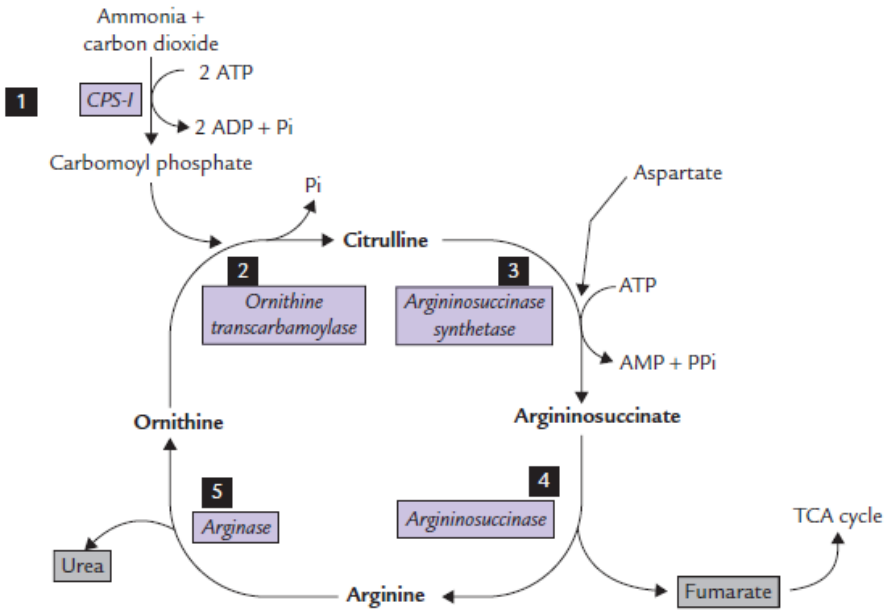
Metabolism of purines and pyrimidines
And Metabolism of purines and pyrimidines disorders

The urea:

The only organ where urea synthesis occurs is **liver**. Urea is the major excretory product in humans, accounting for an average of **86 %** of nitrogen eliminated. The rest of the nitrogen is eliminated as follows: 4.5% by creatinine, 2.8 % as ammonium ions, 1.7% as uric acid, and 5.0% as other compounds. About *30 g urea is excreted per day*; the amount excreted is dependent on protein intake. Higher the protein intake more is the urea synthesis and excretion.

Reactions of Urea Cycle

The sequence of reactions leading to urea synthesis was first proposed by *Krebs* and *Henseleit* in 1932, five years before the elucidation of TCA cycle. Urea cycle was the first cyclic pathway to be identified. All the reactions of this pathway are shown in Figure below *The first two reactions take place in the mitochondria, and the rest occur in cytosol*.



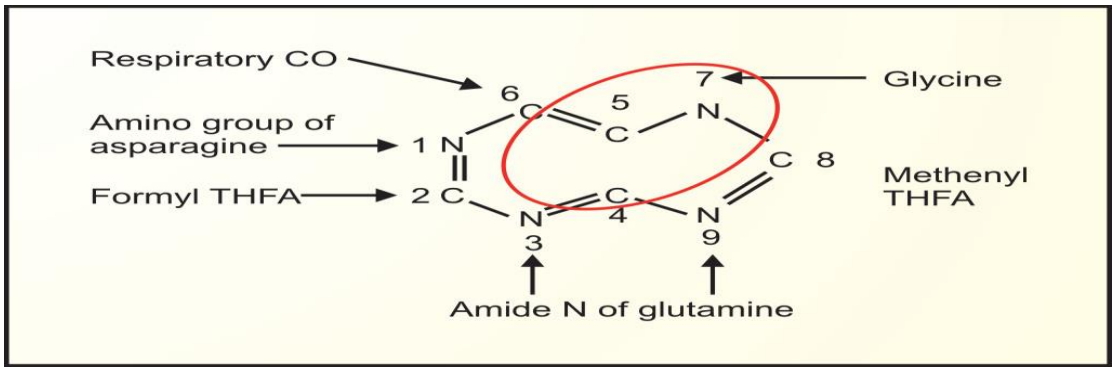
.7. Reactions of the urea cycle.

BIOSYNTHESIS OF PURINE NUCLEOTIDES:

i. The purine nucleotides are synthesized by most of the tissues. However the major site is the liver. This pathway operates in the cytoplasm.

ii. The major pathway is denoted as de novo synthesis, because the purine ring is synthesized from different small components.

iii. There are ten steps in the de novo synthesis pathway. The enzymes catalyzing these reactions are existing as a multienzyme complex in eukaryotic cells; this arrangement increases the efficiency of the pathway.



Salvage Pathway

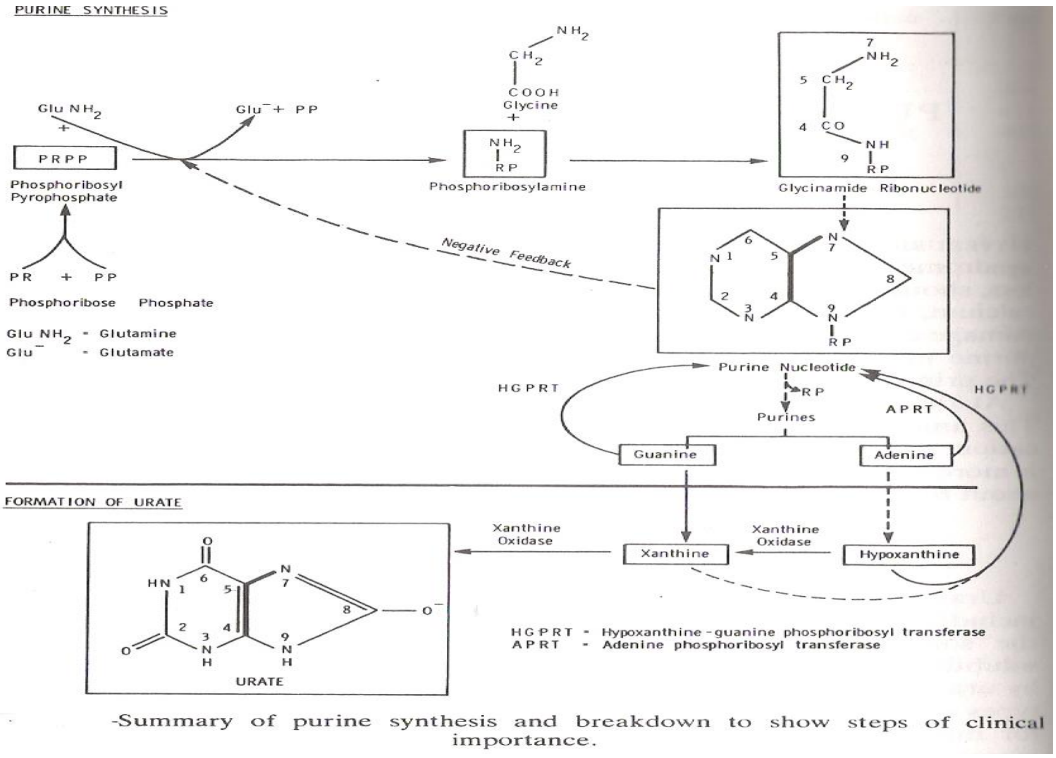
i. This pathway ensures the recycling of purines formed by degradation of nucleotides.
ii. PRPP is the starting material in this pathway.
iii. The free purines are salvaged by two different enzymes; adenine phosphoribosyl transferase (APRTase) and hypoxanthine guanine phosphoribosyl transferase (HGPRTase).
iv. The pathway is of special importance in tissues like RBCs and brain where the de novo pathway is not operating. Salvage pathway is summarized below:

$$\text{Adenine} + \text{PRPP} \xrightarrow{\text{APRTase}} \text{AMP} + \text{PPi}$$

$$\text{Guanine} + \text{PRPP} \xrightarrow{\text{HGPRTase}} \text{GMP} + \text{PPi}$$

Uric acid

i. The normal blood level of uric acid ranges from 2-5 mg/dl in females and 3-7 mg/dl in males.
ii. The daily excretion varies from 500-700 mg. Nucleic acid content is more in non-vegetarian diet.
iii. Uric acid is sparingly soluble in water.



-Summary of purine synthesis and breakdown to show steps of clinical importance.

Disorders of Purine Metabolism

The most common abnormality is an elevation of uric acid level in blood referred to as hyperuricemia. It is defined as serum uric acid concentration exceeding 7 mg/dl in male and 6 mg/dl in female. Increased excretion of uric acid in urine is called uricosuria. The manifestations are due to the low solubility of uric acid in water.

GOUT

i. It is due to accumulation of urate crystals in the synovial fluid resulting in inflammation leading to acute arthritis.

ii. At 30 ^{o}C, the solubility of uric acid is lowered to 4.5 mg/dl. Therefore, uric acid is deposited in cooler areas of the body to cause tophi. Thus tophi are seen in distal joints of foot.
iii. Increased excretion of uric acid may cause deposition of uric acid crystals in the urinary tract leading to calculi or stone formation with renal damage. Gout may be either primary or secondary.

Clinical Findings of Gout

Gouty attacks may be precipitated by high purine diet and increased intake of alcohol. Often the patients have a few drinks, go to sleep symptomless, but are awakened during the early hours of morning by excruciating joint pains.

Ammonia :

Formation of ammonia:

The first step in the catabolism of amino acids is to remove the amino group as ammonia. This is the major source of ammonia. However, small quantities of ammonia may also be formed from catabolism of purine and pyrimidine bases. Ammonia is highly toxic especially to the nervous system. Detoxification of ammonia is by conversion to urea and excretion through urine. Ammonia is produced in most tissues and must be transported to live without causing ammonia toxicity. **Glutamate, glutamine and alanine are the transport forms of ammonia** from peripheral tissues to liver.

1. *Glutamate* (α-ketoglutarate plus ammonia) may be considered as the major participants in such inter transport of ammonia. Concentration of glutamate in blood is about tenfold higher than other amino acids.

2. *Glutamine* is the transport form of ammonia from brain. Since brain is extremely sensitive to ammonia, it possesses a special mechanism for its immediate detoxification by combining it with glutamate to blood stream is capable of rendering the experimental animal. Ammonia can readily diffuse through cell membranes and enter tissues so that little is left in blood circulation (its concentration in peripheral blood is 30–60 μg/dL).

Other Sources of Ammonia

Although major source of ammonia is amino acids, additional sources are also known. These are:

1. Bacterial degradation of urea in the intestinal lumen.
2. Action of renal *glutaminase* on glutamine in renal tubular cells.
3. Action of intestinal *glutaminase* on glutamine in intestinal mucosal cells.
4. Release of amino groups of purines and pyrimidines as ammonia during catabolism of these nitrogen bases.

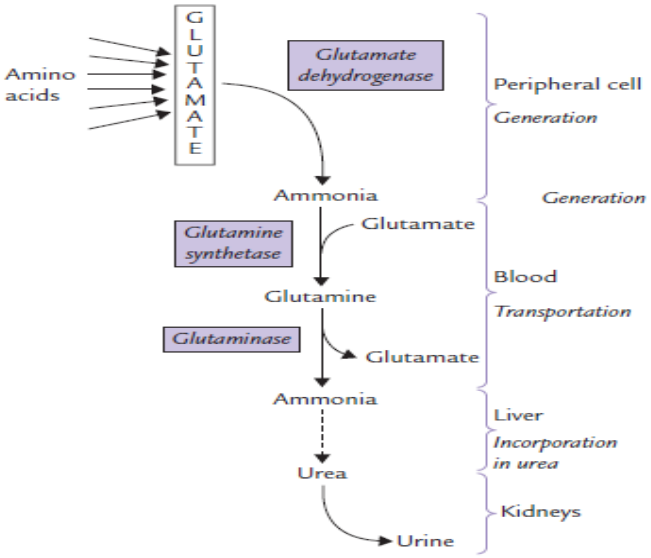


Fig. 13.6. Generation, transport and hepatic incorporation of ammonia into urea.

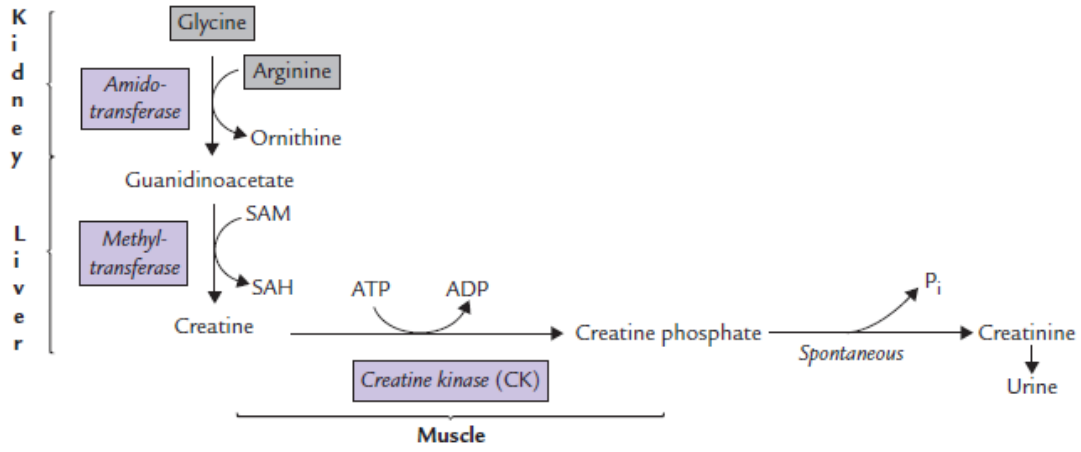
Creatinine:

Creatine Phosphate and Creatinine Creatine (N methylguanidinoacetate) is an amino acid derived product that is present in muscle tissue and to a lesser extent in nervous tissue. It plays a pivotal role in the metabolism of high-energy phosphates. Creatinine is a dead end metabolite formed by spontaneous cyclization of creatine and creatine phosphate.

Biosynthesis: Creatine phosphate is synthesized from three amino acids: glycine, arginine and methionine. The biosynthetic pathway consists of three sequential reactions, one reaction each occurs in kidney, liver and muscle

Functions: In vertebrate muscles, creatine phosphate occurs as a reservoir of high-energy phosphate groups. It helps generation of ATP in exercising muscles by substrate level phosphorylation. Nearly 1% of the weight of skeletal muscle is accounted for by this compound. Small amount is present in smooth muscles, testes, liver and kidneys as well. Creatine phosphate

possesses an energy rich phosphate bond, with a standard free energy of hydrolysis of 10.3 kcal/mole. This value is much higher than the standard free energy of ATP hydrolysis (7.3 kcal/mole). Therefore, hydrolysis of creatine phosphate can be coupled with concomitant generation of ATP (i.e. **substrate level phosphorylation**). Creatine phosphate ADP ATP Creatine During muscle contraction, when the ATP: ADP ratio declines, ATP is regenerated rapidly by the above reaction. This reaction, called the reversible creatine kinase reaction is the most important source of ATP during the first few seconds of muscle contraction. For more sustained muscular activity, however, ATP has to be regenerated by (anaerobic) glycolysis or oxidative metabolism. The compound corresponding to creatine phosphate in invertebrates is **arginine phosphate**. These high energy compounds of muscles are termed **phosphogens**.



Creatine –phosphate synthesis

Clinical implications:

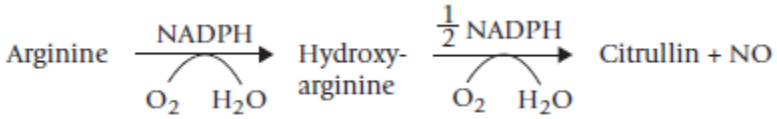
Creatine phosphate is relatively unstable at the pH prevailing in the sarcoplasma, and is non-enzymatically converted to creatinine in this organelle. This conversion takes place continuously in healthy muscle. The creatinine so produced is released in circulation transported to kidneys and excreted in the urine. Creatine phosphate Creatinine Spontaneous Pi Urine This has two important implications in clinical medicine:

1. The amount of creatinine excreted in the urine over a 24-hour period, which correlates with the muscle mass, is constant for a given individual (about 15 mg/kg of body weight). The quantity of creatinine is measured in the 24-hour urine specimens to validate that the collection was complete.
2. It is a useful kidney function test because its blood level is remarkably constant. It is neither secreted nor reabsorbed in the tubular system, and so

its excretion during a specified time in a **creatinine clearance test** serves as a measure of glomerular filtration rate. Small quantity of creatine is also excreted in urine. Muscle wasting due to any cause, such as muscular dystrophy, starvation, diabetes, fever and thyrotoxicosis Results in increased excretion of creatine in urine.

Nitric oxide:

Nitric oxide causes vasodilatation and is an important regulator of blood pressure. It acts by stimulating the cytoplasmic GC, thereby increasing cGMP. NO is unique intracellular messenger (Box 29.3) for being membrane soluble. It readily diffuses to nearby cells and increases cGMP level in them. Such a phenomenon occurs in vascular endothelial cells and the nearby smooth muscles: NO synthesized in the endothelium diffuses into the smooth muscle cells where it increases cGMP. The latter relaxes smooth muscles to cause vasodilatation. NO is synthesized by the enzyme, *NO synthase* in the endothelial cells from one of the nitrogen molecules in the side chain of arginine.



The reaction sequence involves NADPH and the products include NO, citrulline and $NADP^+$. Activity of *NO synthase* is stimulated by calcium-calmodulin. Therefore, the agents that elevate cytoplasmic calcium concentration increase the *NO synthase* activity.