Learning Objectives

- Ammonia metabolism
- Ammonia Intoxication
- Biosynthesis of Urea cycle
- Disorders related to Urea cycle

Introduction

- Ammonia is produced by all tissues during the metabolism of variety of compounds and is it disposed by formation of urea in the liver
- Blood ammonia level must be low because even slight elevation leads hyperammonemia (toxic to CNS).
- Normal blood ammonia is 30-60µM
- Therefore, mechanism required for transport of nitrogen from peripheral tissues to liver for disposal as urea

Sources/Formation of Ammonia

From glutamine

- Catabolism of branched-chain aa in skeletal muscle

- This glutamine is taken up by cells of intestine, liver , and kidney

- Liver and kidneys generate ammonia from glutamine by glutaminase and glutamate dehydrogenase

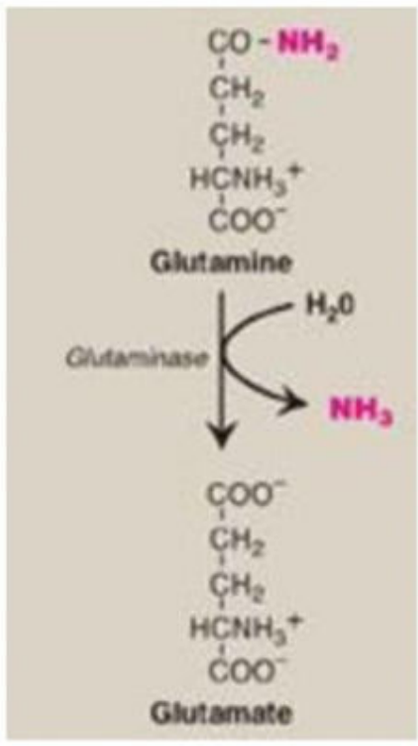

Fig 19.17. Lippincott's Illustrated Reviews, Biochemistry, 6th Ed

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- In kidneys, most of this ammonia is excreted into urine as $NH4_+$, provides an important mechanism for maintaining body's acid-base balance through excretion of protons

- In liver, ammonia is detoxified to urea and excreted

From bacterial action in intestine

- Ammonia is formed from urea by bacterial urease in lumen of intestine

- This ammonia is absorbed from intestine by way of portal vein, and all is removed by liver via conversion to urea

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From amines

Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of monoamine oxidase

From purines and pyrimidines

In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia

Glutamine Transport Ammonia in Bloodstream

- Free ammonia produced in tissues is combined with glutamate to yield glutamine by glutamine synthetase and it requires ATP
- Glutamate and ATP react to form ADP and γ-glutamyl phosphate intermediate which reacts with ammonia to produce glutamine and inorganic phosphate (Pi).

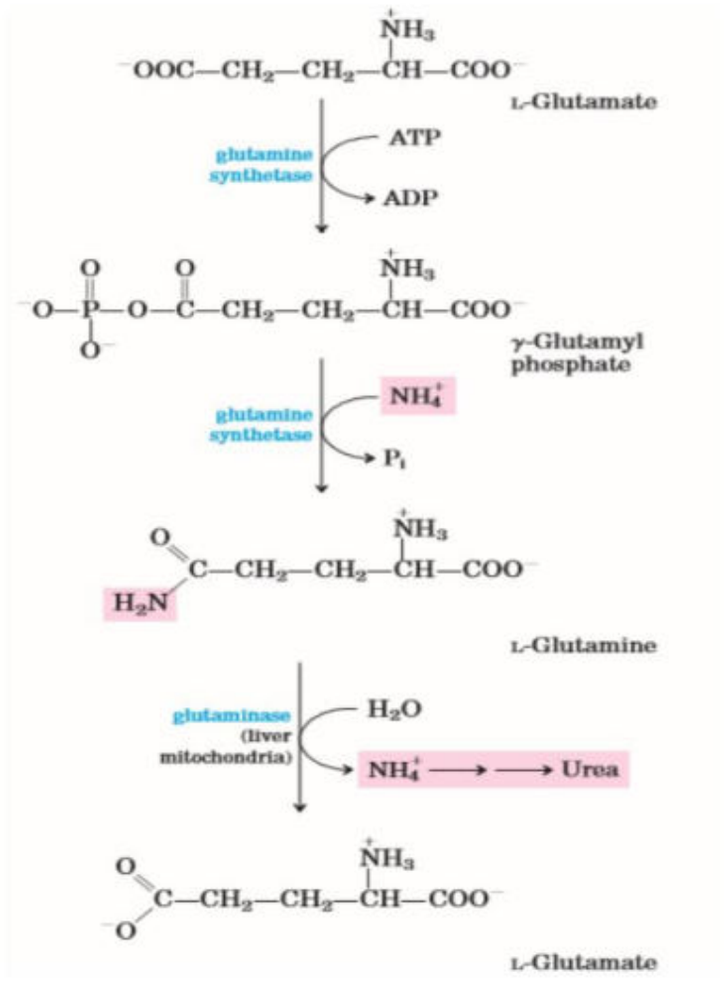

Fig18.8: Lehninger Principles of Biochemistry by David L Nelson

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- Glutamine is a nontoxic transport form of ammonia
- In microorganisms, the glutamine synthetase serves as an essential portal for the entry of fixed nitrogen into biological systems

Glucose-Alanine Cycle: Alanine transport ammonia from skeletal muscle to liver

Two mechanisms are available in humans for transport of ammonia from peripheral tissues to liver for its ultimate conversion to urea

1) Combine ammonia with glutamate to form glutamine (nontoxic transport form of ammonia) by glutamine synthetase

- Glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia which is converted to urea

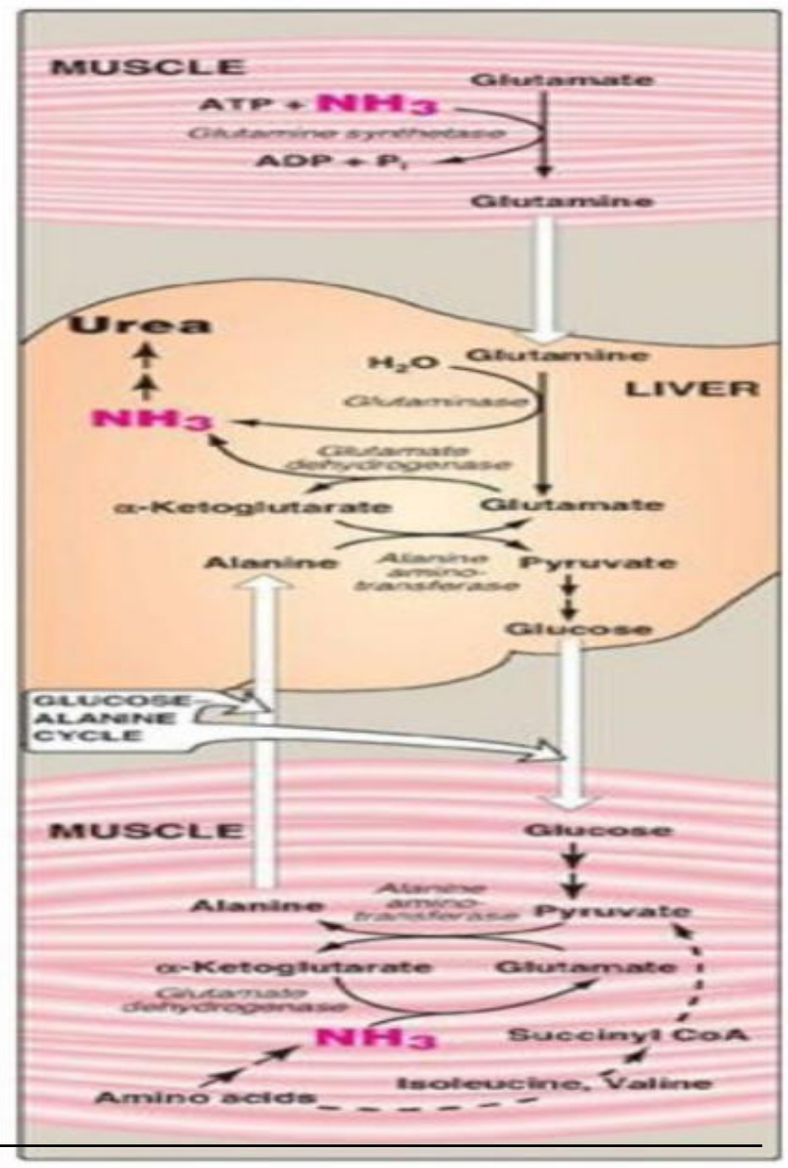

Fig 19.13. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.
Lippincott's Illustrated Reviews, Biochemistry, 6th Ed

2) Formation of alanine by transamination of pyruvate produced from both aerobic glycolysis and metabolism of succinyl CoA generated by catabolism of branched-chain aa isoleucine and valine

- Alanine is transported by blood to liver, where it is converted to pyruvate by transamination
- Pyruvate is used to synthesize glucose, which can enter blood and be used by muscle

Ammonia Metabolism

- Formation of urea in the liver is quantitatively the most important disposal route for ammonia
- Urea travels in blood from liver to kidneys, where it passes into the glomerular filtrate
- Glutamine provides a nontoxic storage and transport form of ammonia

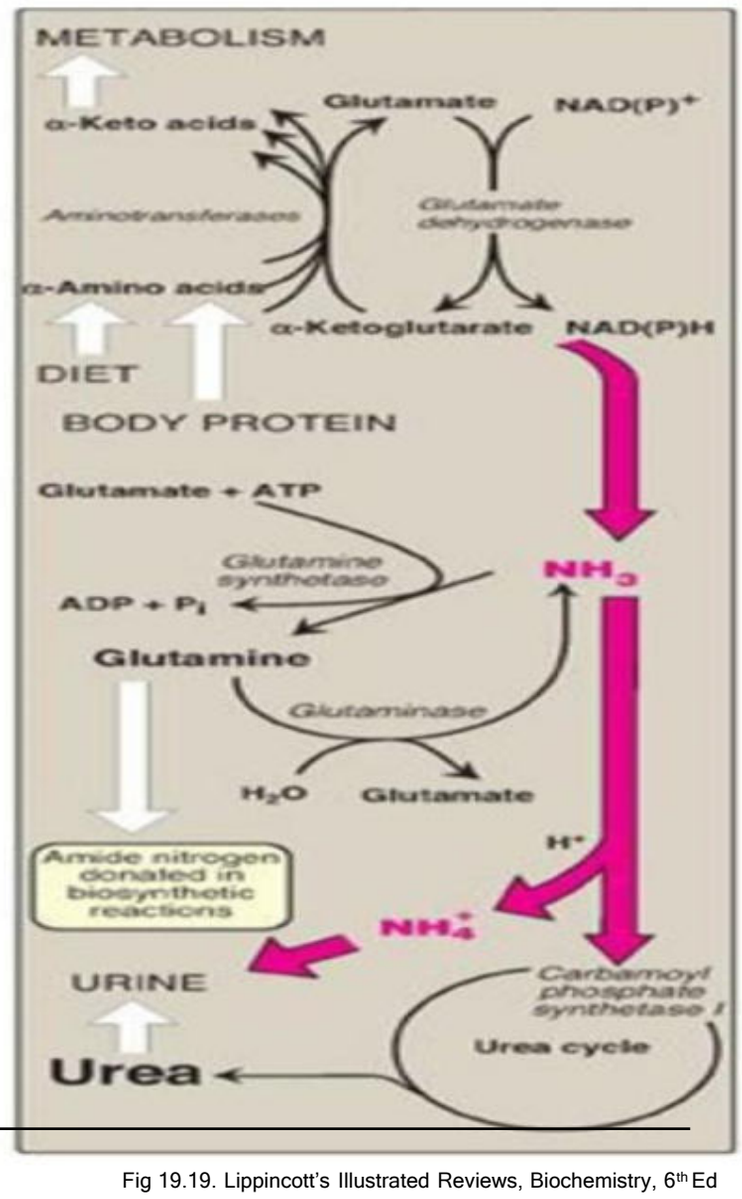

Fig 19.19. Lippincott's Illustrated Reviews, Biochemistry, 6th Ed

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- The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs in skeletal muscle and liver

- Its imp. in central nervous system (CNS), where it is major mechanism for removal of ammonia in the brain

Ammonia Intoxication

- Ammonia produced by enteric bacteria and absorbed into portal venous blood and ammonia produced by tissues are rapidly removed from circulation by liver and converted to urea

- Only traces (10-20 µg/dL) normally are present in peripheral blood

- This is essential, since ammonia is toxic to CNS

- Should portal blood bypass liver, systemic blood ammonia may attain toxic levels

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- This occurs in severely impaired hepatic function or development of collateral links between portal and systemic veins in cirrhosis
- Symptoms of ammonia intoxication include tremor, slurred speech, blurred vision, coma, and ultimately death
- Ammonia may be toxic to brain in part because it reacts with α-ketoglutarate to form glutamate
- Resulting depletion of levels of α-ketoglutarate then impairs function of tricarboxylic acid (TCA) cycle in neurons.

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Hyperammonemia: Elevated levels of ammonia in blood cause symptoms of ammonia intoxication, which include tremor, slurring of speech, and blurring of vision

- High levels result in coma and death
- There are two types of conditions: Acquired and Hereditary

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Acquired: Cirrhosis of liver may result in formation of collateral circulation around liver

- As a result, portal blood is shunted directly into systemic circulation and does not have access to liver
- Therefore, conversion of ammonia to urea is severely impaired, leading to elevated levels of ammonia.

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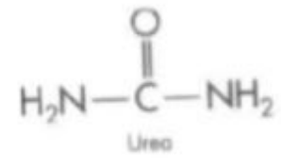
Hereditary: Genetic deficiency of each of five enzymes in urea cycle pathway

- X-linked ornithine transcarbamoylase/ornithine carbomyl transferase deficiency is common of these disorders
- In each case, failure to synthesize urea leads to hyperammonemia during first weeks of birth
- Treatment included restriction of dietary protein in presence of sufficient calories to prevent catabolism

Urea Cycle

Introduction



- Urea is major disposal form of amino groups derived from aa.
- It accounts for about 86-90% of the nitrogen containing components of the urine
- One nitrogen group of urea is supplied by free NH_3 and the other nitrogen from aspartate

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- Glutamate is the immediate precursor of both ammonia groups by the following reactions:

Transamination: transfer of ammonia to oxaloacetate to form aspartate transaminase (AST)

Oxidative deamination: removal of free ammonia, glutamate dehydrogenase

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- Carbon and oxygen of urea are derived from CO2

- Urea is produced by Liver and then transported in blood to kidneys for excretion in kidneys

- Blood urea level is measured as blood urea nitrogen (BUN). Levels are 8-20 mg/dl

- BUN is low in liver failure and is very high in patients with renal failure in uremia.

Urea Biosynthesis

- Synthesis of 1 mol of urea requires 3 mol of ATP, 1 mol each of NH_4^+ and of aspartate, and five enzymes

- Of the six participating aa, N-acetylglutamate as an enzyme activator, others serve as carriers of atoms that become urea

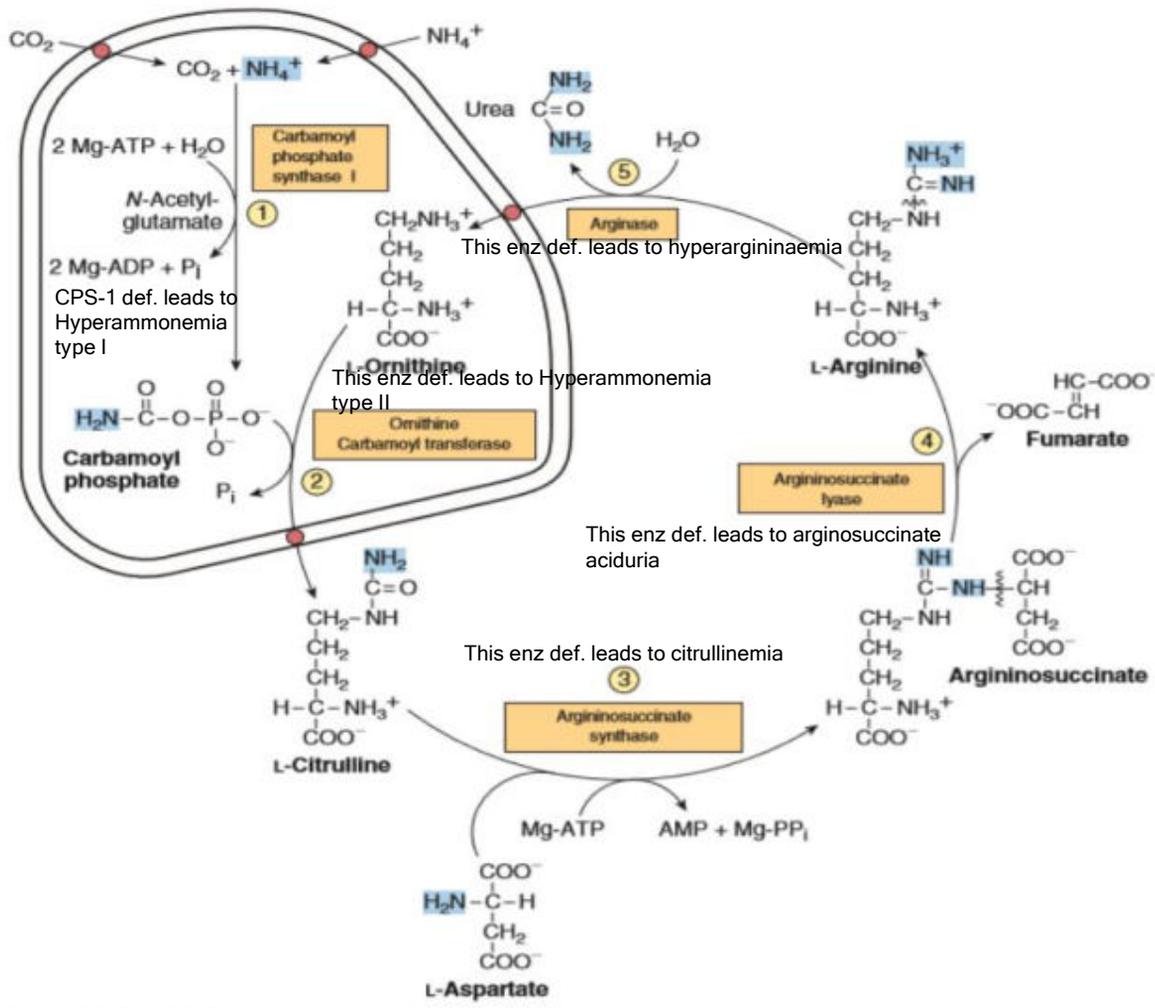

FIGURE 28–16 Reactions and intermediates of urea biosynthesis. The nitrogen-containing groups that contribute to the formation of urea are shaded. Reactions ① and ② occur in the matrix of liver mitochondria and reactions ③, ④, and ⑤ in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers (see red dots) present in the inner membrane of liver mitochondria.

Fig 28.16. Harper's Illustrated Biochemistry 30th Edition

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- While ammonium ion, CO2, ATP, and aspartate are consumed, the ornithine consumed in reaction 2 is regenerated in reaction 5

- Thus is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine

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- Ammonia, which is very toxic in humans, is converted to urea, which is nontoxic, very soluble, and readily excreted by the kidneys

- The enzymes of the urea cycle are induced if a high-protein diet is consumed for several days

- When the nitrogen of aa is converted to urea in the liver, their carbon skeletons are converted either to glucose (in the fasting state) or to fatty acids (in the fed state)

Regulation of the urea cycle

- Carbamoyl phosphate synthetase I (CPS-I), is allosterically activated by N-Acetylglutamate

- Steady-state levels of N-acetylglutamate are determined by concentrations of glutamate and acetyl-CoA and arginine

- Arginine is an activator of N-acetylglutamate synthase, and thus an activator of the urea cycle

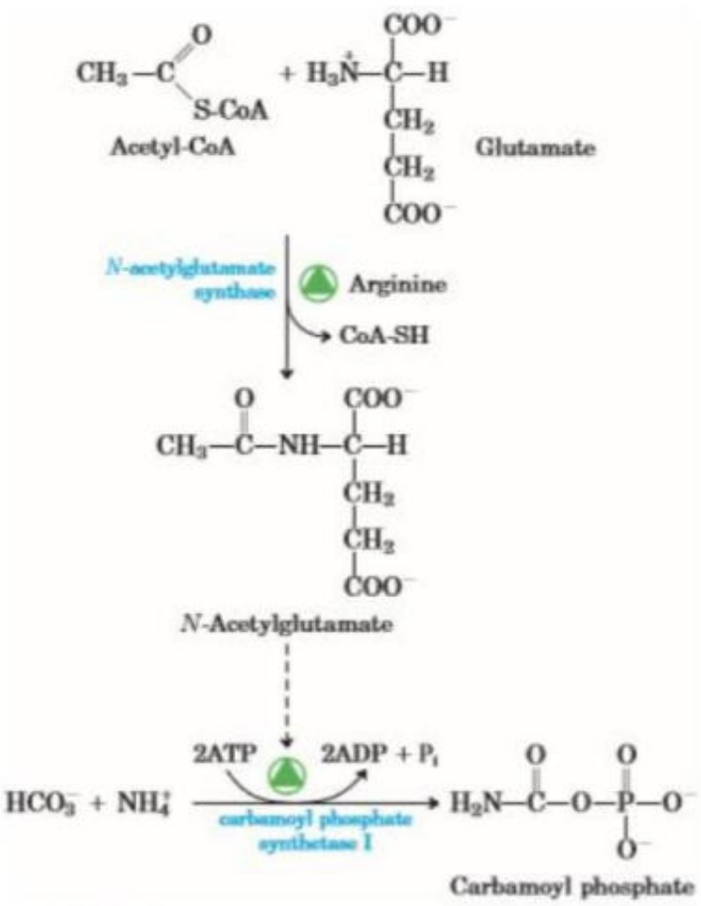

FIGURE 18–13 Synthesis of *N*-acetylglutamate and its activation of carbamoyl phosphate synthetase I.

Fig18.13: Lehninger Principles of Biochemistry by David L Nelson

Disorders of Urea cycle

- Urea cycle disorders are characterized by hyperammonemia, encephalopathy, and respiratory alkalosis

- Deficiencies of CPS-1, ornithine carbamoyl transferase, argininosuccinate synthase, and argininosuccinate lyase, result in accumulation of precursors of urea, principally ammonia and glutamine

- Ammonia intoxication is most severe when metabolic block occurs at reactions 1 or 2 of urea cycle

- Leads to feeding difficulties, vomiting ataxia, lethargy, irritability, poor intellectual development

Cont--

- Hyperammonemia Type I: Deficiency of CPS-1, infants die in the neonatal period

- Hyperammonemia Type II: Deficiency of ornithine transcarbamoylase, Levels of glutamine are elevated in blood, cerebrospinal fluid, and urine, result of enhanced glutamine synthesis in response to elevated levels of tissue ammonia

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Citrullinemia: Patients who lack detectable argininosuccinate synthase activity, citrulline levels elevated

- Plasma and cerebrospinal fluid citrulline levels are elevated, and 1 to 2 g of citrulline are excreted daily.

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Argininosuccinic aciduria: The metabolic defect is in argininosuccinate lyase

- Elevated levels of argininosuccinate in blood, CSF, and urine, is associated with friable, tufted hair.

- Diagnosis by the measurement of erythrocyte argininosuccinate lyase activity can be performed on umbilical cord blood or amniotic fluid cells

Hyperargininemia: is an autosomal recessive defect in the gene for arginase

- Blood and CSF levels of arginine are elevated
- Urinary aa pattern, which resembles that of lysine-cystinuria may reflect competition by arginine with lysine and cysteine for reabsorption in renal tubule

Gene Therapy for Correcting Defects in Urea Biosynthesis:

- Gene therapy for rectification of defects in the enzymes of the urea cycle is an area of active investigation
- Animal models using an adenoviral vector to treat citrullinemia

Reference Books

1) Text Book of Medical Biochemistry by Chatterjee & Rana Shinde, 8th Ed
2) Biochemistry, Lippincott's Illustrated Reviews, 6th Ed
3) Harper's Illustrated Biochemistry-30th Ed
4) Lehninger Principles of Biochemistry

Group Discussion

- Subtopics of previous and today's class discussed in groups.

Thank you