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## Orotic Aciduria

Aliah L. Fonteh

*Liberty University*, [afonteh@liberty.edu](mailto:afonteh@liberty.edu)

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## OROTIC ACIDURIA

By Aliah Fonteh

### CASE REPORT I<sup>1</sup>

#### Overview

Uridine monophosphate synthase (UMPS) is a bifunctional enzyme with the catalytic sites for orotate phosphoribosyl pyrophosphate transferase (OPRT) and orotidine monophosphate decarboxylase (OMPDC). The conversion of orotic acid to uridine-5'PO<sub>4</sub> is catalyzed by UMPS. Orotic aciduria can occur whether one or both of these enzymes are impaired. In 1968 when this case was presented, only three previous patients homozygous for a defect in the enzymes OPRT and OMPDC had been reported. A pedigree analysis was utilized to focus on the genotype of family members four generations before the patient. The pedigree revealed family members heterozygous for hereditary orotic aciduria type I. The pattern of inheritance for hereditary orotic aciduria type I is autosomal recessive. The enzyme activity for the patient's father, siblings, and niece revealed "borderline" enzyme activity.

#### History, Initial Symptoms, and Laboratory Examination

On April 1, 1965, a 6 lb. 7 ¾ oz. female patient was born to normal 21-year old parents. Abnormalities in patient hemoglobin leading to anemia were first evidenced after two months of life. Administration of 30 milligrams (mg) of iron and 50 mg of Vitamin C daily did not improve the patient's anemic conditions. After hospital admission to North Carolina Baptist Hospital in Winston-Salem, NC, the six-month old patient was found to have pallor and bilateral strabismus. Laboratory analysis, bone marrow analysis, and a peripheral blood smear revealed erythrocyte dysfunction. Erythroid hyperplasia was also discovered after the bone marrow analysis. The peripheral blood smear revealed anisocytosis, poikilocytosis, hypersegmentation of granulocytes, and mild hypochromia. The initial treatment plan was intramuscular administration of 15 mg and 7.5 mg of folic acid for five days each, respectively. Oral administration of folic acid for two weeks, then 30 microgram (µg) rotations of Vitamin B12 every other day were administered. Vitamin B12 supplementation was repeated three times.

#### Diagnostics

Diagnostic exams included erythrocyte enzyme assays and urinary screening tests. The patient's OMPDC and OPRT activity was 0.02 and 0.96, respectively. Control values for OMPDC activity were 0.79 and 1.9, while control values for OPRT were 5.4, 4.1, and 3.6. The measure of the conversion of orotic acid to uridine-5'PO<sub>4</sub> was also studied. Measurement of these values was 0.02 compared to control values of 0.83, 1.5, and 2.4. Overall, the patient's enzyme activity for both OMPDC and OPRT was very low compared to controls.

#### Symptoms

The patient's main symptoms included megaloblastic anemia, orotic aciduria and orotic acid crystallization, growth delays, and developmental delays. Early patient symptoms required an erythrocyte transfusion twice, and by early 1966, the patient was first observed to have orotic acid crystalluria. The crystalluria became observable following dehydration and gastroenteritis. Orotic acid excretion for the patient was 3.89 gm per gram of creatinine, compared to other cases which may have up to 1,000 times the orotic acid excretion quantity compared to normal adults. The patient was experiencing growth and length delay because the patient's weight and length were in the 10th and 50th percentiles, respectively.

### Management and Results of Treatment

At eleven months, the administration of 1.5 gm/day of oral uridine began. Reticulocyte development spiked within two days, hemoglobin concentration rose from 7.8 gm/100 mL at the first hospital admission to 12.3 gm/100 mL on the fifth day of therapy, and the leukocyte count increased to a range of 3900 to 12000 per m<sup>3</sup> during therapy. Bone marrow analysis after two months did not reveal any abnormalities. In addition, the child's development was evident due to increased activity and appetite. Compared to the weight and height percentiles at six months, by eighteen months the patient was in the 50th and 90th percentiles, respectively. Mental delays were not present when the patient was assessed at age 2.5.

The daily dosage of 1.5 gm was significant for the reduction in orotic acid output, and this measure helped to reduce the risk of urinary tract obstruction due to the crystallized orotic acid. Oral uridine is responsible for reticulocyte development and correction of megaloblastic changes of the bone marrow. Upon recognition that Vitamin B12 and iron were not correcting the anemia, the administration of oral uridine may have occurred early enough to prevent further damage such as mental retardation. Physician's knowledge of the patient's history also provided an earlier diagnosis for this rare disease. Rogers et al. speculate that early therapeutic management is important for future cases as uridine administration may have a role in reducing the abnormal effects that can occur in patients. They hypothesize that such effects as mental retardation and physical growth disturbances can be reversed with early treatment. The patient clearly exemplifies the benefit of early therapy because by the age of 2.5, the patient had achieved all developmental milestones for her age. Other cases do not always generate these results, even with oral uridine treatment.

## CASE REPORT II<sup>2</sup>

### Overview

Imaeda et al. was the first to identify a case of hereditary orotic aciduria in Japan. Though previous patients heterozygous for the condition were phenotypically "normal" except for orotic aciduria, the case report for the patient (introduced below) exhibited severe symptoms. Pyrimidines can be found in mother's milk and are crucial in the neonatal period and in the duration of breast-feeding. For normal patients, *de novo* pyrimidine synthesis will provide sufficient nucleotides important in cellular function and development. However, in a homozygote for hereditary orotic aciduria, pyrimidine therapy must be administered. This patient

remained deficient in pyrimidines from breast milk and by *de novo* synthesis for over a week. After nine days, administration of dietary pyrimidine was added to the milk of the patient.

### History, Initial Symptoms, and Laboratory Examination

The family history of the patient in this case includes members heterozygous for hereditary orotic aciduria (type not specified). The patient's father demonstrated normal enzyme activity and was not found to be a carrier for a mutation in UMPS. However, though the patient's mother was phenotypically normal, she was found to be a heterozygote carrier. The patient was also found to be a heterozygote carrier. The patient is a three-year old male born to nonconsanguineous parents. Born prematurely, the patient was admitted into the NICU (newborn intensive care unit). Asphyxia and low blood sugar were determined after Apgar test score results and laboratory examination, respectively. The newborn had low birth weight and was difficult to feed. Initial computed tomography of the cranium did not signify any abnormal mental developments.

### Symptoms

The patient's symptoms included orotic aciduria, neurological defects, and developmental delays. In some cases of orotic aciduria, megaloblastic anemia is affiliated with orotic aciduria. In the case of this patient, megaloblastic anemia was not identified. By eight months old, the patient began to experience developmental abnormalities and impaired motor milestones. At thirteen months, the patient was diagnosed with cerebral palsy (spastic quadriplegia). In addition, mental retardation progressed in this time frame, becoming most apparent at age 3. Imaeda et al proposes that insufficient pyrimidine nucleosides in the neonatal period may have led to severe neurological symptoms in this patient.

### Diagnostics

Upon urinary analysis, high levels of orotic acid and orotidine were discovered. Creatinine excretion is based on 20 mg per kg of body weight. Control patients have orotic acid levels of 1.1-1.9  $\mu\text{mol}$  per mmol of creatinine, but the patient had levels of 10.5  $\mu\text{mol}$ . Compared to control values of 0.3 to 1.5 for orotidine, the patient had a value of 2.6  $\mu\text{mol}$ . Both OPRT and OMPDC activity was very low with a value of 8.3%. Though the father had higher levels of activity for both enzymes (94.3% and 88.6% respectively), the heterozygous mother had very low activity. Her activity was documented as 4.4% for OPRT and 7.9% for OMPDC.

### Therapy/Management

Since the newborn had difficult feeding and low birth weight, therapy included intravenous infusion of glucose and minerals. Imaeda et al proposes that for future treatment of similar cases, oral uridine therapy must be introduced as soon as possible. If possible, treatment should begin from birth and leading up to the month after birth for the ability to mitigate the effects of insufficient pyrimidine levels in the cell.

## INTRODUCTION

The organic acid 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid is most commonly known as Orotic acid or “Vitamin B13”. The body can obtain orotic acid exogenously from dairy products and root vegetables (i.e. carrots, beets) or synthesize orotic acid endogenously through the pyrimidine synthetic pathway commonly found in hepatocytes, erythrocytes, and the kidney (See **Figure 1**).<sup>3-6</sup> The mitochondrial enzyme dihydroorotate dehydrogenase (DHODH) converts dihydroorotate into orotic acid so that uridine monophosphate synthase (UMPS) can catalyze the conversion of orotic acid to uridine monophosphate (UMP).<sup>6</sup> Orotic acid is mainly found within the cytoplasm of cells.

As an intermediate in pyrimidine synthesis, orotic acid is important for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) production, diphosphosugar formation within basement membranes, and the formation of uridine nucleotides. Though the body can attain pyrimidines from the diet, a sufficient amount of pyrimidines to regulate the above cellular processes is required. Moreover, it is the endogenous production of pyrimidines that generates the quantity of pyrimidines needed to meet the cellular requirement. Thus, when orotic acid is present but not able to continue on in the pathway for the synthesis of pyrimidines, DNA/RNA production, basement membrane stability, and uridine nucleotide formation is compromised.

Orotic acid plays a role in the stability of tissues because it is a precursor for uridine diphosphate formation. The link between orotic acid and uridine diphosphate is due to the role that diphosphosugars have in the abundant connective tissue, collagen. Collagen, which is an important protein for strength within connective tissues, requires glycosylation of uridine diphosphate to form UDP sugar moieties. Thus, uridine diphosphosugars, which are important components of collagen, require sufficient pyrimidine synthesis to provide normal structural stability for tissues in the body. Additionally, membranes within the kidney depend on orotic acid for a similar reason. Both the glomerular and tubular basement membrane depend on the formation of uridine nucleotides for the formation of thicker membranes. If orotic acid (the precursor to uridine nucleotides), is not able to enter the pyrimidine pathway, then the strength of these membranes is reduced. Furthermore, orotic acid is also involved in other key metabolic pathways, some of which are implicated in certain disease states. Orotic acid is a compound found to be associated with the pathways linked to  $\beta$ -Ureidopropionase deficiency, Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) and dihydropyrimidinase deficiency.<sup>3</sup> Orotic acid may also be connected with the following diseases: Canavan disease, colorectal cancer, Crohn’s disease, and ulcerative colitis.<sup>3</sup>

Though normal amounts of orotic acid are essential for pyrimidine synthesis and other crucial biochemical processes, excessive amounts of orotic acid can lead to metabolic acidosis and have toxic effects on tissues. Excess orotic acid causes harm to tissues because of the role that orotic acid plays as an acidogen and a metabotoxin.<sup>3</sup> An acidogen is a compound that increases acid concentration, which in large quantities can lead to metabolic acidosis.<sup>3</sup> An increase in hydrogen ion concentration correlates with a decrease in blood pH. If pH levels decrease, then the range necessary for normal enzyme function is altered. Thus, acidosis will lead to a compromise of enzyme function and also an impairment of cellular function. Based on the composition of cells, tissues, organs, and organ systems, when function is impaired at the enzymatic level, it will also affect the body’s ability to maintain homeostasis and thus normal function. Acidosis is dangerous because it will change the environment for optimal function of enzymes within many bodily systems, particularly the cardiovascular and the nervous system. As

a metabolite, an accumulation of orotic acid suggests the role that it can play as a metabotoxin. A metabotoxin is a compound that releases metabolites that could be damaging or toxic to the cell.<sup>3</sup> When orotic acid donates a proton to a conjugate base, it can form a salt and water in this neutralization reaction. Nonetheless, as an acid, the release of protons will contribute to the decrease of pH and the increase of toxicity within the plasma.

## OROTIC ACID TRANSPORTERS

The solute carrier gene 22 family codes for a subfamily of at least ten transmembrane organic anion transporters (OATs), organic cation transporters (OCTs), and organic carnitine transporters (OCTNs).<sup>7</sup> The structural components of this subfamily of transporters includes twelve transmembrane domains, and approximately 540-560 amino acids (See **Figure 2**).<sup>7</sup> OATs are transport proteins responsible for influx of anions between epithelial barriers and fluids throughout the body. Previously, orotic acid transport in the liver and kidney was unknown. Evidence that the transporters OAT1, OAT2, OAT4, and hURAT1 play a role in orotic acid transport has been discovered.<sup>7-9</sup>

OAT1 is found primarily on the basolateral surface of the proximal tubule of the kidney as well as in the choroid plexus. Substrates of OAT1 include numerous small xenobiotics, polycyclic aromatic hydrocarbon (PAH), cyclic nucleotides,  $\alpha$ -ketoglutarate, folate, diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), indoxyl sulfate, prostaglandin E2, toxins, and mercurials.<sup>7</sup> The mechanism of transport for OAT1 appears to function mainly as an efflux transporter in drug removal from the plasma. Metabolomic analysis of wild-type OAT and knock-out OAT mice were extracted and further investigated by reverse-phase liquid chromatography and mass spectrophotometry.<sup>7</sup> The purpose of this research was to determine the substrates of OAT in vivo. Findings revealed that if OAT1 (SLC22A6) is affected, then there is a reduction in the urinary excretion of orotic acid and orotate.<sup>7</sup>

OAT2 is found in greatest concentration in the liver, however, it is also found in the kidney. The substrates for OAT2 includes antivirals, cGMP, acetylsalicylate, prostaglandin E2, dicarboxylates, glutamate, PAH, and salicylate.<sup>7</sup> Orotic acid has also been identified as a specific substrate for OAT2 (SLC22A7).<sup>7</sup> Results from Fork et al. suggest that OAT2 has the following roles: 1) it is responsible for the efflux of hepatic glutamate from the cytosol 2) it allows the liver to maintain homeostasis for plasma concentrations of glutamate, and 3) transports orotic acid by influx (See **Figure 3**).<sup>10</sup> Research studies have confirmed that this function of glutamate efflux from cells is conserved across the following organisms: human, rat, pig, and mouse.<sup>10</sup> Orotic acid may also be effluxed from hepatocytes by OAT2, and this efflux may be accelerated by glutamate. If liver failure is occurring, it can impact ureagenesis and the maintenance of glutamate concentrations in the plasma. OAT2 may be important to investigate for therapeutic intervention in acute glutamate brain toxicity or to increase glutamate efflux by orotic acid infusion.

Two other isoforms are of interest to researchers for their role in orotic acid transport—OAT4 and hURAT1.<sup>9</sup> OAT4 can be found in the placenta, kidney, and brain and uses estrone sulfate, dehydroepiandrosterone sulfate, prostaglandin E2, urate, NSAIDs, antihypertensives, uric acid, and ochratoxin A as a substrate.<sup>7</sup> However, Anzai et al also discovered that the isoform OAT4 (SLC22A11) transports orotic acid into renal proximal tubular cells.<sup>7-9</sup> Nigam et al. proposes that OAT4 may allow reabsorption of organic acids on the apical membrane of these proximal tubular cells.<sup>7,8</sup> Regulation of OAT4 transport can be mediated by strong inhibitors

such as estrone sulfate, DHEA sulfate, probenecid and benzbromarone.<sup>8</sup> Clinically, such inhibitors must be further investigated due to the impact that they have on the transport of the substrates listed above.

Lastly, the human urate transporter 1 (hURAT1) can be found in the kidney and utilizes urate and orotate as a substrate. The gene SLC22A12 codes for the hURAT1, formerly known as renal specific transporter (Rst).<sup>7,9</sup> This transporter was first reported to facilitate the transport of orotate in renal proximal tubular cells by Miura et al.<sup>9</sup> The hURAT1 can be compared to OAT1 due to functional similarities.<sup>7</sup> Studies on orotic acid transport are relevant for understanding the mechanism for orotic acid excretion. Particularly, since orotic acid crystal formation could lead to urinary obstruction, these transporters may be further investigated as possible drug targets in cases of orotic aciduria.<sup>1</sup>

## ETIOLOGY OF OROTIC ACIDURIA

The causes of orotic aciduria can be divided into four main categories. First, orotic aciduria caused by a defect in pyrimidine synthesis; second, orotic aciduria caused by a defect in the urea cycle; third, drug-induced orotic aciduria; and lastly, idiopathic orotic aciduria. The primary cause of orotic aciduria is also known as hereditary orotic aciduria. Hereditary orotic aciduria, which has three subtypes (type I, II, and III), is due to a genetic defect which significantly impairs uridine monophosphate synthetase (UMPS) activity. Reduction in this activity impairs the production of pyrimidines, important biological macromolecules that are essential for life. Urea cycle defects which cause orotic aciduria are due to enzymes and transporters within the urea cycle which are not functioning properly. The urea cycle is the process used to remove nitrogenous substances from the body. Since an accumulation of nitrogenous substances will impair the brain and other organ systems, the urea cycle is a very important process for homeostasis. Drugs such as allopurinol, 5-fluorouracil, and 6-azauridine are main inhibitors of the pyrimidine pathway and will also induce orotic aciduria. Idiopathic orotic aciduria occurs when other diseases cause an increase in orotic acid excretion in the urine. Regardless of the cause generating orotic aciduria, similar symptoms such as motor and mental impairment may result. Thus, early diagnosis of the condition and the appropriate therapy can reduce the damage which may be caused by sustained orotic aciduria.

## OROTIC ACIDURIA DUE TO DEFECTS IN PYRIMIDINE METABOLISM

A pyrimidine is a six-membered heterocyclic ring containing carbon, nitrogen, and oxygen. DNA contains pyrimidines such as the nitrogenous bases cytosine (2-hydroxy-4-aminopyrimidine) and thymine (2, 4-dihydroxy-5-methyl pyrimidine). RNA contains mainly cytosine and uracil (2, 4-dihydroxypyrimidine). The combination of the nitrogenous base, a deoxyribose sugar, and a phosphate group form the backbone of DNA. Ribonucleic acid is a linear structure which contains a nitrogenous base, ribose sugar, and a phosphate group. Phosphorylation of these bases will generate a nucleotide. A nucleotide is incorporated into the double helical structure within DNA, versus a single, linear strand for RNA. These bases can also be mono, di, and triphosphorylated for chemical reactions in the body. Many tissues need to synthesize pyrimidines for the regulation of cellular identity and function.

## Biochemical Significance of Pyrimidines

The cellular machinery within the body maintains a universal code that is essential for the proper development of organ systems, organs, tissues, cells, and the cellular organelles governing cell activity. Ultimately, this universal code is affiliated with the macromolecules deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) which are mandatory for life. The central dogma of life orchestrates how the instructions for life contained in DNA can be converted into a message that the cell uses. First, DNA is important for making more copies of the same cell, and DNA to DNA copying occurs through a process called replication. Second, the DNA must be converted to RNA through a process known as transcription. Transcription allows the genetic information (genes) to be prepared for translation to proteins. The translation of RNA to gene products is vital for the regulation of processes involved in metabolism, growth, development, aging, disease, etc.

Pyrimidines are important compounds necessary within the cell cycle for the synthesis of genetic information. Genetic information regulates the activity of all cells in the body because it contains the manuscript for cellular machinery, is regulated to maintain homeostasis within the body, and allows for cellular growth and development. Defects in the synthesis of these macromolecules will impact the quantity of bases available for DNA and RNA synthesis. When DNA and RNA synthesis is not sufficient, cellular growth and stability is impacted. The phosphate groups on these nucleotides may also serve to be used in phosphate transfer reactions. The release of phosphate from adenine triphosphate (ATP) and other nucleotides is an important source of cellular energy which is required for various biochemical reactions. Biochemical reactions including the active transport of substances across the cell membrane, the activation of enzymes, the contraction of skeletal muscle, and the inhibition of certain pathways depend on phosphate transfer. Specifically, guanine triphosphate (GTP) is a main regulator in protein synthesis, uridine triphosphate (UTP) provides energy of activation in the reaction of glucose and galactose, and cytidine triphosphate (CTP) plays a role for energy in lipid metabolism.<sup>11,12</sup> Disruption of the pathway causes problems because the body cannot synthesize these compounds, and the normal recycling of these compounds for re-incorporation into DNA or RNA (“salvage pathway”) may also be disrupted or insufficient to provide the needs of the cell.

In addition,  $\beta$ -alanine and  $\beta$ -aminoisobutyrate are formed from the catabolism of pyrimidines. Both compounds can be converted into substrates in the energy-producing tricarboxylic acid (TCA) cycle.<sup>3,11,12</sup> The TCA cycle produces GTP, NADH, and FADH<sub>2</sub>. The TCA cycle is a main producer of 3 molecules of the reducing agents NADH and 2 molecules of the compound FADH<sub>2</sub> which serve as donors to the electron transport enzymes. For each molecule of NADH, the electron transport chain (ETC) can generate 2.5 moles of ATP. For each mole of FADH<sub>2</sub>, about 1.5 moles of ATP. The electron transport enzymes function to generate ATP through the electrochemical gradient generated in the mitochondrial matrix. ATP, as discussed previously, is the energy investment required for many biochemical processes to proceed.

Pyrimidine synthesis is not only important for DNA and RNA production, but also for other biochemical processes. Pyrimidines are used as nucleotide-lipid cofactors to generate stable erythrocyte membranes, for cell proliferation, and for the biosynthesis of glycogen and phospholipids. The role of pyrimidines can also be identified in cell-mediated immunity.<sup>6,13</sup> In early development, rapid pyrimidine synthesis is required for neural maturation and organogenesis. Thus, mutations with a severe effect on the activity of UMPS can lead to death.<sup>14</sup>



## OVERVIEW OF TYPE I, TYPE II, AND TYPE III OROTIC ACIDURIA

A mutation that disrupts the bifunctional activity of uridine 5'-monophosphate synthase (UMPS) is the most frequent inborn error of pyrimidine nucleotide synthesis.<sup>5</sup> Orotic aciduria (type I, II, or III) occurs due to this defect in pyrimidine synthesis. This disease was discovered as a rare autosomal recessive disorder in 1959.<sup>5,15,16</sup> Hereditary orotic aciduria can be divided into three subtypes, two of which are clinically indistinguishable (type I and II) and a third that does not present with macrocytic hypochromic megaloblastic anemia (See **Table 1**).<sup>16</sup> In type I, both orotate phosphoribosyl pyrophosphate transferase (OPRT) and orotidine monophosphate decarboxylase (OMPDC) are defective, whereas in type II, only OMPDC is impacted.<sup>16</sup> OPRT and OMPDC have been found defective in erythrocytes, leukocytes, fibroblasts, and saliva due to a mutation in the individual catalytic sites of UMPS.<sup>13</sup> UMPS activity also plays an important role in the brain, liver, skeletal muscle, and spleen.<sup>14</sup> Defects in pyrimidine synthesis commonly lead to mental retardation.

### Epidemiology of Disease

Hereditary orotic aciduria is a rare autosomal recessive disorder with a prevalence in infants and children of 1:1,000,000.<sup>17</sup> There have been only about twenty identified cases worldwide.<sup>14</sup> Out of these cases, point mutations were discovered in three Japanese families.<sup>18</sup> Further information about this rare disorder is limited.

### Biochemical Pathology

There are three subtypes of hereditary orotic aciduria. In the most common type, type I orotic aciduria, 90% of patients experience severe loss of both OPRT and OMPDC activities due to a deficiency in UMPS.<sup>19,20</sup> Studies show that deficient quantities of wild-type UMPS activity are responsible for the variation in levels of orotate to orotidine in type I orotic aciduria. Erythrocyte activity of OPRT is absent in type I.<sup>13</sup> Type II is clinically indistinguishable from type I but includes specific inactivation of OMPDC which occurs through an unknown mechanism.<sup>16,19</sup> Cells will have less than 1% activity of OMPDC and an increase in OPRT activity with the type II subtype.<sup>20</sup> The orotate levels are comparable to that of type I, but there is an increase in the excretion of orotidine. Type III, or hereditary orotic aciduria without megaloblastic anemia (OAWA), is correlated with a deficiency in OMPDC. The case where this subtype first presented revealed a homozygous missense mutation within a conserved region of the OMPDC domain of the UMPS gene (c.928 T4>G; p. Phe310Val).<sup>13</sup> In a type III patient, the levels of OPRT activity are present but remain low.

Carbamoyl phosphate is formed intracellularly from glutamine, bicarbonate, and ATP by CAD, a cytoplasmic enzyme with active sites for carbamoyl phosphate synthetase II (CPS II), aspartate transcarbamoylase, and dihydroorotase (See **Figure 5**).<sup>5</sup> The reaction proceeds with CPS II forming carbamoylaspartate, with enzyme-catalyzed cyclization by aspartate transcarbamoylase (ATC) to form dihydroorotate, and with dihydroorotate dehydrogenase (DHODH) oxidation of dihydroorotate to orotic acid. Orotic acid is converted to uridine

monophosphate (UMP) by UMPS.<sup>21</sup> The formation of UMP can also occur via a “salvage” pathway from uracil phosphoribosyltransferase using uracil and 5-phosphoribosyl-1-pyrophosphate ([PRPP] See **Figure 4**).<sup>22</sup>

The enzyme uridine monophosphate synthase (UMPS) is essential in this process because of its bifunctional regulation of pyrimidine synthesis. This enzyme is involved in the ribosylation of orotate to orotidine monophosphate (OMP) via orotate phosphoribosyl pyrophosphate transferase (OPRT). UMPS also decarboxylates OMP to uridine monophosphate with OMPDC. When the enzyme uridine monophosphate synthase is impaired, there will be a buildup of orotate (OA) or orotidine monophosphate (OMP). Though OA is permeable, once it crosses the cell membrane it does not undergo any modifications and is excreted. OMP is impermeable and is dephosphorylated to form the compound orotidine prior to secretion. Normally, the UMPS reaction promotes UMP formation and only generates small amounts of cellular OA and urinary OA. In hereditary orotic aciduria, though more cellular orotate and urinary orotate are being produced, OA is not able to complete the pathway of pyrimidine synthesis.<sup>13</sup>

Consecutive phosphorylation of UMP to UDP and UTP occurs by the enzymes uridylate kinase and nucleoside diphosphate kinase (NDPK) (See **Figure 4**). Cytidine triphosphate (CTP) and thymidine triphosphate (TTP) can be formed from uridine triphosphate (UTP). Pyrimidine synthesis is important for the provision of high energy molecules for biochemical processes and for DNA and mRNA synthesis. The regulatory processes that govern pyrimidine synthesis include negative feedback by UTP and positive regulation by PRPP upon the CPS II active site of CAD (See **Figure 4**).<sup>5,23</sup> Low pyrimidine concentration due to a defect in UMPS will impact erythrocyte formation and cause megaloblastic anemia.<sup>20</sup> Megaloblastic anemia is usually the first manifestation that will occur for this condition because a defect in synthesis of the pyrimidine UTP will decrease the synthesis of the other pyrimidines, CTP and TTP as well.<sup>18</sup> This condition results in an inadequate amount of nucleotides for DNA synthesis, causing the bone marrow to produce large cells that are unable to undergo cell division. Since cell division is impaired, the patient will not be able to develop normal shaped erythrocytes.<sup>6,17</sup>

### Genetic Basis of Disease

Orotic aciduria is a rare, autosomal recessive disorder that can occur due to an inborn error in metabolism. Hereditary orotic aciduria occurs with an autosomal recessive mutation of 3q21.<sup>17,24</sup> The UMPS gene is 480 amino acids long with six exons. While the amino acid sequence for the UMPS gene has an N-terminal of 214 amino acids and contains OPRT, the C-terminal of the enzyme is 258 amino acids and contains OMPDC. The underlying genetic defect may occur due to biallelic missense mutations which lead to amino acid substitutions.<sup>24</sup> These mutations decrease the steady-state levels of UMPS, impair UMPS binding ability, and reduce UMPS catalytic activity. Variants may include null alleles and missense changes. Upon investigation of two type I families, researchers discovered that polymorphisms within exon regions may not reduce the function of OPRT or OMPDC, as discovered with G231A and 440Gpoly.<sup>14</sup>

### Clinical Presentation

Patients with hereditary orotic aciduria may present with symptoms primarily before twelve months of life.<sup>13</sup> Though an alteration in pyrimidine synthesis disrupts many biological processes, clinical presentation of UMPS defects mainly includes phenotypic expression of mental retardation and neurological dysfunction.<sup>13,25</sup> Deficient quantities of pyrimidines will impact the stability of erythrocytes, causing macrocytic hypochromic megaloblastic anemia in type I and II.<sup>19</sup> Neurological deficits occur in type I, II, and III because of a decrease in pyrimidine nucleosides which are converted into nucleotides (CTP, UTP, and TTP). Nucleotides are important in metabolic reactions and DNA and mRNA synthesis.<sup>14</sup> Both type I and II hereditary orotic aciduria will present with megaloblastic anemia. Type III does not present with megaloblastic anemia, but it is still unclear as to why the bone marrow does not produce abnormal erythrocytes in type III patients.<sup>22</sup> Heterozygous UMPS-mutations can lead to orotic aciduria that appears mild or isolated and does not cause clinical abnormalities.<sup>24</sup>

### Signs/Symptoms

When nucleotide synthesis is impaired, as for patients with UMPS-mutations, then the functions of the nervous system, the renal system, the immune system, and the integumentary system become compromised. First, insufficient pyrimidines will lead to the manifestation of symptoms within the nervous system which include growth retardation, developmental delay, intellectual disability, strabismus, motor impairment, and hypotonia.<sup>13,24</sup> Symptoms that may occur include delayed growth and developmental/psychomotor retardation (type I, II, III), congenital malformations (type II), and immune deficiencies (type I,II).<sup>20</sup> A patient with type III may often suffer from neurological abnormalities, congenital development concerns, and developmental delay. However, neurological abnormalities and developmental delays are also possible in type I and II. Besides neurological symptoms, the abnormal function of the renal, immune, and integumentary system will also generate symptoms within some patients.

For the renal system, orotic acid crystallizes in the urine because there are large amounts of insoluble orotic acid present in the kidneys.<sup>2,26,27</sup> Moreover, crystallization can lead to renal failure due to the obstruction of the ureters and urethra by crystallized orotic acid. Other signs affiliated with this disease include hematuria and splenomegaly.<sup>13,27,28</sup> Defects in the immune system will occur because nucleotides promote the synthesis of cell types that are necessary in cell-mediated immunity and for defense from bacterial and fungal invasion.<sup>29</sup> If nucleotide synthesis is impaired, then immune function will also become impaired and lead to a reduced ability to fight infection. Defective pyrimidine synthesis can vary from normal to low T cell number and T-cell mediated cell death, defects in the delayed type hypersensitivity response, and reduced T levels of serum IgG and IgA.<sup>28</sup> Immunological involvement may also cause neutropenia, leukopenia, and lymphopenia (particularly due to infections of candidiasis, fatal varicella, and meningitis).<sup>13,22,24,30</sup> In regards to the integumentary system, impaired pyrimidine synthesis will lead to the generation of scant hair and reduced nail growth. Heterozygous individuals exemplify orotic aciduria but are asymptomatic.<sup>2</sup>

### Diagnosis

Most inherited defects of pyrimidine metabolism are difficult to diagnose due to the rarity of this condition, heterogeneous presentations which may appear similar to other disorders, and their varied phenotypic spectrum which is still under investigation.<sup>9</sup> Type I, II, and III orotic

aciduria can be differentiated from a urea cycle disorder, as it is not characterized by hyperammonemia or an altered amino acid profile. Definitive diagnosis of type I or type II orotic aciduria is based on megaloblastic anemia without a deficiency in B12 or folic acid as well as abnormal OMPDC/OPRT activity and hyperoroticaciduria.<sup>4</sup> Type III diagnosis will be due to motor or neurological impairment, as well as to low enzyme activity and hyperoroticaciduria.

Since pyrimidines will be quickly cleared from blood and CSF by the renal system, the best diagnostic marker for pyrimidine deficiencies is through urine analysis.<sup>20</sup> Upon urinary evaluation, homozygotes may present with more than one millimole (mmol) of orotic acid per millimole creatinine, whereas disease free individuals have ~1 micromole ( $\mu\text{mol}$ ) orotic acid excreted per mmol creatinine.<sup>5</sup> Elevated orotic acid levels include concentrations greater than 60 nanomoles (nmoles) of orotic acid per milligram (mg) of creatine.<sup>31</sup> Urinary orotic acid concentrations of 500-1000x normal in homozygotes with a UMPS mutation will lead to orotic acid crystals, especially during dehydration.<sup>2,5,18</sup> Orotate (OA) will be great in concentration when the wild-type UMPS is functional. If OMPDC is inhibited, then this factor indicates that orotidine monophosphate (OMP) is great in concentration. Such tests determine the concentration of both intracellular amounts of OA and OMP.<sup>16</sup> An orotate to orotidine ratio above 10 will occur in type I patients. Type II presents with a orotate to orotidine ratio that is lower than type I due to increased orotidine levels. The urinary analyses of patients with type III include equimolar orotate to orotidine concentrations, or a ratio of about 1. It is best to diagnose this disease within the neonatal or infancy period because if pyrimidine nucleoside supply is reduced, it may be the main cause of neurological symptoms.<sup>13,28</sup>

### Prognosis

If hereditary orotic aciduria is detected early, treatment can be administered for the lifetime of the patient. The prognosis for a patient with type I, II, or III orotic aciduria is good if the UMPS defect is detected early and if oral uridine treatment is also started early. In addition, due to early uridine treatment, then normal psychomotor development may be attainable (See Case Study 1).<sup>18</sup> Pyrimidine replacement therapy can lead also to remission and reduced urinary orotic acid levels.<sup>13</sup> Late detection of the disorder may not allow neurological deficits to be reversed. Late detection may lead to coma, seizures, or death.

### Management

Early diagnosis and a distinction between type I and II orotic aciduria, vitamin B12, and folate deficiency must be made for proper treatment. Hereditary orotic aciduria type I and II causes hypochromic erythrocytes or megaloblastic bone marrow that are not responsive to common hematonic treatments of pyridoxine, iron, folic acid, or vitamin B12.<sup>13,22</sup> One of the early cases of congenital orotic aciduria was managed with an oral cytidylic-uridylic compound. Case Study I discussed earlier also revealed that oral administration of uridine can reduce symptoms and restore the patient to normal developmental milestones.<sup>1</sup> In 2015, the United States Food & Drug Administration approved the drug Uridine triacetate (Xuriden), which was developed by Wellstat Therapeutics, Inc. for treatment of hereditary orotic aciduria.<sup>32</sup> Xuriden is the current drug for lifelong treatment of orotic aciduria type I and II.<sup>13,33</sup> Oral uridine is an effective therapeutic agent because it can be ingested in granules with milk, formula, applesauce, pudding, or yogurt, so it is in a usable form for pyrimidine synthesis.<sup>32</sup> The prescription is a

single daily dosage of 60 mg/kg, though as long as the dosage is not more than 8g, the patient may take up to 120 mg/kg daily.<sup>33</sup> Other research discusses the administration of the nucleoside uridine in doses of 50–300 mg/kg/ daily as effective therapy. Xuriden provides uridine as the substrate for tissue kinase to form UMP. UMP is the precursor for uracil, cytosil, and thymine nucleoside synthesis.<sup>19</sup> The effectiveness of uridine triacetate to accomplish the completion of the *de novo* pyrimidine synthesis pathway is due to its ability to be transported across the blood-brain-barrier and into cells throughout the body. Additionally, pyrimidine replacement reduces urinary excretion of orotic acid and generates clinical and hematological remission. Treatment has not yet been established for type III.

## OROTIC ACIDURIA DUE TO UREA CYCLE DEFECTS

### Defects in Urea Cycle Enzymes

Orotic aciduria can occur in urea cycle disorders when there is a defect in an enzyme such that carbamoyl phosphate builds up. The most common defect in ureagenesis is an X-linked deficiency which correlates with impaired activity of the mitochondrial matrix enzyme ornithine transcarbamoylase (OTC).<sup>34,35</sup> The prevalence of OTC deficiency has been reported as 1:40,000.<sup>34</sup> A defect in ornithine transcarbamoylase (OTC) will generate orotic aciduria because within the urea cycle, OTC is normally responsible for coupling carbamoyl phosphate with ornithine to form citrulline.<sup>35</sup> If there is a defect in this enzyme, then the levels of carbamoyl phosphate build up and are shuffled to an alternate pathway, the pathway for orotic acid synthesis (See **Figure 6**).<sup>23</sup> The more carbamoyl phosphate accumulates, the more carbamoyl phosphate is pushed into the pathway for the formation of orotic acid. With a deficiency in OTC, upon a liver biopsy there is low enzyme activity of OTC after enzymatic assay, low levels of citrulline in serum, and hyperammonemia.<sup>35,36</sup> In addition, orotic acid can be found in the urine of OTC deficient patients.<sup>34</sup> The clinical manifestations of OTC deficiency also include lethargy and neurological deficits such as coma.<sup>37</sup>

With early diagnosis, particularly if family history is revealed, and treatment of the hyperammonemia occurs early, then the life expectancy of the patient may increase. When knowledge of the family history reveals affected individuals, patients should be monitored in the neonatal period because of an increased risk for coma or death.<sup>35</sup> Mental retardation may present for boys with OTC deficiency forms milder than others. Girls heterozygous for the mutation may present with milder symptoms compared to boys.<sup>34,35,37</sup> Hyperammonemia can generate coma, ataxia, seizures, and cerebral edema in more serious cases.<sup>37</sup> Since hyperglutaminemia may also accompany the hyperammonemia, it may also be used as a diagnostic tool.<sup>35,37</sup> In the case of a 2-day-old infant whose mother was heterozygous for OTC deficiency, death of the child occurred due to the impacts of hyperammonemia.<sup>34</sup> Symptoms that can confirm OTC deficiency after birth include lethargy, difficulty feeding, respiratory alkalosis, and encephalopathy.<sup>34</sup> Treatment for this disorder includes the intravenous administration of sodium benzoate, sodium phenylacetate, or arginine hydrochloride.<sup>37</sup>

There is a slight elevation in urinary orotic acid levels when the urea cycle enzymes argininosuccinate lyase (AL), arginase, argininosuccinic synthetase (AS), and arginase are defective. (See **Figure 6**).<sup>23</sup> The OTC gene is found on chromosome Xp21.1, primarily in the liver. The AS gene is found on chromosome 9q34 and is expressed in the liver and the skin. AL can be found on the AL gene of chromosome 7cen-q11.2. Expression of the AL gene is

predominant in the liver, skin, and erythrocytes. The ARG1 gene of chromosome 6q23 codes for Arginase.

AS is an autosomal recessive deficiency which is characterized by both orotic aciduria and citrullinemia. With normal AL activity, AL breaks arginosuccinate to fumarate (for the TCA cycle) and arginine. Abnormal activity will also lead to an accumulation of orotic acid, due to an accumulation of the precursor products in the pathway (See **Figure 6**).<sup>23</sup> Orotic aciduria also occurs with the autosomal recessive defect in arginase. Instead of hydrolyzing arginine to ornithine and urea in the completion of the urea cycle, this rare disorder leads to an accumulation of ammonia, arginine, and orotic acid.<sup>38</sup> Therapy for AS and AL deficiency includes supplementation of arginine. Arginine supplementation will promote the removal of amino acids and nitrogen waste products because arginine will allow citrulline and arginosuccinate to be synthesized.<sup>39</sup>

In conclusion, it is important to determine whether orotic aciduria is due to a primary or secondary factor. To distinguish the specific cause of orotic aciduria, defects in urea cycle enzymes may initially be considered. However, laboratory measurements of orotate and orotidine can be used in the diagnosis of type I, II, and III hereditary orotic aciduria. Diagnosis of primary orotic aciduria (type I, II, III) can be confirmed when the patient does not have hyperammonemia. A lack of hyperammonemia eliminates disorders of urea cycle enzymes such as ornithine transcarbamoylase (OTC), arginosuccinate lyase (AL), arginosuccinic synthetase (AS), and arginase as the precipitating cause for excess orotic acid in the urine.

#### Defects in Urea Cycle Transporters

A functional deficiency in the transporters Citrin (aspartate-glutamate transporter) and ORNT-1 (ornithine transporter) will also cause elevated orotic acid levels. There will be normal arginosuccinic acid (ASA) levels and increased citrulline and arginine plasma levels if either transporter is defective. The aspartate-glutamate transporter is coded by the gene CITRIN, found on 7q21.3, and is expressed mainly in the liver. ORNT-1 is coded by the ORNT1 gene which is found on chromosome 13q14 and expressed in the liver and skin. A second ORNT transporter, the hepatic ORNT-2, may also impact orotic acid excretion if it is also defective.

#### DRUG-INDUCED OROTIC ACIDURIA

Patients should avoid the following pharmacogenetic drugs which further affect pyrimidine metabolism: nifedipine and nimodipine. Nifedipine and nimodipine are calcium channel blockers that competitively inhibit uridine kinase and OMPDC (See **Figure 4**).<sup>20</sup> Drugs such as allopurinol, 5-fluorouracil, and 6-azauridine will lead to an increase in urinary orotic acid content by inhibiting the final steps of the pyrimidine pathway. Allopurinol will compete with orotic acid for binding to orotate phosphoribosyltransferase. Uracil and *de novo* pyrimidine levels reduce because orotate phosphoribosyl transferase generates the oxypurinol nucleotide from allopurinol, and this nucleotide inhibits orotidylate decarboxylase. The influence of allopurinol causes a reduction in pyrimidine levels and simultaneous accumulation of orotic acid and orotidine in the cell, thus spilling over in high concentration to the urine. The anticancer drug 5-fluorouracil will worsen orotic aciduria because it is phosphoribosylated by orotate phosphoribosyl transferase.

The anticancer drug 6-azauridine (6-AZUR) is converted to 6-azauridylate, and 6-azauridylate will inhibit orotidylate decarboxylase and promote increased orotic acid and orotidine excretion (See **Figure 7,8**).<sup>19,40-42</sup> ATP and Mg<sup>2+</sup> are required for phosphorylation of 6-AZUR.<sup>41</sup> Phosphorylated 6-AZUR is responsible for the competitive inhibition of OMPDC.<sup>41</sup> Fallon et al. performed urine analyses of orotic acid on six leukemia patients receiving intravenous 6-AZUR dosages for treatment.<sup>42</sup> Their investigation of plasma and urine levels of orotic acid suggest that highest orotic acid excretion occurs with high dosage of 6-AZUR. These dosages are 120-200 mg per kg.<sup>42</sup> A second discovery was that orotic acid excretion reduced after three days, whereas orotidine excretion in the urine was still evidenced one to three days after orotic acid excretion stopped.<sup>42</sup> With administration of 6-AZUR, orotidine levels did not decline constantly as the orotic acid levels, but instead continued at the same maximum excretion value, even when 6-AZUR was removed.<sup>42</sup> Since feedback pathways regulate pyrimidine synthesis, if 6-AZUR is administered as an anticancer agent, it blocks pyrimidine synthesis and increases orotic acid production (See **Figure 7,8**).<sup>41,42</sup> Excess orotic acid may be converted to orotidylic acid. As a result, after conversion to orotidylic acid (orotidine 5'-monophosphate), orotidylic acid pyrophosphorylase will generate the product orotidine.

### IDIOPATHIC OROTIC ACIDURIA

Mitochondrial disorders, hepatic fibrosis, lysinuric protein intolerance, Reye syndrome, malignancies, and trauma can also induce orotic aciduria.<sup>21</sup> Reye syndrome correlates with damaged mitochondria such that carbamoyl phosphate is channeled into orotic acid synthesis. Mother's milk consists of pyrimidine nucleosides, and if feeding is delayed or if an infant formula is given without nucleosides to an infant with hereditary orotic aciduria, this could correlate with neurological symptoms (See Case Study II).<sup>2</sup>

### CONCLUSION

Though the first identified cases for this disease were in 1959, laboratory examinations, early diagnosis, analysis of family history, and prompt treatment with oral uridine is crucial for reducing potential motor and neurological impairment. For case study I and II presented above, since both the enzymes OPRT and OMPDC had very low activity compared to controls, this is why the physicians could propose an inborn error of metabolism. Reflecting on the patients in case I and case II, both newborns had low birth weight, difficulty feeding, and low enzyme activity. In case I, the presence of megaloblastic anemia suggests a type I or type II hereditary orotic aciduria (See **Table I**). Interestingly, studies have shown that not all cases will present with megaloblastic anemia (type III orotic aciduria). Though not specified, this may have been the subtype for the male patient in case II (See **Table I**). Other considerations may be needed when discussing the phenotypic severity of disease. For example, when comparing a female to a male patient with this disorder, whether or not lyonization has inactivated an X chromosome for the female and thus reduced the phenotypic severity of the disease should also be considered.

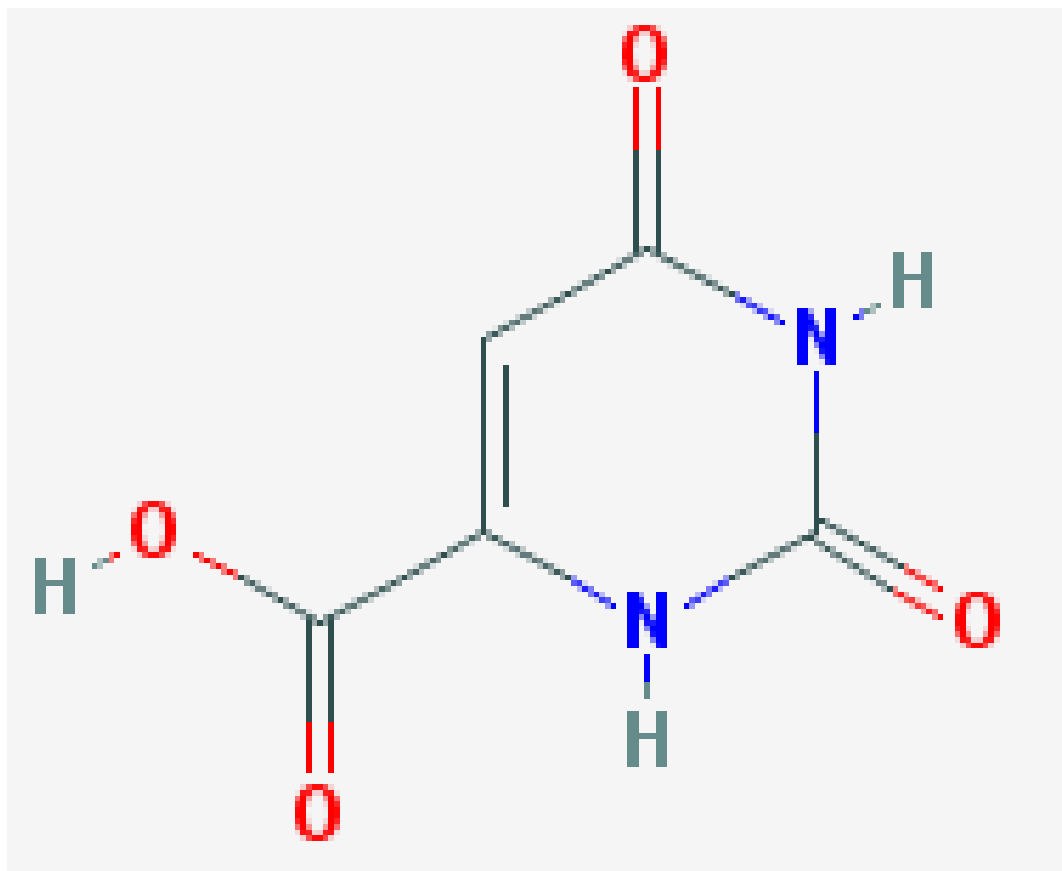
Dangers of excess orotic aciduria include acidosis in three forms: organic acidemia, organic aciduria, and metabolic acidosis. Acidosis causes mental and physical impairment, so a delay in diagnosis and treatment could lead to abnormalities in key organ systems such as the brain or heart, seizures, coma or death.<sup>3</sup> Children who are treated and live may have developmental and intellectual delays. However, as exhibited in case study I, some patients are

able to attain normal developmental milestones, birth weight, and birth height. If therapy with oral uridine is not administered after an early diagnosis, then in patients with severe phenotypes, coma or death may ensue. Early diagnosis of moderate phenotypes can reduce significant mental abnormalities. However, whether early diagnosis can reverse any neurological impairment in severe phenotypes may need to be studied further. Though hereditary orotic aciduria is a rare disease, the impact on *de novo* pyrimidine synthesis embodies the reason why early symptoms should be investigated, why an early diagnosis and analysis of family history is crucial for proper therapy, and how combinations of both may increase the potential of life for the patient.

Several other inborn errors of metabolism besides hereditary orotic aciduria can also lead to excess orotic acid in the urine. These inborn errors include the following: argininemia, lysinuric protein intolerance/lysurinic protein intolerance syndrome, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH), ornithine transcarbamoylase (OTC) deficiency, citrullinemia type I, and purine nucleoside phosphorylase deficiency.<sup>3</sup> However, hereditary orotic aciduria (type I, II, III) is principally investigated because it is the most common of these errors. Impacts of anticancer drugs on the pyrimidine synthesis pathway could be studied further, since addition of these compounds impairs pyrimidine synthesis. Treatment for type III orotic aciduria, which is still unknown, should be investigated. Improved databases for identification of genetic mutations and categorization of these mutations based on phenotypic severity are needed for earlier detection of future cases. Currently, databases are not up to date with the genomic deletions involving UMPS. Different databases record deletion and missense variations but provide clinicians with little to no information on which mutation is pathogenic or benign. Future research should focus on finding better screening methods and thus improved detection of type I, II, and III orotic aciduria. Researchers should explore treatment options for type III orotic aciduria and further analyze the biochemistry affiliated with the question “Why does type III not cause megoblastic anemia?”. Studies of the role of the OAT transporters may help in designing a therapy to remove excess orotic acid and to reduce crystalluria.

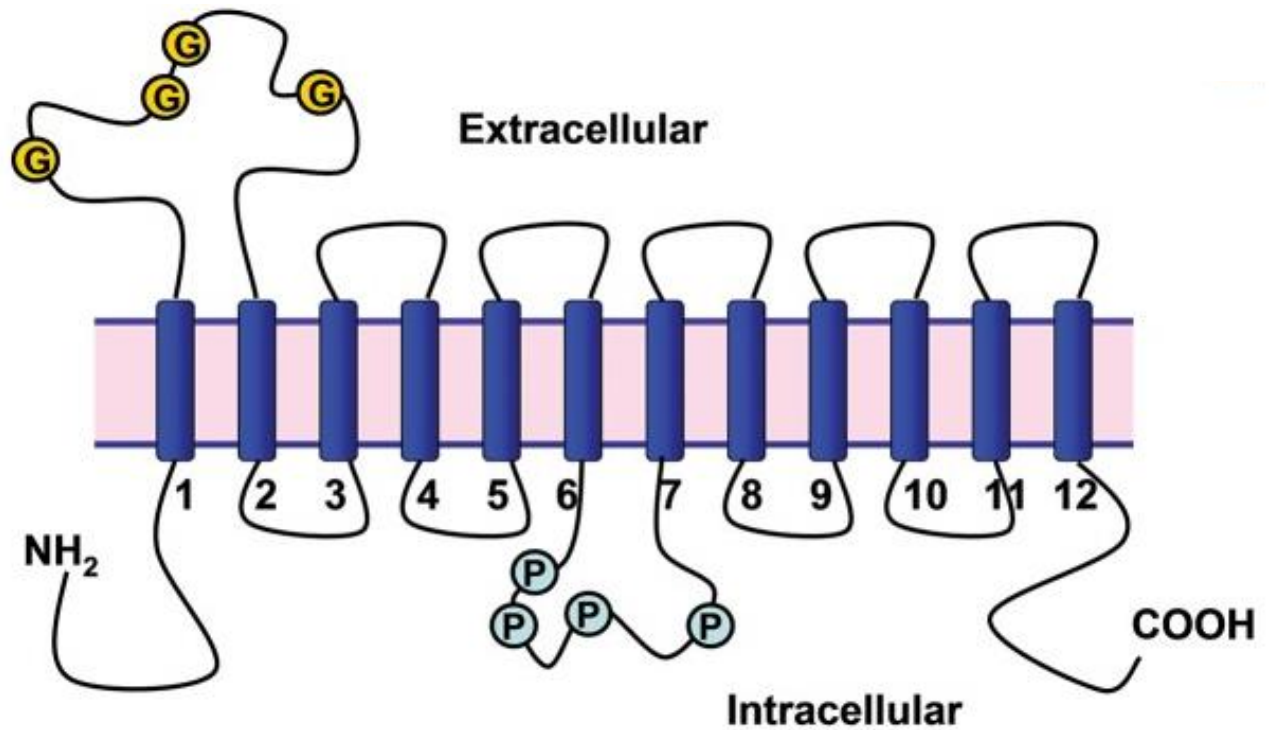


FIGURES



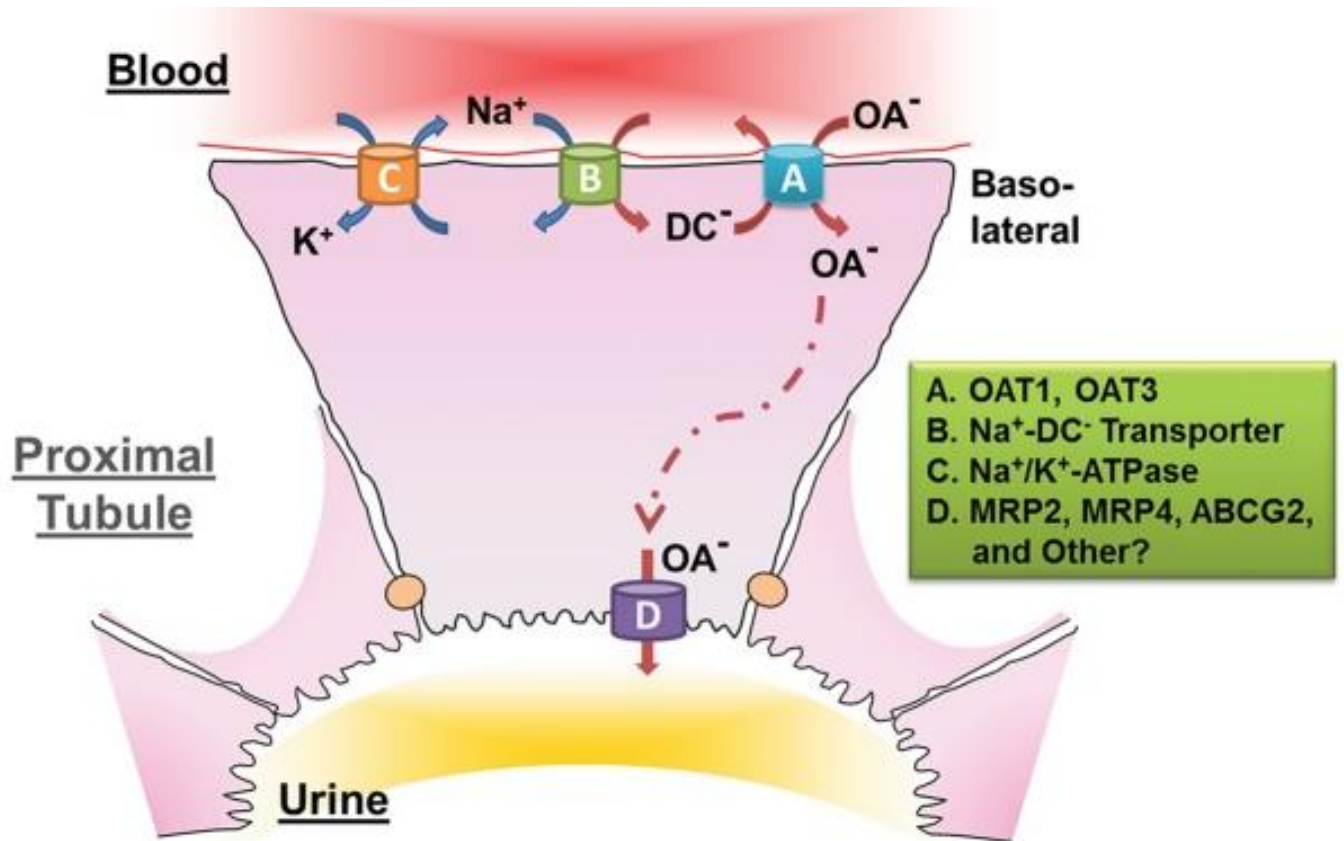
**Figure 1. Orotic Acid (C<sub>5</sub>H<sub>4</sub>N<sub>2</sub>O<sub>4</sub>) Structure.<sup>3</sup>**

Orotic acid is a precursor to pyrimidine synthesis. It is a 156.1 g/mol compound that functions as an acid and a metabolite. In excess, orotic acid can spill over into the urine and form orotic acid crystals.



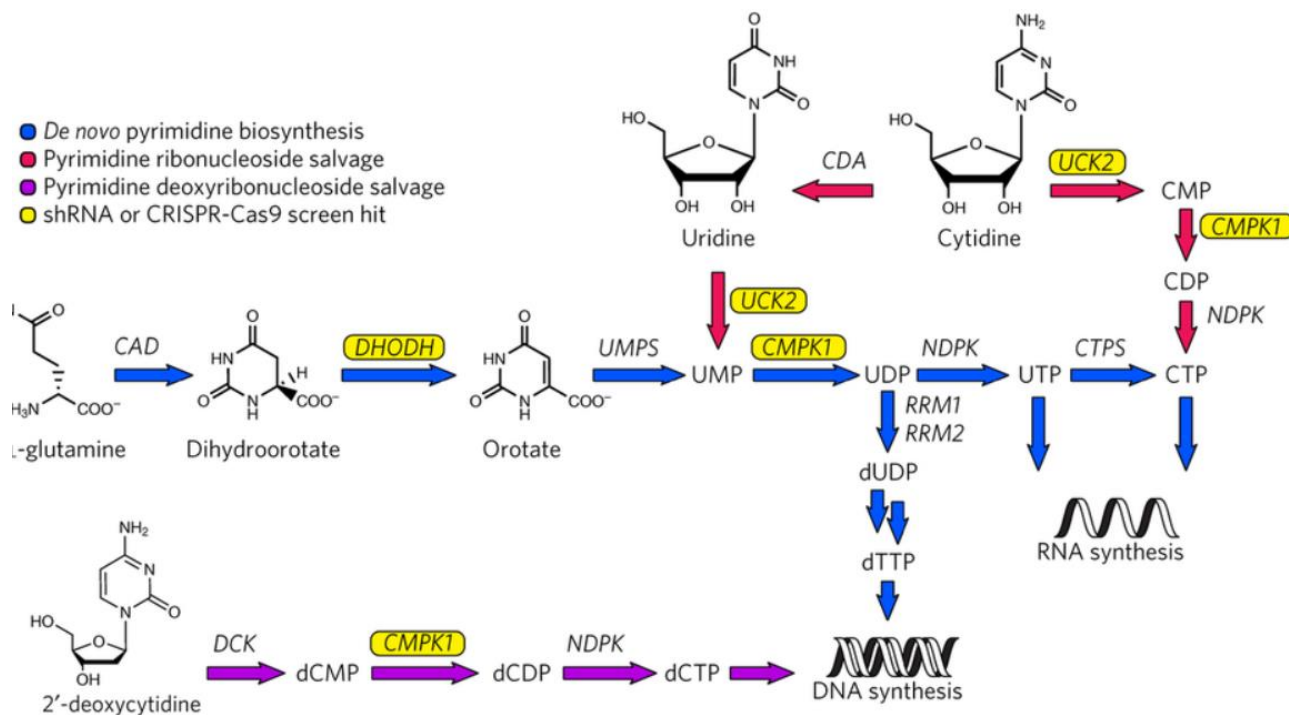
**Figure 2. Structural Composition of OAT and proposed transport mechanism from blood to urine.<sup>7</sup>**

The above figure is the proposed structure of organic anion transporters (OAT). A total of twelve transmembrane loops (two pairs of six domains) are present. Two major loops are formed in the transporter- the first loop is located extracellularly while the second loop is located intracellularly. The first loop contains sites for glycosylation, whereas the second loop between sites 6-7 represents the substrate for protein kinase C (PKC) phosphorylation. The termini of the protein include an amino (NH<sub>2</sub>) and carboxy (COOH) terminus which is located within the cell.



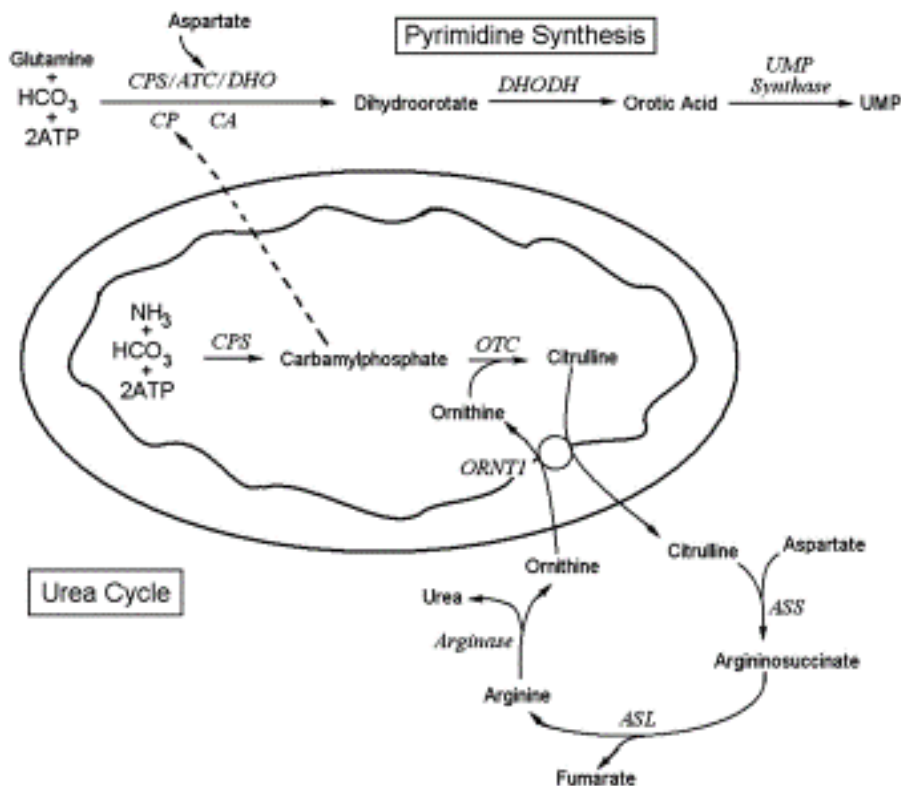
**Figure 3. Renal Proximal Tubule Transport of Organic Anions.<sup>7</sup>**

The above illustration attempts to explain the mechanism of secondary active transport of organic anions from the blood to the urine by renal proximal tubule cells. As organic anions enter the basolateral side of the tubule from the plasma, the OAT transport system exports dicarboxylates from the intracellular space to the plasma. A second transporter, a Na<sup>+</sup>/dicarboxylate symporter, brings both Na<sup>+</sup> and carboxylates in from the plasma to the tubule. The concentration of dicarboxylate and Na<sup>+</sup> couple to allow OAT transport of organic anions, but since dicarboxylates are transported against their concentration gradient, the energy for this process is also dependent upon the electrochemical gradient established by Na<sup>+</sup>-K<sup>+</sup>-ATPase. Organic anions can be transported into the urine across the apical membrane.



**Figure 4. De Novo Pyrimidine Synthesis.**<sup>43,45-52</sup>

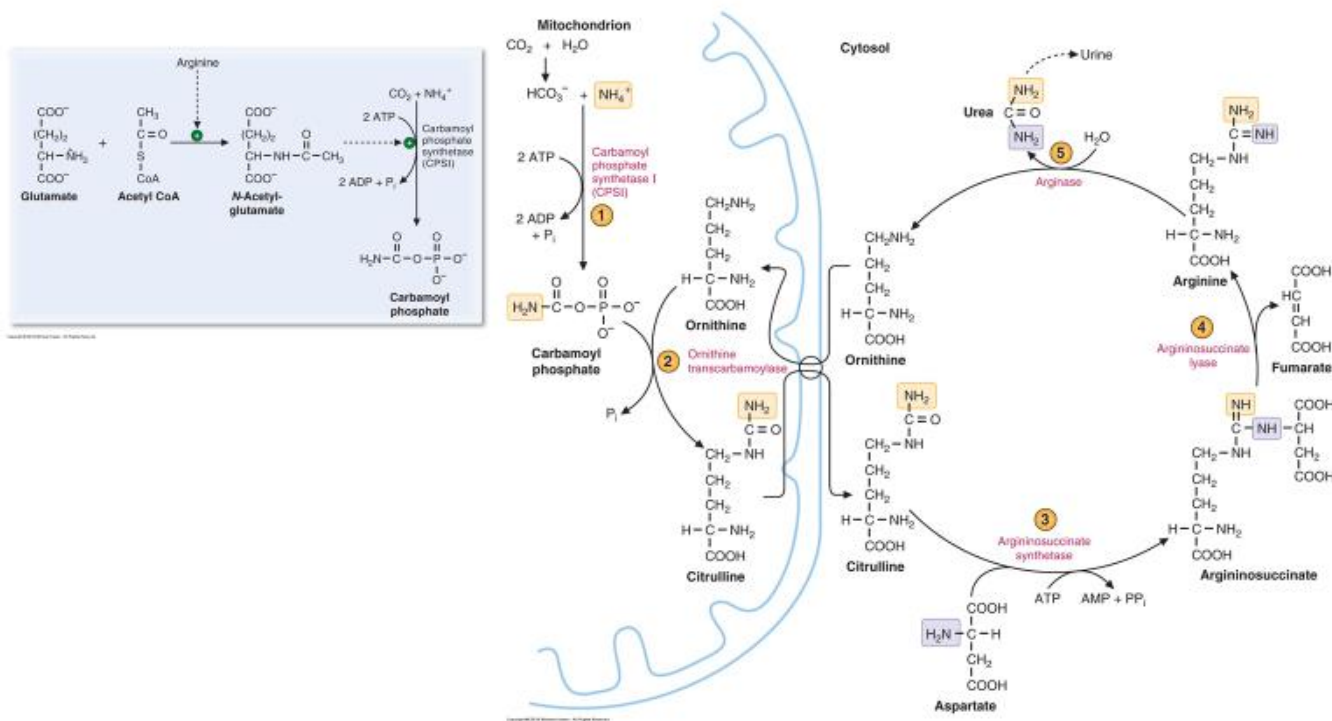
The pathway for pyrimidine synthesis is illustrated according to the blue arrows. Important enzymes for this pathway include: CAD, DHODH, UMPS, CMPK1, NDPK, CTPS, and RRM1 and RRM2. CAD is the rate-limiting enzyme for pyrimidine synthesis and generation of dihydroorotate.<sup>45</sup> Dihydroorotate dehydrogenase (DHODH) converts dihydroorotate to orotate.<sup>46</sup> Uridine Monophosphate synthase (UMPS) forms UMP.<sup>47</sup> Cytidine/Uridine Monophosphate Kinase 1 (CMPK1) is responsible for phosphoryl transfer from ATP to UMP, to CMP, and to dCMP.<sup>48</sup> Nucleoside diphosphate kinase (NDPK) phosphorylates targets and transfers the  $\gamma$ -phosphate from GTP, UTP, and ATP.<sup>49</sup> Cytidine Triphosphate Synthase (CTPS) is responsible for the rate-limiting step of cytidine triphosphate (CTP) synthesis from uridine triphosphate.<sup>50</sup> Ribonucleotide Reductase Catalytic Subunit M1 (RRM1) and Ribonucleotide Reductase Catalytic Subunit M2 (RRM2) convert ribonucleotides into deoxyribonucleotides.<sup>51,52</sup>



From: Orotic Acid Excretion and Arginine Metabolism  
 J Nutr. 2007;137(6):1656S-1661S. doi:10.1093/jn/137.6.1656S  
 J Nutr | © 2007 American Society for Nutrition

**Figure 5. Pyrimidine Synthesis from Carbamoyl Phosphate** <sup>5,20,44</sup>

This figure illustrates that carbamoyl phosphate will be translocated to the cytosol for the formation of carbamoylaspartate by aspartate transcarbamoylase. The amide group of glutamine will be used to donate nitrogen and the cyclization of carbamoylaspartate forms dihydroorotate. Dihydroorotate dehydrogenase(DHODH) will convert dihydroorotate to orotic acid by oxidation with  $\text{NAD}^+$ . 5-phosphoribosyl-1-pyrophosphate (PRPP) will donate a ribose phosphate group to orotic acid to form orotidylate by pyrimidine phosphoribosyltransferase. The decarboxylation of orotidylate generates uridylate by the activity of orotidylate decarboxylase. Uridine monophosphate synthase (UMPS) will convert orotic acid to uridine monophosphate (UMP). Not shown is the conversion of UMP to UDP by UMP kinase. Next, UDP is converted to UTP, then CTP. The active forms of nucleotides exist as triphosphates, so the activity of a monophosphate kinase (uridylate kinase) and nucleoside diphosphate kinase is necessary.<sup>22</sup>



**Figure 6. Urea Cycle**<sup>23</sup>

This figure illustrates the key enzymes of the urea cycle. A deficiency in N-acetylglutamate synthase (NAGS) or carbamoyl phosphate synthetase I (CPSI) will not cause orotic aciduria. Only a defect in an enzyme within this pathway that leads to an accumulation of carbamoyl phosphate will generate the transport of excess carbamoyl phosphate to the cytoplasm to be utilized in the pyrimidine cycle. Within the urea cycle, it is deficiencies in the mitochondrial ornithine/citrulline transporter, the cytosolic enzymes arginase, argininosuccinate synthetase, and argininosuccinate lyase that can lead to an accumulation of carbamoyl phosphate within the hepatic mitochondria.<sup>23</sup> Arginase deficiency can also lead to orotic aciduria.

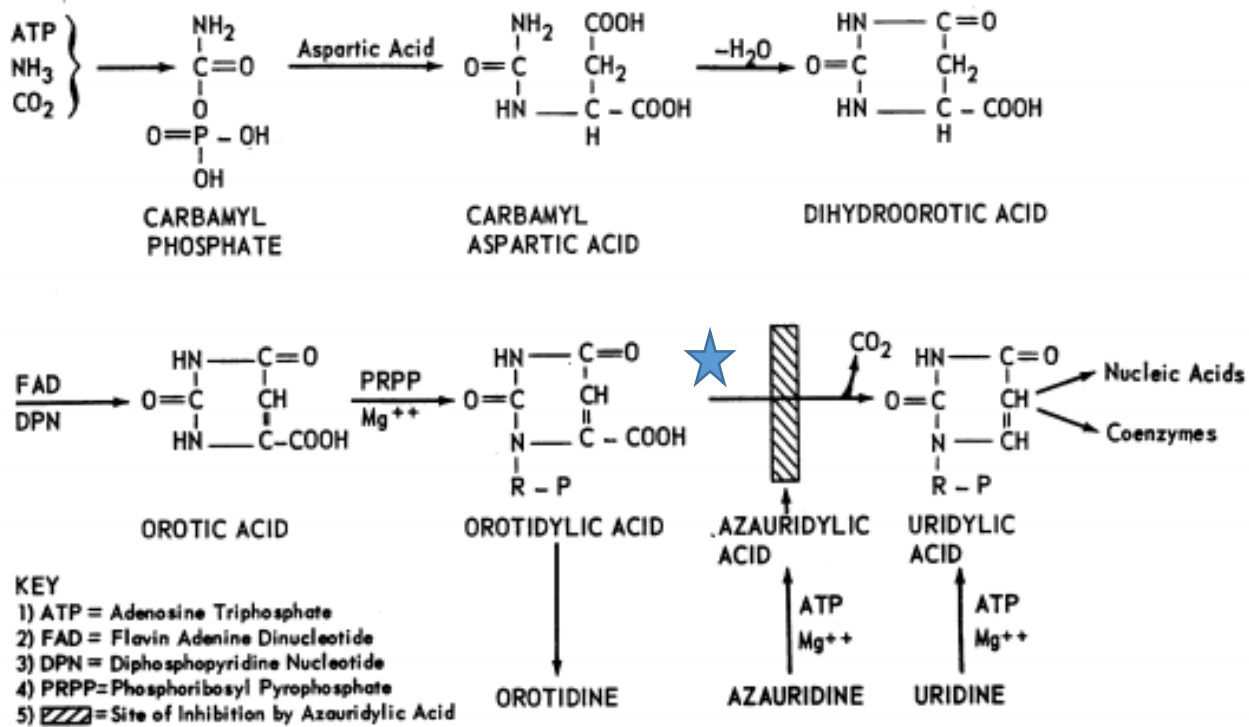
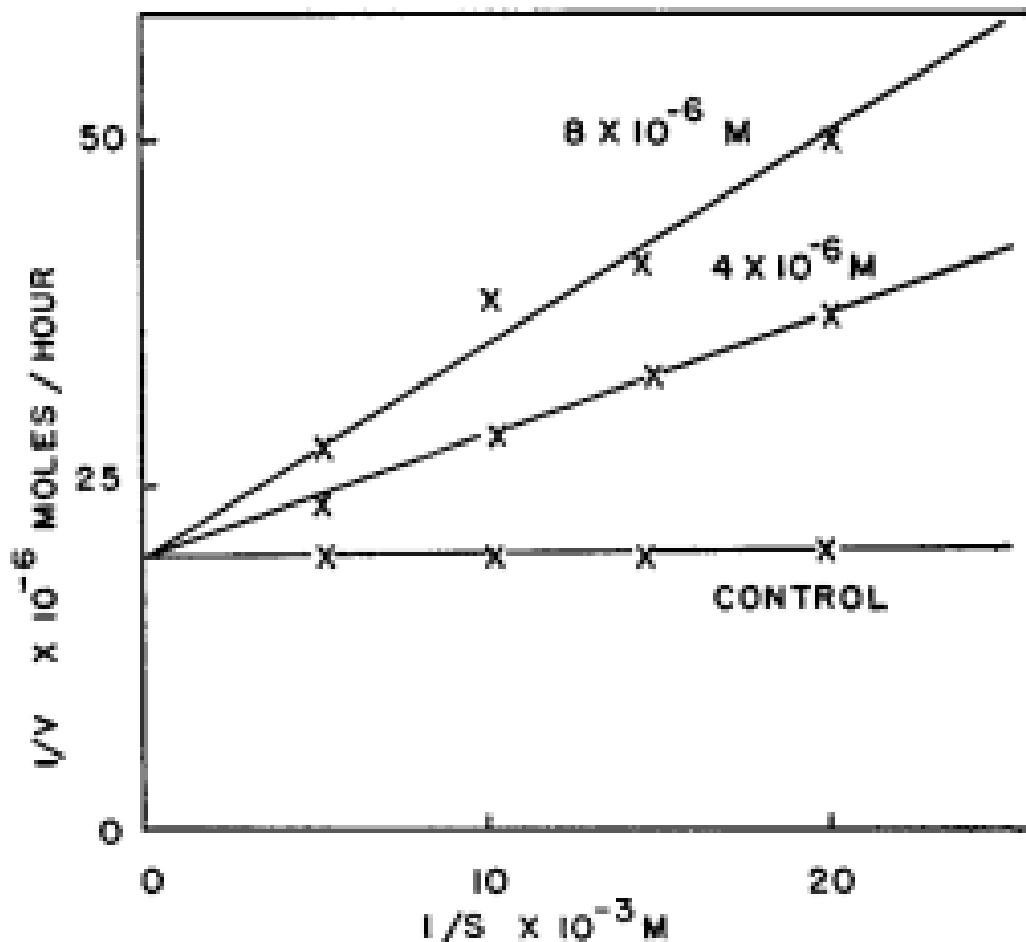


Figure 7. Synthesis of Pyrimidines is Inhibited by 6-AZUR.<sup>42</sup>

The drug 6-azauridine (6-AZUR) is able to competitively inhibit enzyme activity of orotidylic acid decarboxylase (represented by the blue star), thus preventing the decarboxylation of orotidylic acid to uridylic acid. Additionally, the completion of the *de novo* pyrimidine synthetic pathway is disturbed.



**Figure 8. Lineweaver-Burk Plot of Competitive Inhibition of Orotidylic decarboxylase by Azauridine 5' Phosphate.** <sup>41</sup>

A kinetic study conducted with 0.05 M Tris Buffer at pH 8 and 25° C generated enzyme units for the enzyme that was 0.05/mL. Earlier pH studies determined that at pH 8, the enzyme activity was interrupted most significantly by 6-AZUR. The active form of 6-AZUR occurs when the compound is in a solution at a pH above 7, and ionized at the carboxylic position.



**Table 1. Distinguishing features between Type II and Type III Hereditary Orotic Aciduria<sup>13,16</sup>**

<b>Distinguishing Features</b>	<b>Type I</b>	<b>Type II</b>	<b>Type III aka Orotic Aciduria Without Anemia (OAWA)</b>
<b>Enzyme Impacted</b>	loss of UMPS activity- both OPRT and OMPDC are defective	specific inactivation of OMPDC, mechanism is unknown.  an increase in OPRT activity	deficiency in OMPDC that can lead to a qualitative change in UMPS  OPRT activity is present but low
<b>Type of mutation</b>	autosomal recessive mutation	autosomal recessive mutation	the case where this subtype first presented revealed a homozygous missense mutation within a conserved region of the ODC domain of the UMPS gene(c.928 T4>G; p.Phe310Val)
<b>Megaloblastic anemia</b>	macrocytic hypochromic megaloblastic anemia occurs	macrocytic hypochromic megaloblastic anemia occurs	does not present with macrocytic hypochromic megaloblastic anemia
<b>Urinary levels of orotate, orotidine</b>	orotate to orotidine ratio above 10	orotate levels similar to type I, but there is an increase in orotidine levels so the ratio is lower	equimolar orotate to orotidine concentrations, or a ratio of about 1
<b>Clinical distinguishability (compared to Type I)</b>	N/A	clinically indistinguishable from type I	able to be distinguished from type I
<b>Signs/Symptoms</b>	Delayed growth/development Psychomotor retardation Congenital malformations Immune deficiencies	Delayed growth/development Psychomotor retardation Congenital malformations Immune deficiencies	delayed growth/development Psychomotor retardation neurological abnormalities
<b>Therapy/Effectiveness</b>	Therapy includes the administration of Xuriden	Therapy includes the administration of Xuriden	Treatment is not yet established for type III

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