Synthesis of deoxyribonucleotides

The nucleotides described so far all contain ribose (ribonucleotides). The nucleotides required for DNA synthesis, however, are

2'-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase.

Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is compos e d of two subunits, R1 and R2, and is specific for the reduction of purine nucleoside diphosphates (ADP and GDP) and pyrimidine nucleoside diphosphates (CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are **two sulfhydryl groups** on the enzyme itself, which, during the reaction, form a disulfide bond (Figure 2 and Figure 2).

Regeneration of reduced enzyme

In order for ribonucleotide reductase to continue to produce deoxyribonucleotides, the disulfide bond created during the production of the 2'-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is **thioredoxin**, a peptide coenzyme of ribonucleotide reductase. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, forming a disulfide bond in the process (Figure 2).

Regeneration of reduced thioredoxin:

Thioredoxin must be converted back to its reduced form in order to continue to perform its function. The necessary reducing equivalents are provided by NADPH + H^+, and the reaction is catalyzed y thioredoxin reductase (Figure 2).

Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. And this enzyme is regulated by **two mechanisms; 1st;** Activity sites on the enzyme molecule: The binding of dATP to active sites on

the enzyme inhibits the overall catalytic activity of the enzyme and effectively prevents DNA synthesis. In contrast, ATP bound to these sites activates the enzyme.

2nd **Substrate specificity sites**: The binding of nucleoside triphosphates to additional allosteric sites (known a s the substrate specificity sites) on the enzyme regulates substrate specificity, causing an increase in the convers ion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis. For example, deoxythymidine triphosphate binding at the specificity sites causes a conformational change that allows reduction of GDP to dGDP at the catalytic site.

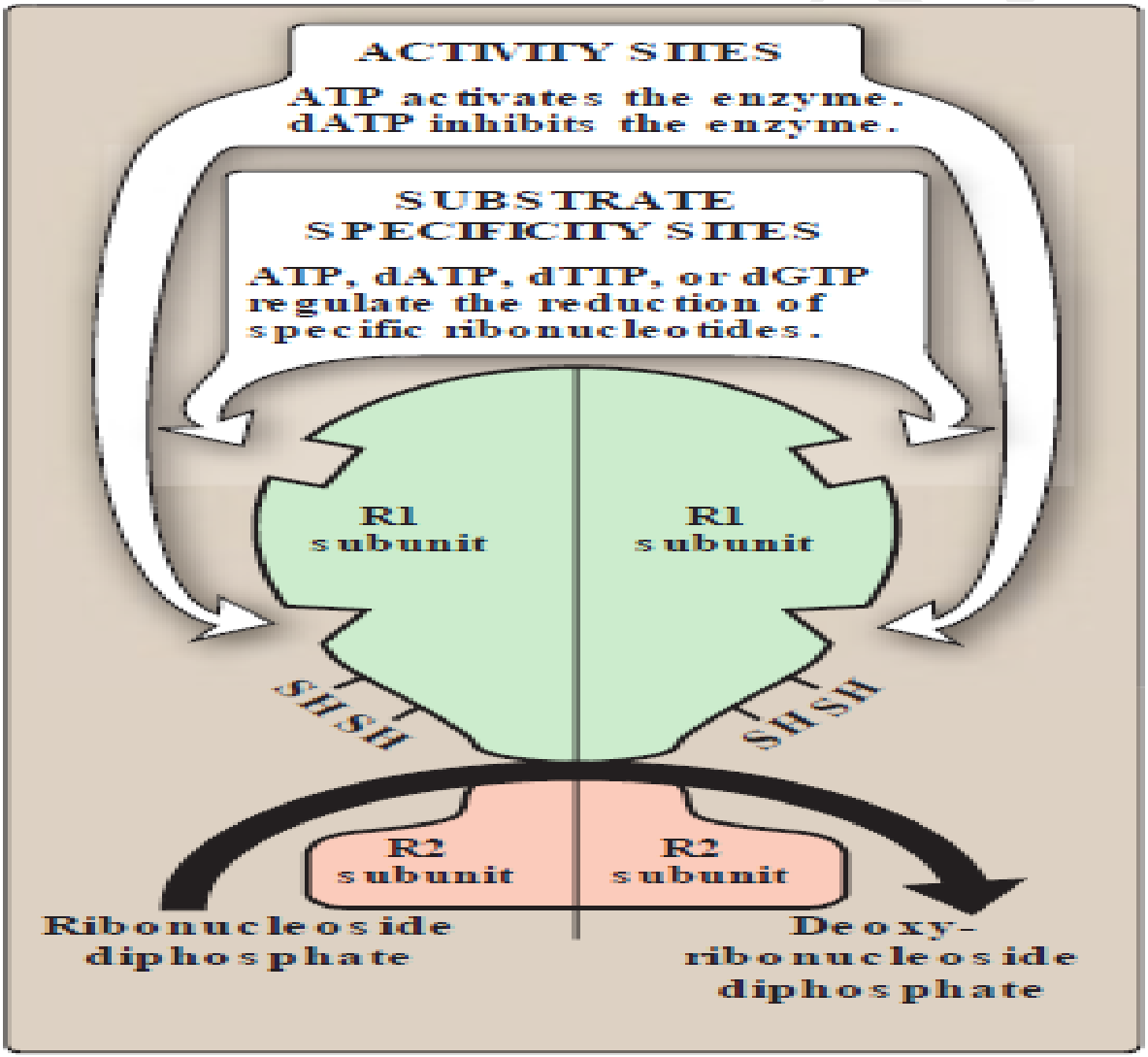

Figure 1: structure and regulation of ribonucleotide reductase.

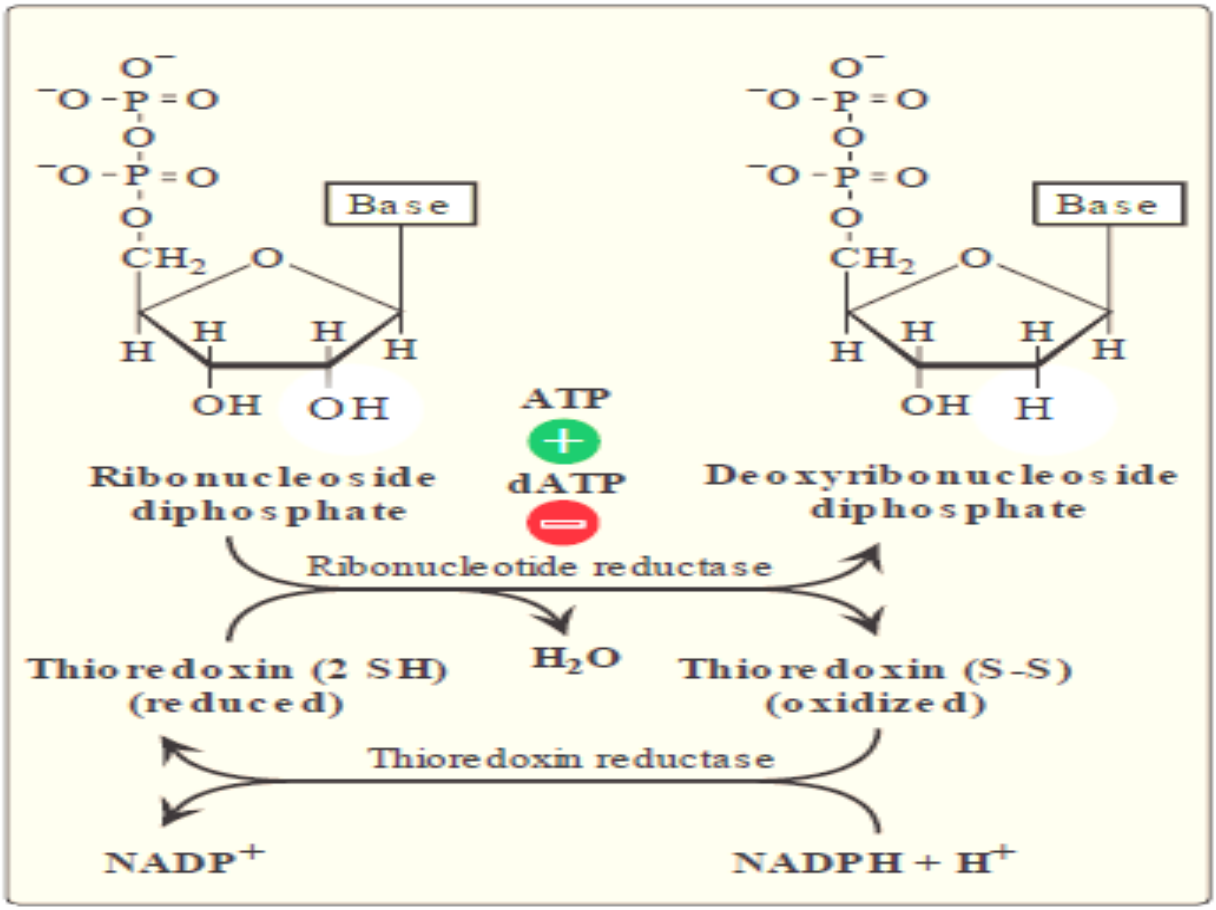

Figure 2: Convers ion of ribonucleotides to deoxyribonucleotides. NADP (H) = nicotinamide adenine dinucleotide phosphate; dATP = deoxyadenosine triphosphate.

Pyrimidine synthesis and degradation

As with the purines, the pyrimidines (uracil, cytosine and thymine) are also synthesized through a complex series of reactions using raw materials readily available in cells. One important difference is that the pyrimidine base is made first and the sugar added late, whereas purines are assembled on a ribose-5-P scaffold. Uridine monophosphate (UMP) is the precursor of all pyrimidine nucleotides. The *de novo* pathway produces UMP, which is then converted to cytidine triphosphate (CTP) and thymidine triphosphate (TTP). Salvage pathways also recover preformed pyrimidines. The sources of the atoms in the pyrimidine ring are glutamine, CO2, and aspartate (Figure 3).

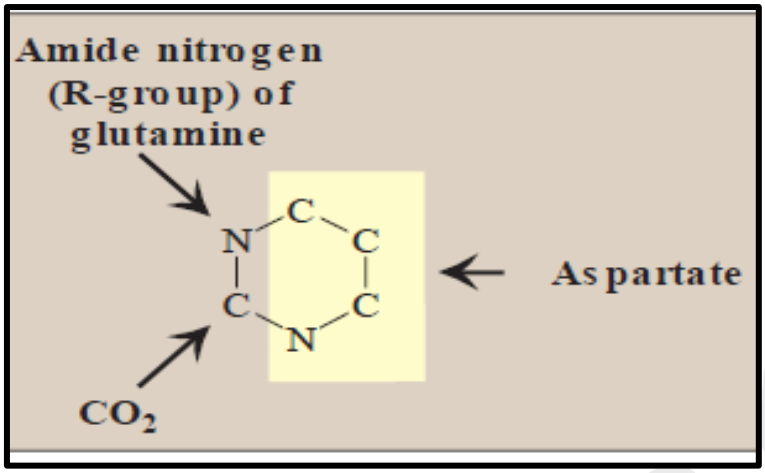

Figure 3 : Source s of the atoms in the pyrimidine ring.

De novo pathway Pyrimidine synthesis

A. Synthesis of carbamoyl phosphate

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine, CO2 and 2 moles of ATP, catalyzed by **carbamoyl phosphate synthetase (CPS) II**. CPS II is inhibited by uridine triphosphate (**UTP**) the end product of this pathway, (which can be converted into the other pyrimidine nucleotides), and is activated by PRPP. Regeneration of THF is crucial for DNA synthesis; inhibitors of dihydrofolate reductase (such as methotrexate) are potent inhibitors of cell growth.

B- Synthesis of orotic acid

Most of the atoms required for formation of the pyrimidine ring are derived from aspartate, added in a single step, catalyzed by aspartate transcarbamylase. The pyrimidine ring is then closed by dihydroorotase. The resulting dihydroorotate is oxidized to produce orotic acid (orotate) as shown in Figure 4**.** The enzyme that produces orotate, dihydroorotate dehydrogenase, is a flavoprotein associated with the inner mitochondrial membrane. All other enzymes in pyrimidine biosynthesis are cytosolic.

Note: **Leflunomide**, a drug represents a specific inhibitor of the enzymes used in pyrimidine ring biosynthesis **(CPS II, aspartate transcarbamylase, and dihydroorotase)**, is used for treatment of

rheumatoid arthritis because blockage of this step inhibits lymphocyte activation and thereby limits inflammation.

C. Formation of a pyrimidine nucleotide

The completed pyrimidine ring is converted to the nucleotide orotidine monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis (Figure 4). PRPP is again the ribose 5-phosphate donor under the action of the enzyme orotate phosphoribosyl transferase produces OMP. OMP, the parent pyrimidine mononucleotide, is converted to uridine monophosphate (UMP) by orotidylate decarboxylase, which removes the carboxyl group.

Note: Both purine and pyrimidine synthesis require **glutamine, aspartic acid, and PRPP as essential precursors.**

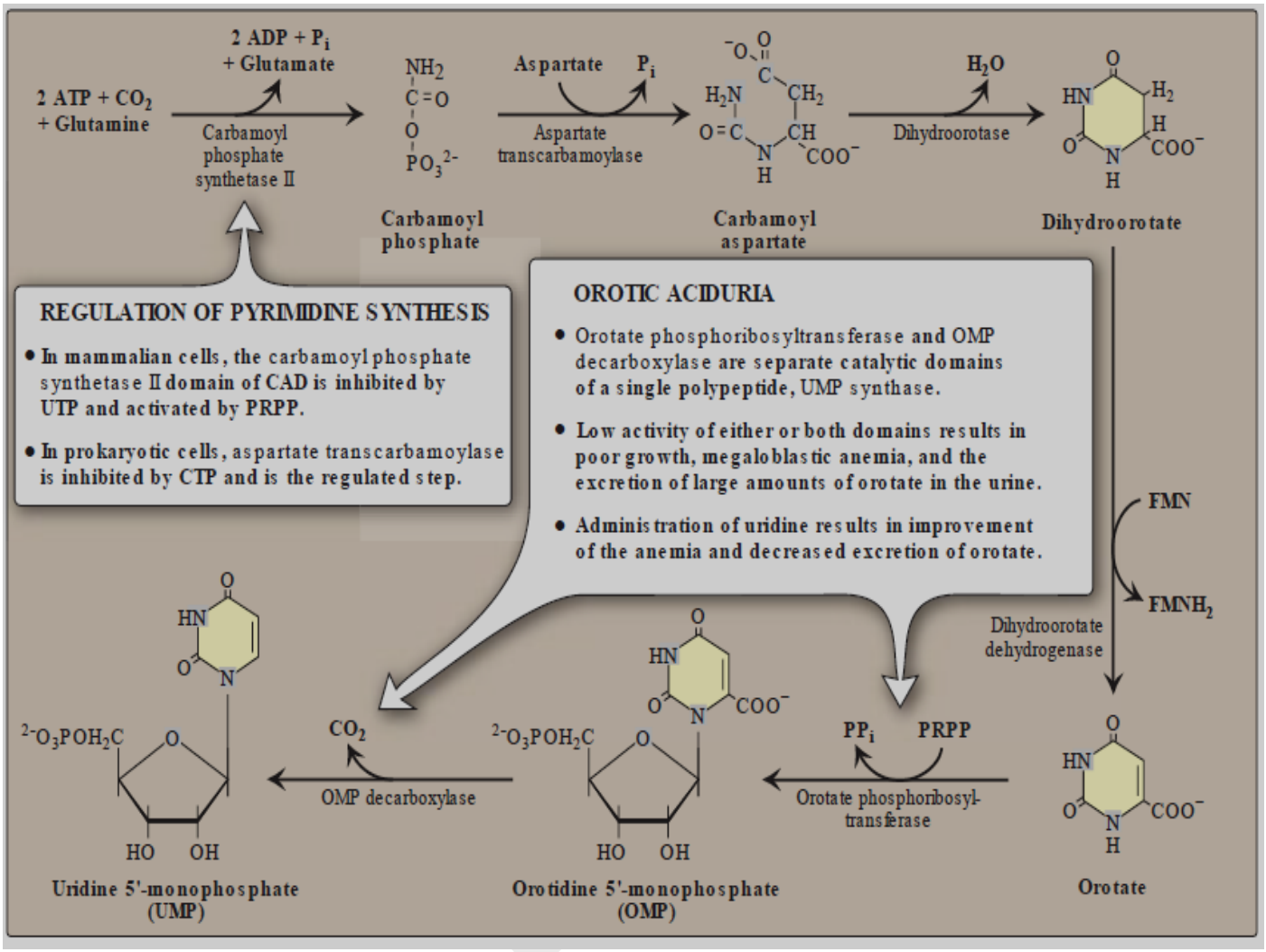

Figure 4: The metabolic pathway for synthesis of pyrimidines. Formation of orotic acid and UMP, the first pyrimidine nucleotide,.

UMP is the first "completed" product. UMP can then be phosphorylated to produce UDP. UDP acts as a branch point; it can be converted to UTP and used as a nucleotide, or it can serve as a substrate for the synthesis of the two other major pyrimidine nucleotides.

Synthesis of cytidine triphosphate

Cytidine triphosphate (CTP) is produce d by amination of UTP by CTP synthetase with glutamine providing the nitrogen (Figure 5).

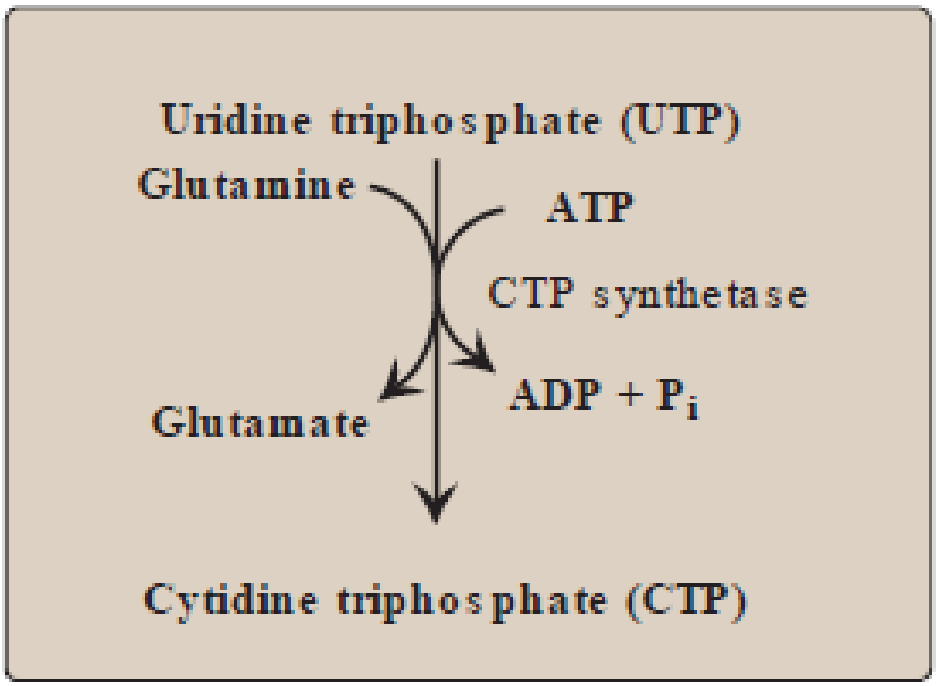

Figure 5: Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.]

Synthesis of deoxythymidine monophosphate

Since thymidine is only present in DNA, the first step in the conversion of **UMP to dUMP is catalyzed by ribonucleotide reductase, which removes the 2´-hydroxyl, to create the deoxynucleotide.**

The methylation of **dUMP requires N5, N10-methylene tetrahydrofolate.** Furthermore, dUMP is converted to deoxythymidine monophosphate (dTMP) by the enzyme thymidylate synthase, which needs tetrahydrofolate (THF) (Folic acid) as co-enzyme. Thus, inhibitors of thymidylate synthase serve as antitumor agents such as 5-fluorouracil (cytotoxic drug).

By decreasing the supply of THF (folic acid) or inhibition of its action by drugs like methotrexate , not only inhibit purine but, preventing methylation of dUMP to dTMP, they also decrease the availability of this essential component of DNA. DNA synthesis is inhibited and cell growth slowed. Thus, these drugs are used to decrease the growth rate of cancer cells.

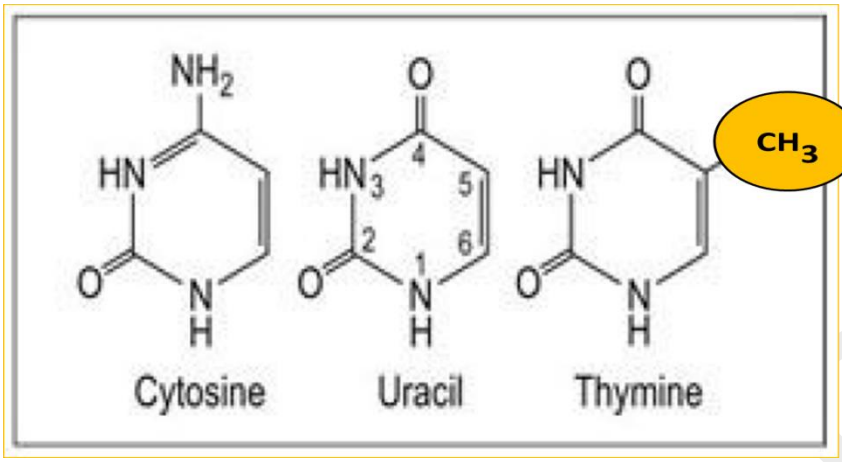

Figure 6: pyrimidine bases.

Degradation and salvage of pyrimidines

Unlike the purine ring, which is not cleaved in humans, the pyrimidine ring is opened and degraded to highly soluble products, β-alanine (from the degradation of CMP and UMP) and β-aminoiosobutyrate (from TMP degradation), with the production of NH3 and CO2. Pyrimidine bases can be salvaged to nucleosides, which are phosphorylated to nucleotides. However, their high solubility makes pyrimidine salvage less significant clinically than purine salvage.

Disorders of Pyrimidine Metabolism

Pyrimidine catabolic product molecules are almost soluble, so few disorders result from excess levels of their synthesis or catabolism. Two inherited disorders affecting pyrimidine biosynthesis are the result of deficiencies in the bio functional enzymes catabolizing the two last steps of UMP synthesis (Orotate phosphoribosyl transferase and OMP decarboxylase).

Blocks in steps leading to pyrimidine synthesis may result in deficient production of pyrimidine nucleotides. There may be anemia and immune deficiency (from decreased red and white cell production) and excess orotic acid which may precipitate in the urine.

Orotic Aciduria:

Orotic aciduria is a rare metabolic genetic disorder characterized by excess production of orotic acid and accumulation in urine. In this disorder, both orotate phosphoribosyl transferase and oritidine phosphate decarboxylase activities are markedly deficient

It causes a characteristic form of anemia, immune deficiency and may be associated with mental and physical retardation. In addition to the characteristic excessive orotic acid in the urine, patients typically have megaloblastic anemia which cannot be cured by administration of vitamin B12 or folic acid. It also can cause inhibition of RNA and DNA synthesis.

1- Primary Orotic Aciduria.

A- (Type I):

It represents the most severe form of the disease. It is hereditary genetic metabolic disorder characterized by megaplastic anemia with many large immature red blood cells, low white blood cell count, retarded growth, delay in physical development, leukopenia and the urinary excretion of large quantities of orotic acid. The disease is caused by deficiency of both enzymes orotate pyrophosphorylase and orotidine mono phosphate decarboxylase. These enzymes normally catalyze the formation of uridine monophosphate from orotic acid during pyrimidine synthesis.

The disorder is extremely rare; it can be detected by an examination of their white blood cells.

This disorder can be treated with pyrimidine nucleotide (uridine and/ or cytidine) (2-4 g/d), which increase UMP production via the action of nucleotide kinase. Uridine treatment results in marked improvement in the hematological abnormalities, in growth and development, and in decreased excretion of uric acid.

B- (Type II):

Deficient in only OMP decarboxylase. It is autosomal recessive, the urinary excretion of orotidine mono phosphate in higher concentrations than orotate; it is characterized by megaloblastic anemia.

Administration of cytidine monophosphate and uridine monophosphate reduces urinary orotic acid and the anemia.

.2- Secondary orotic aciduria:

It is an acute metabolic disorder due to liver mitochondrial failure (Reye's syndrome) to utilize carbamoyl Phosphate due to the deficiency of ornithine trans carbamolyase enzyme which is arising primarily in children ,is a serious disease that can be fatal. It is a form of hepatic destruction following recovery from a viral infection. Shortly after the infection, the patients develops neurologic abnormalities such as coma, liver functions are always abnormal.

Administration of cytidine monophosphate and uridine monophosphate reduces urinary orotic acid and the anemia.

Administration of uridine, which is converted to UMP, will bypass the metabolic block and provide the body with a source of pyrimidine.