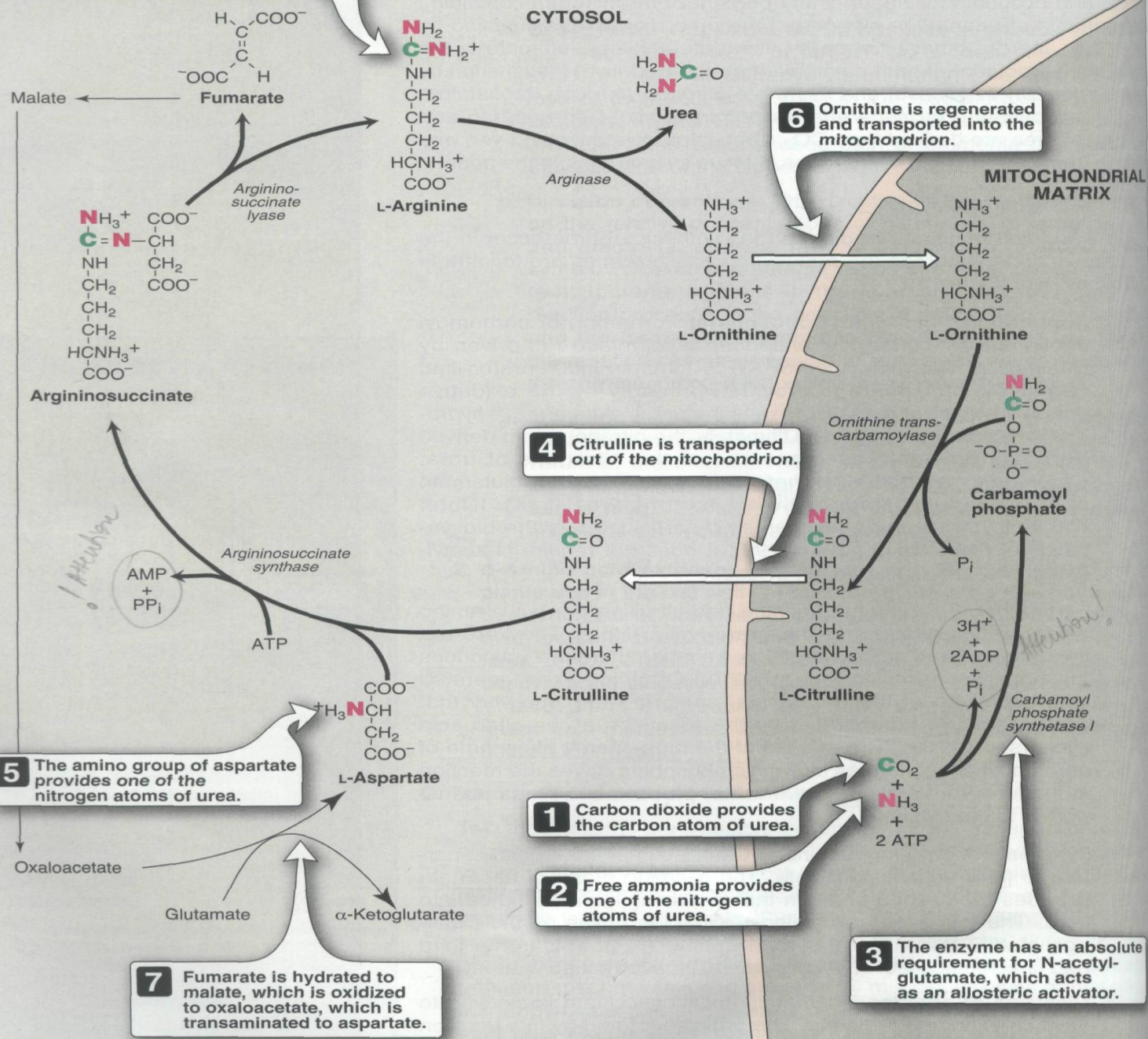
- **4. Cleavage of argininosuccinate:**
- Is cleaved by **argininosuccinate lyase** to yield arginine and fumarate
- Arginine formed by this reaction serves as the immediate precursor of urea
- Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways,

- e.g malate can be transported into the mitochondria and re-enter the TCA cycle. Alternatively cytosolic malate can be oxidized to OAA, which can be converted to aspartate or glucose
- **5. Cleavage of arginine to ornithine and urea:**
- **Arginase** cleaves arginine to ornithine and urea
- Arginase occurs almost exclusively in the liver



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Tissues in addition to the liver use this pathway to make arginine.



Fate of urea

- Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine
- A portion of urea diffuses from the blood into the intestine, and is cleaved to CO_2 and NH_3 by bacterial **urease**
- This ammonia is partly lost in the feces, and is partly reabsorbed into the blood

Overall stoichiometry of the urea cycle

- Aspartate + NH_3 + CO_2 + 3ATP \rightarrow urea + fumarate + 2ADP + AMP + 2Pi + PPi + $3\text{H}_2\text{O}$
- Four high-energy phosphates are consumed in the synthesis of each molecule of urea
- Therefore, the synthesis of urea is irreversible, with large negative ΔG

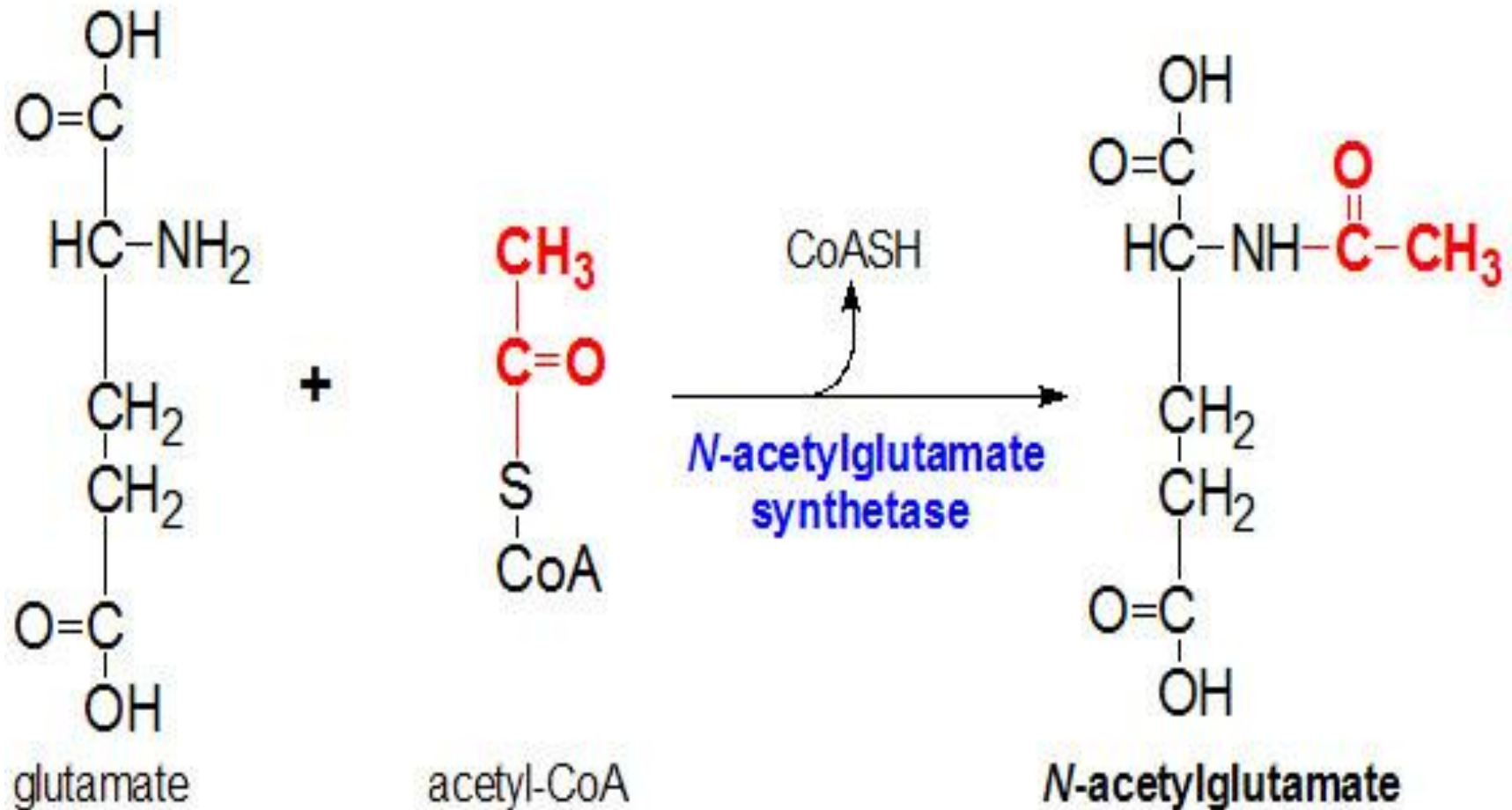
- In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut
- The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients
- Oral administration of **neomycin** reduces the number of intestinal bacteria responsible for this NH_3 production

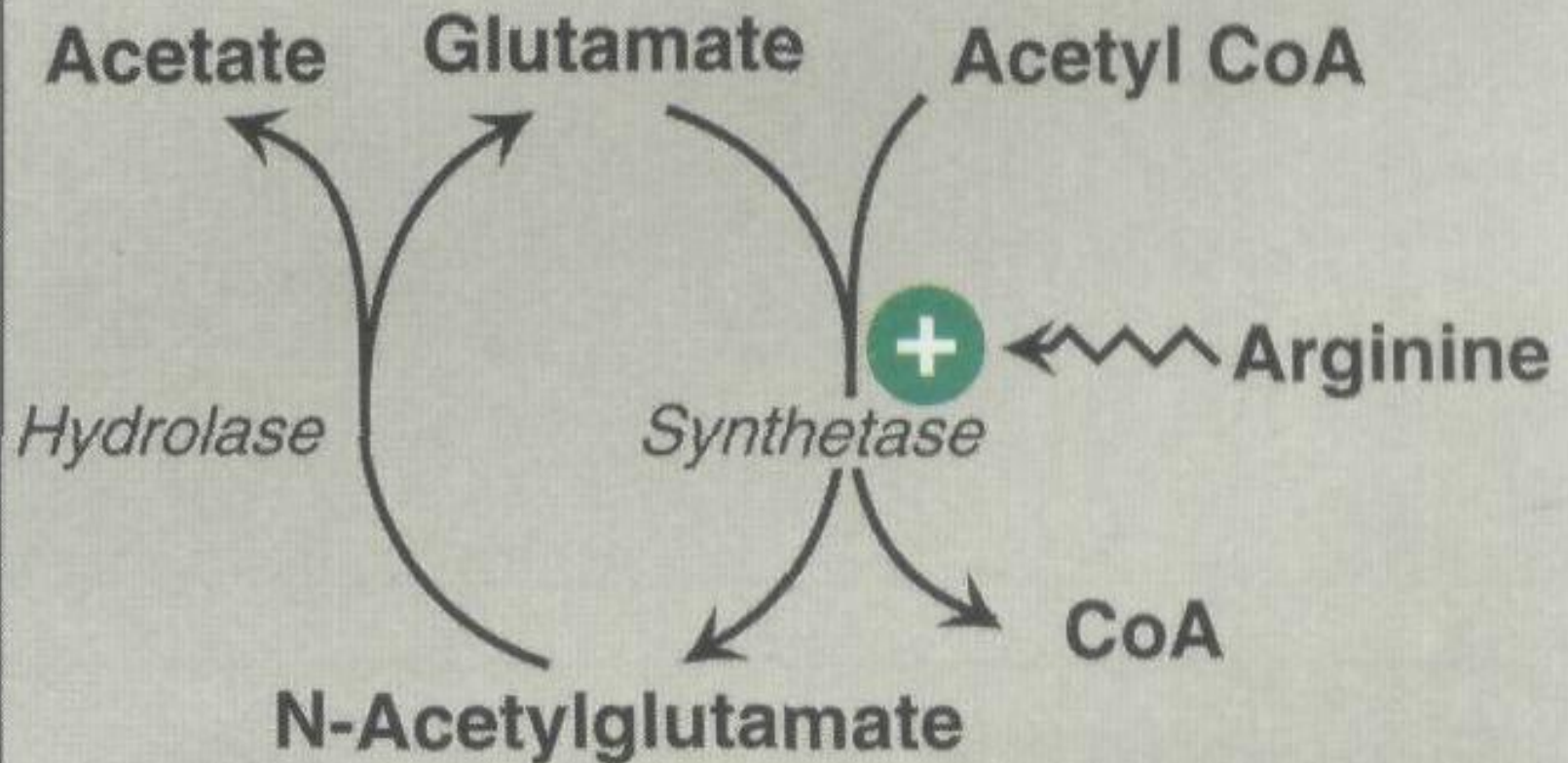
- One nitrogen of the urea molecule is supplied by free NH_3 , and the nitrogen by aspartate
- Glutamate is the immediate precursor of both NH_3 (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of OAA by aspartate aminotransferase)
- Glutamate, in turn, gathers nitrogen from other amino acids

Regulation of the urea cycle

- **N-Acetylglutamate** is an essential activator for carbamoylphosphate synthase – the rate limiting step in the urea cycle
- N-Acetylglutamate is synthesized from acetyl CoA and glutamate in a reaction for which arginine is an activator
- Therefore, the intrahepatic concentration of N-Acetylglutamate increases after ingestion of a protein-rich meal

Regulation of the Urea Cycle





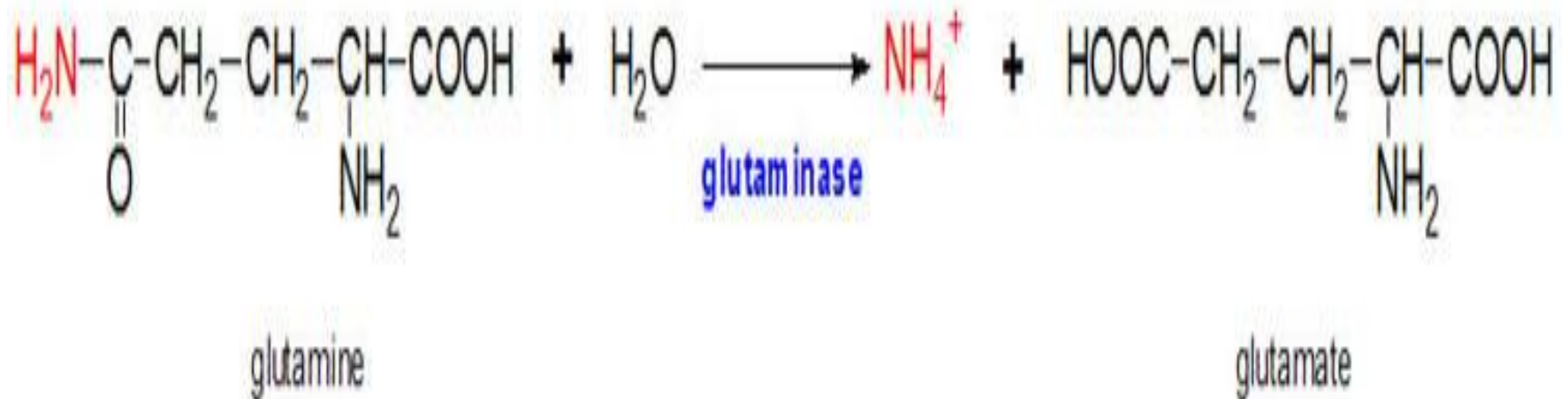
Metabolism of ammonia

- Ammonia is produced by all tissues during the metabolism of the variety of compounds
- However, the level of ammonia in the blood must be kept very low because even slightly elevated concentrations (**hyperammonemia**) are toxic to the central nervous system (CNS)

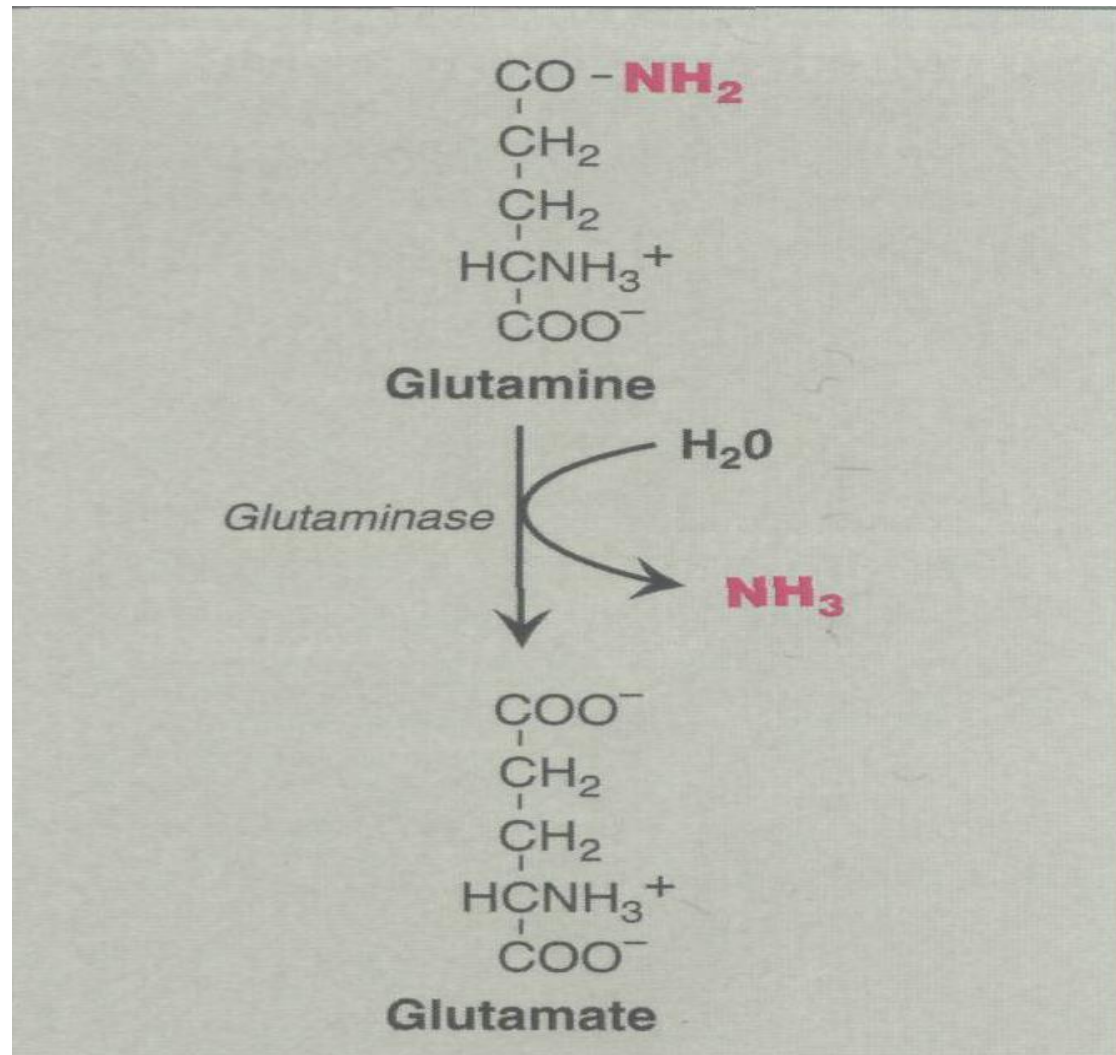
Sources of ammonia

- Amino acids are quantitatively the most important source of ammonia
- 1) **From amino acids:** Many tissues, particularly the liver, form ammonia from amino acids by aminotransferase and glutamate dehydrogenase reactions
- 2) **From glutamine:** the kidneys form ammonia from glutamine by the action of renal glutaminase

Hydrolysis of Glutamine to form Ammonia



Hydrolysis of glutamine to form ammonia



- Most of this ammonia is excreted into the urine as NH_4^+ , which is an important mechanism for maintaining body's acid-base balance
- Ammonia is also obtained from the hydrolysis of glutamine by intestinal glutaminase
- The intestinal mucosal cells obtain glutamine either from the blood or from digestion of dietary protein

- **3) From bacterial action in the intestine:**
Formed from urea by the action of bacterial urease in the lumen of the intestine
- This ammonia is absorbed from the intestine by way of the portal vein
- **4) From amines:** Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of **amine oxidase**

- **5) From purines and pyrimidines:** In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia

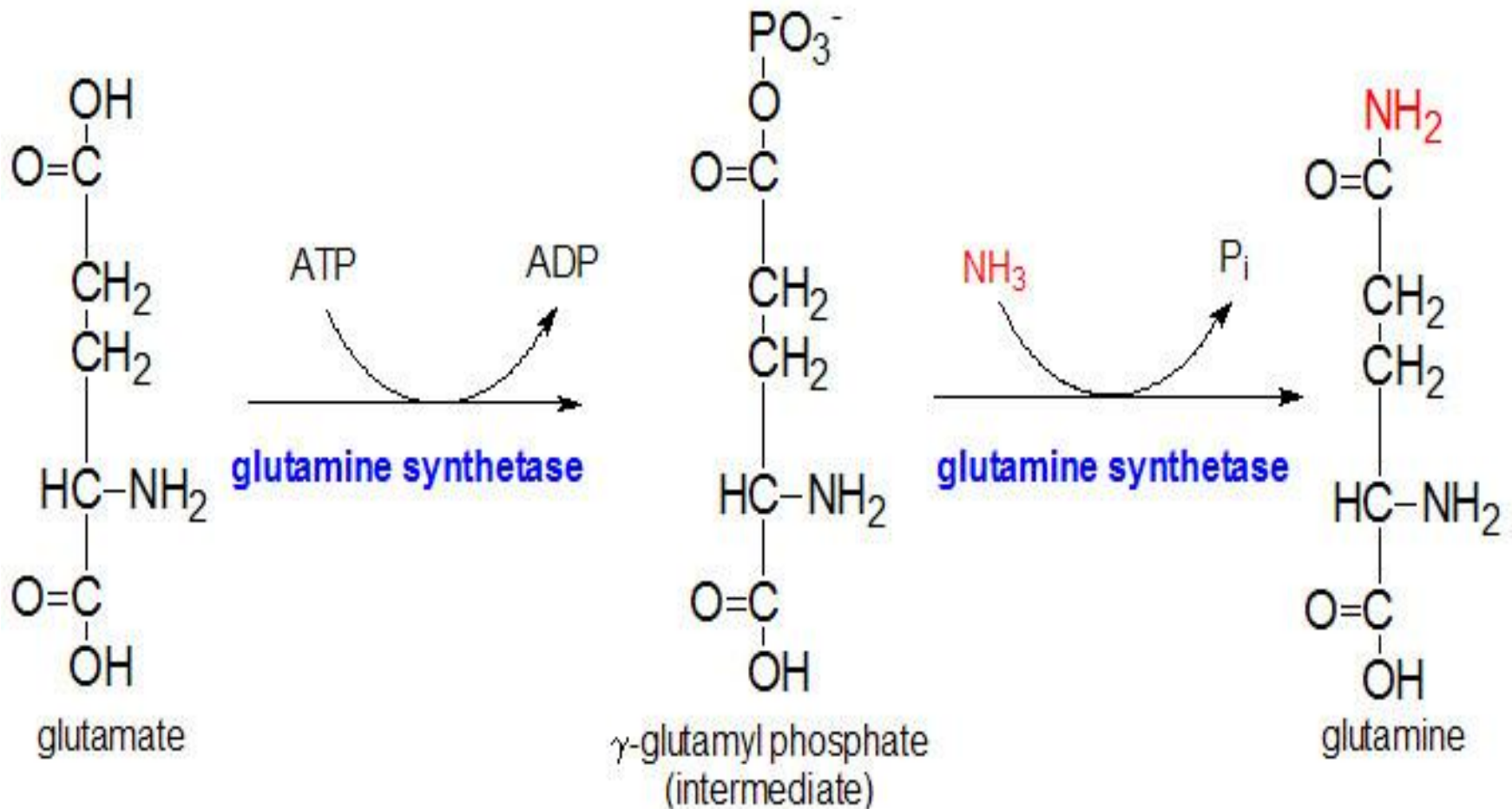
Transport of ammonia in the circulation

- Although ammonia is constantly produced in the tissues, it is present at very low levels in blood
- This is due to both, the rapid removal of blood ammonia by the liver, and the fact that many tissues, particularly muscles, remove amino acid nitrogen in the form of glutamine or alanine, rather than as free ammonia

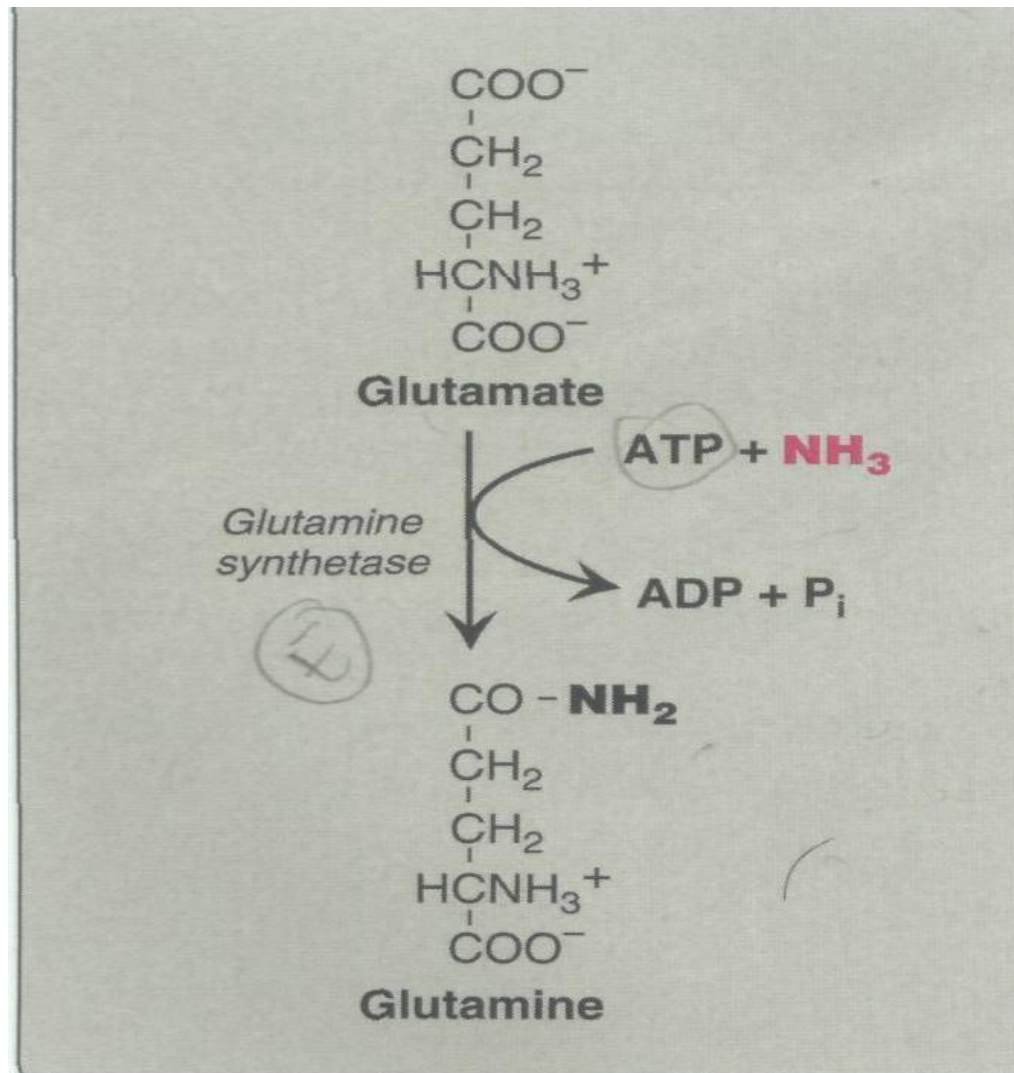
- 1) **Urea**: Formation of urea in the liver is quantitatively the most important disposal route for ammonia
- 2) **Glutamine**: provides nontoxic storage and transport form of ammonia
- Glutamate + ATP + NH_4^+ \rightarrow Glutamine + ADP + Pi

- Formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in the muscle and liver, but is also important in the nervous system, where it is the major mechanism for the removal of ammonia in the brain
- Circulating glutamine is removed by the kidneys and deaminated by glutaminase

The Glutamine Synthetase Reaction



Synthesis of glutamine



Hyperammonemia

- The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low (5 to 50 $\mu\text{mol/L}$)
- When the liver function is compromised, due either to genetic defects of the urea cycle, or liver disease, blood levels can rise above 1000 $\mu\text{mol/L}$

- Ammonia has a direct neurotoxic effect on the CNS
- Elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence, vomiting, cerebral edema and blurring of vision
- At high concentrations, ammonia can cause coma and death

- The two major types of hyperammonemia are:
- 1) **Acquired hyperammonemia**: Liver disease is a common cause in adults
- It may be a result of acute process, e.g, viral hepatitis, ischemia or hepatotoxins
- Cirrhosis of the liver caused by alcoholism, hepatitis or biliary obstruction may result in formation of collateral circulation around the liver

- As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver
- The detoxification of ammonia is therefore, severely impaired
- 2) **Hereditary hyperammonemia**: Genetic deficiencies of each of the five enzymes of the urea cycle
- Ornithine transcarbamoylase deficiency is the most common of these disorders

UCD	Enzyme Deficiency	Symptoms/Comments
Type I Hyperammonemia , CPSD	Carbamoylphosphate synthetase I	with 24h–72h after birth infant becomes lethargic, needs stimulation to feed, vomiting, increasing lethargy, hypothermia and hyperventilation; without measurement of serum ammonia levels and appropriate intervention infant will die: treatment with arginine which activates <i>N</i> -acetylglutamate synthetase
N-acetylglutamate synthetase Deficiency	<i>N</i> -acetylglutamate synthetase	severe hyperammonemia, mild hyperammonemia associated with deep coma, acidosis, recurrent diarrhea, ataxia, hypoglycemia, hyperornithinemia: treatment includes administration of carbamoyl glutamate to activate CPS I
Type 2 Hyperammonemia , OTCD	Ornithine transcarbamoylase	most commonly occurring UCD, only X-linked UCD, ammonia and amino acids elevated in serum, increased serum orotic acid due to mitochondrial carbamoylphosphate entering cytosol and being incorporated into pyrimidine nucleotides which leads to excess production and consequently excess catabolic products: treat with high carbohydrate, low protein diet, ammonia detoxification with sodium phenylacetate or sodium benzoate
Classic Citrullinemia , ASD	Argininosuccinate synthetase	episodic hyperammonemia, vomiting, lethargy, ataxia, seizures, eventual coma: treat with arginine administration to enhance citrulline excretion, also with sodium benzoate for ammonia detoxification
Argininosuccinic aciduria , ALD	Argininosuccinate lyase (argininosuccinase)	episodic symptoms similar to classic citrullinemia, elevated plasma and cerebral spinal fluid argininosuccinate: treat with arginine and sodium benzoate
Hyperargininemia , AD	Arginase	rare UCD, progressive spastic quadriplegia and mental retardation, ammonia and arginine high in cerebral spinal fluid and serum, arginine, lysine and ornithine high in urine: treatment includes diet of essential amino acids excluding arginine, low protein diet

- In each case, the failure to synthesis urea leads to hyperammonemia during the first weeks following birth
- All inherited deficiencies of the urea cycle enzymes result in mental retardation
- Treatment includes limiting protein in the diet, and administering compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine

- For example, phenylbutyrate given orally is converted to phenylacetate
- This condenses with glutamine to form phenylacetylglutamine, which is excreted

NEUROTOXICITY ASSOCIATED WITH AMMONIA

- Marked brain damage is seen in cases of failure to make urea via the urea cycle or to eliminate urea through the kidneys
- The result is a buildup of circulating levels of ammonia
- Aside from its effect on blood pH, ammonia readily traverses the brain blood barrier

- In the brain it is converted to glutamate via glutamate dehydrogenase, depleting the brain of α -KG
- As the α -KG is depleted, OAA falls correspondingly and ultimately TCA cycle activity comes to a halt
- In the absence of oxidative phosphorylation and TCA cycle activity, irreparable cell damage and neural cell death ensue

- In addition, increased glutamate leads to glutamine formation
- This depletes glutamate stores needed in neural tissue since glutamate is both a neurotransmitter and a precursor for the synthesis of **γ -aminobutyrate (GABA)**, another neurotransmitter

- Therefore, reductions in brain glutamate affect energy production as well as neurotransmission