

Metabolic and Genetic Studies of a Family with Ornithine Transcarbamylase Deficiency

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Extract

We have described a patient with ornithine transcarbamylase (OTC) deficiency. The clinical course and pedigree substantiate the X-linked transmission of the defect with varying degrees of illness in females. Because orotic aciduria accompanied hyperammonemia in the patient, orotic acid was measured as an indication of a partial OTC deficiency after ammonia and protein loading in members of the patient's family. Both the mother, an obligate carrier, and an aunt, the only symptomatic female in this pedigree, had hyperammonemia after the ingestion of ammonium chloride. They also had significant orotic aciduria after a protein load as did the two female cousins whose ammonium tolerance was normal. The maternal grandmother excreted a large amount of orotic acid in her urine relatively consistently. These data suggest that the two female cousins and the maternal grandmother are asymptomatic female heterozygotes despite their normal ammonium loading tests.

Speculation

Protein loading in the form of a relatively palatable and innocuous meal, followed by determination of orotic acid content in urine, may prove to be a sensitive, noninvasive, and easy means to identify female carriers of ornithine transcarbamylase deficiency.

Introduction

Hyperammonemia which resulted from a deficiency of hepatic ornithine transcarbamylase (carbamoyl phosphate: L-ornithine carbamoyl transferase; EC. 2.1.3.3) has been recognized with increasing frequency since the defect was first described by Russell and coworkers in 1962 [4-7, 10, 12-15, 18, 19, 22-24, 26, 29]. The known pedigrees suggest that the gene coding for this enzyme may be X-linked, and that the deficiency is lethal in males and results in a variable degree of clinical severity in affected females [4-7, 12-15, 22-26, 29]. The only male in which the disorder has not been fatal apparently had a variant form of the disease [14].

Because of the variable manifestations of the disorder in females, and the severity in males, it would be of great value to detect the female carriers of the defect. In previous studies, assays of ornithine transcarbamylase (OTC) in liver tissue obtained by biopsy have been utilized in an attempt to identify the heterozygote, but even in an obligate carrier, the specific activity of the enzyme may be normal [26]. Ammonia determination after oral administration of ammonium chloride has also been employed to detect the carrier state, but the validity of this procedure is subject to the variability intrinsic to the assay of ammonia. Because patients with OTC deficiency have been reported

to excrete increased concentrations of both orotic acid and proline in their urine [5-7, 13, 15, 18, 19, 29], we have investigated the possibility that the determination of these two compounds may be utilized to detect the heterozygous carriers of OTC deficiency.

Patients

A pedigree of the family is illustrated in Figure 1. The proband of this study, *patient III-8*, was delivered after an uneventful pregnancy and weighed 3,430 g at birth. His mother had previously delivered two normal males, and had one spontaneous abortion. Her next pregnancy resulted in the birth of a male infant who was normal until 7 days of age when he began to have seizures and respiratory distress. He expired on the following day. Sixteen months later she delivered another male infant who followed a similar course and expired at 7 days of age. Analysis of his urine by gas chromatography revealed an elevated concentration of orotic acid. Concentrations of amino acids, carbohydrates, and other organic acids in urine and plasma were normal. A tentative diagnosis of OTC deficiency was made despite the fact that the liver of the infant was not available for enzymatic assay.

The mother conceived once again, and a diagnostic amniocentesis was carried out at 14 weeks of gestation. The concentration of orotic acid in amniotic fluid was normal, and the fetus was shown to be a male. Cells cultured from amniotic fluid grew poorly and had to be nurtured in culture media consisting of 30% calf serum. Neither these cultured cells nor cells grown from amniocenteses obtained from other patients were found to have OTC activity. We were unable to assess prenatally whether the infant would or would not be

affected. The parents, cognizant of the data, decided that the mother would carry the infant to term.

The proband was a perfectly healthy appearing male infant who nursed well and demonstrated no clinical abnormalities. Concentrations of ammonia in blood, and urea, orotic acid, proline and ammonia in urine were determined daily, and were within normal limits during the first 6 days of life except that orotic acid started to rise above normal levels on *day 5*. On the 7th day, the infant became lethargic, nursed poorly, and the concentration of orotic acid in urine was found to be increased markedly to 6 mg/24 hr (Fig. 2). That morning the concentration of ammonia was also elevated to 660 $\mu\text{g}/100$ ml blood. Protein feedings were eliminated, but within hours the infant began to have hyperventilation and seizures. Neomycin was given orally and per rectum, and peritoneal dialysis was initiated. The concentration of ammonia in blood began to fall, and the infant's seizures were controlled. Citric acid (300 mg/24 hr) was infused in an attempt to remove ammonia by reaction with the tricarboxylic acid cycle intermediate, α -ketoglutarate. Both phenobarbital and ethinyl estradiol were given to the infant in an attempt to increase the activity of hepatic OTC. Twenty percent glucose with small amounts of free amino acids was infused to prevent protein catabolism. Despite these measures, the patient expired at 25 days of age.

Postmortem examination revealed edema of the peritoneal surfaces, which was secondary to chronic peritoneal dialysis. The liver was grossly pale, but the weight and microscopic features were normal. The thymus was small and depleted of lymphocytes and eosinophiles. The brain and other organs of the body were essentially normal. Besides the apparent distress caused by prolonged peritoneal dialysis, malnutrition

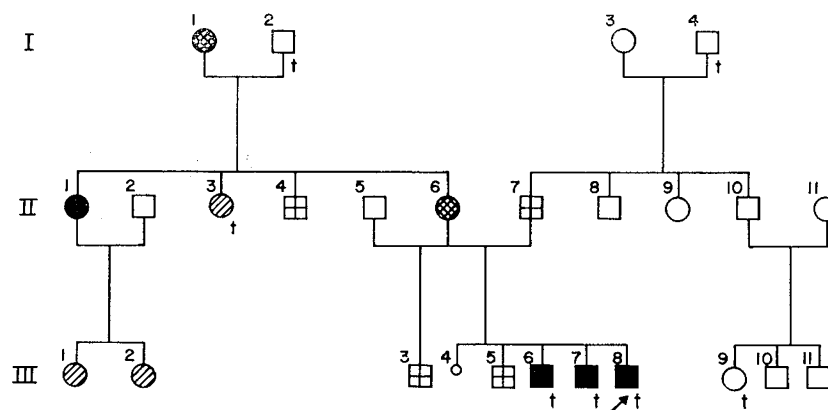


Fig. 1. Pedigree. \circ : female; \square : male; \blacktriangle : proband; \bullet : obligate carrier; \boxplus : tested, normal; \square : male; \dagger : died; $\textcircled{\text{hatched}}$: possible carrier; \blacksquare : proven or presumed ornithine carbamylase deficiency.

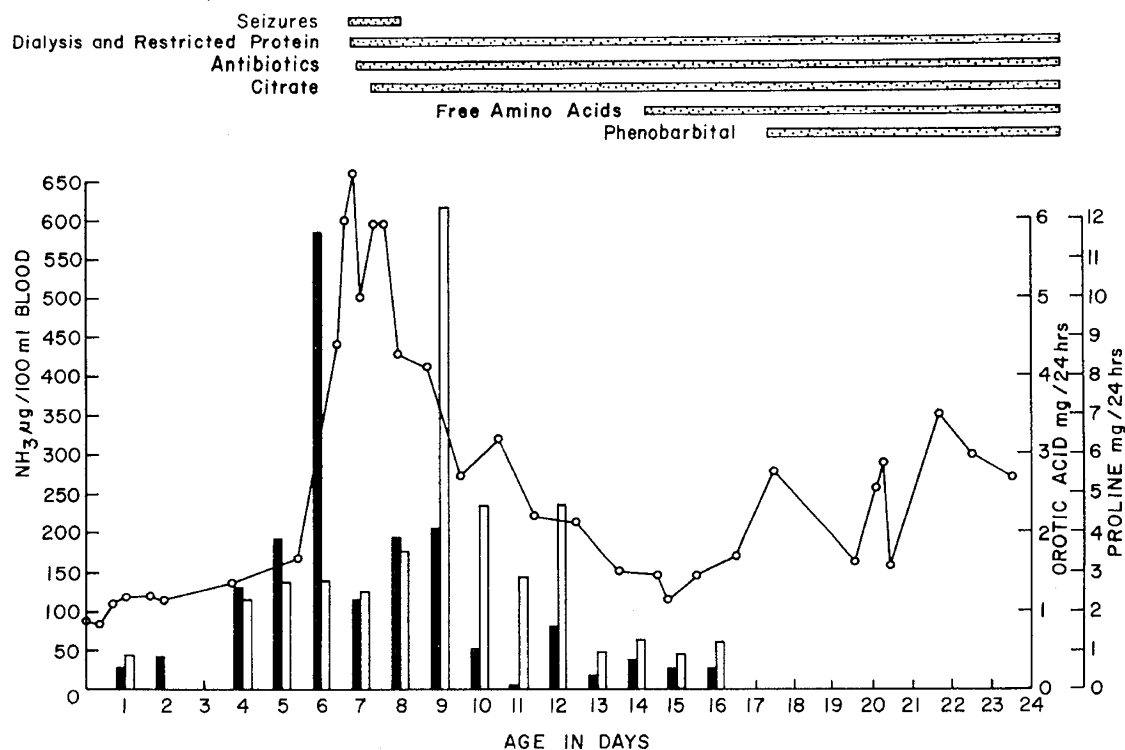


Fig. 2. Summary of clinical and biochemical course of proband. \circ — \circ : ammonia ($\mu\text{g}/100$ ml blood); \blacksquare : orotic acid in urine (mg/24 hr); \square : proline in urine (mg/24 hr).

was also likely to have been an important factor in contributing to the patient's demise.

Family Members

The mother, II-6, is a healthy, 33-year-old woman who has never had any adverse symptoms after protein ingestion.

A maternal aunt, II-3, died at 13 months of age of presumed encephalitis with convulsions and coma. Undiagnosed liver and brain abnormalities were present at autopsy.

Another maternal aunt, II-1, is the only relative with symptoms suggestive of OTC deficiency [10, 13, 22, 25, 29]. She voluntarily limits her protein intake, but when given a large protein meal or when given oral ammonium chloride, she became acutely ill with abdominal pain, nausea, vomiting, dizziness, and migraine-like headaches.

The daughters of this aunt, III-1 and III-2, are 10 and 13 years of age, respectively, and are asymptomatic.

The proband's father, II-7, maternal grandmother, I-1, and maternal uncle, II-4, have never had symptoms likely to be related to OTC deficiency.

A paternal cousin, III-9, died at 3 weeks of age of sudden infant death syndrome.

Methods

Analytical Procedures

1. Blood ammonia was determined on heparinized venous blood. Specimens were immediately placed on ice, and ammonia was measured within 20 min by the Bessman modification of the Seligson method [2].

2. Urine orotic acid was determined using a modification of the method of Adachi *et al.* [1]. Inasmuch as urine contains compounds capable of producing high blanks [21], the method of Adachi *et al.* was modified as follows. (1) It was found necessary to double the amount of *p*-dimethylaminobenzaldehyde used in determining the orotic acid content of urine because of its rapid consumption by urine reactants. Consequently, 2 ml 5% *p*-dimethylaminobenzaldehyde was used per 1 ml urine. (2) The high urine blank obtained in this colorimetric procedure occurs without bromination, whereas color development due to orotic acid does not. Consequently, duplicate samples were processed; one was brominated, the other was not and acted as a blank for the former sample. Using these two modifications, orotic acid could be accurately determined if present at a concentration between 1 and 25 μg orotic acid/ml urine. Results of single specimens

were expressed as a ratio to creatinine (as measured by Jaffe's method [8]).

3. Urine proline was determined by the method of Troll *et al.* [20].

Enzyme Assays

Tissues used for enzyme assays were obtained within 2 hr of death and stored in liquid nitrogen until analyzed.

Urea cycle enzymes were assayed using 10% tissue homogenates prepared in 0.25 M sucrose using a Potter-Elvehjem homogenizer. Carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinic acid synthetase, argininosuccinic acid lyase, and arginase were assayed by methods described previously [29]. Because the colorimetric assay for OTC revealed no activity in tissues from the proband, a more sensitive radiochemical technique was devised. In this method, the conversion of (¹⁴C)carbamyl phosphate [32] to (¹⁴C)citrulline was measured as follows: undiluted homogenates from the proband's tissues were incubated for 5 min at 37° in a total volume of 0.5 ml. The concentration of ornithine was 10 mM, (¹⁴C)carbamyl phosphate concentration was 0.2 mM, and the reaction was carried out in 50 mM glycylglycine buffer, pH 8.3. The reaction was terminated by adding 0.3 ml of 1 M HClO₄, and the resulting mixture was heated for 3 min in a boiling water bath (this will decompose excess (¹⁴C)carbamyl phosphate to ¹⁴CO₂ and inorganic phosphate). Excess ¹⁴CO₂ from the decomposition of (¹⁴C)carbamyl phosphate was removed by adding crushed Dry Ice. (¹⁴C)citrulline formed during the incubation was isolated as follows: 0.3 ml of 10 mM L-citrulline was added as carrier and the mixture was placed on a small column (1 by 2 cm) of Dowex 50-H⁺ in a disposable 5-ml syringe. The column was washed four times with 5 ml water and the citrulline was eluted with 1-ml lots of 0.5 M ammonium acetate. The fractions containing citrulline were combined, mixed, and the radioactive content of a 1-ml aliquot was measured in Aquasol [33] using a Beckman LS 233 liquid scintillation spectrometer [34].

All enzyme assays were linear for the duration of the incubation and less than 5% of the limiting substrate was consumed.

Loading Tests

1. Ammonium loading tests were carried out by oral administration of solid NH₄Cl in gelatin capsules. Adults were given 3 g and the two children, ages 9 and 12 years, 2 g. The concentration of ammonia in

blood was determined before the administration of NH₄Cl and at 1, 2, and 4 hr thereafter. Orotic acid in urine was determined before and in the subsequent two urine samples collected after the ingestion of NH₄Cl.

2. The patients ingested a meal containing 1 g protein/kg body wt. A fasting urine sample and each urine specimen for the ensuing several hours were assayed for the concentration of orotic acid.

Results

Studies on Proband (III-8)

The assays of the activities of urea biosynthetic enzymes of autopsy material obtained from III-8 confirm the diagnosis of OTC deficiency (Table I). The activity of OTC in liver, brain, and intestine was less than 1% of that measured in tissues obtained from normal patients. At pH 7.0, the activity of the enzyme was negligible, being 0.02% of normal values obtained at that pH. The activities of the other enzymes of the urea cycle were comparable with controls.

The chemical abnormalities noted in this infant were similar to those reported in other infants with OTC deficiency. The concentration of ammonia in blood, and the concentrations of orotic acid and proline in urine began to increase on the infant's 5th day of life and became abnormal on the 6th day. Alterations in orotic acid preceded those of ammonia, but were consistently parallel (Fig. 2). The concentrations of proline in urine varied more than those of orotic acid and were not regarded as being valuable indicators of a deficiency of urea biosynthesis. Although

Table I. Activities of urea cycle enzymes¹

	Proband	Control	% of mean control value
	μmol/hr/g wet wt		
Carbamyl phosphate synthetase	84	48/72	140
Ornithine transcarbamylase			
Liver	0.71	10,400/7,680	0.008
Brain	0.06	160	0.04
Intestinal mucosa	0.21	320	0.07
Argininosuccinate synthetase	6.3	5.6/3.4	140
Argininosuccinate lyase	59.4	95.2/75.0	70
Arginase	3,480	3,360/4,704	86

¹ Activity of the enzymes of the urea cycle were measured as described in *Methods*. Control tissues were obtained from two male infants who died from respiratory failure and multiple congenital anomalies, respectively.

Table II. Ammonium chloride loading test¹

	Ammonia in blood, $\mu\text{g}/100\text{ ml}$			Orotic acid in urine, $\mu\text{g}/\text{ml}$ creatinine		
	Fasting	NH_4Cl load	NH_4Cl load minus fasting	Fasting	NH_4Cl load	NH_4Cl load minus fasting
Control subjects	111 ± 9.7	119 ± 4.2	9.6 ± 15.2	4.1 ± 2.31	4.6 ± 1.25	0.3 ± 2.72
<i>II-6</i> (mother)	119	143	24	5.7	5.7	0
<i>II-7</i> (father)	119	121	2		0.9	
<i>I-1</i> (maternal grandmother)	114	126	12	12.1	13.9	1.8
<i>II-1</i> (maternal aunt)	81	211	131	1.7	16.0	14.3
<i>III-1</i> (daughter of <i>II-1</i>)	72	103	31	0.8	8.3	7.5
<i>III-2</i> (daughter of <i>II-1</i>)	61	83	22		9.2	

¹ The ammonia value after loading was the highest of the three samples taken. Control values were obtained from five healthy, unrelated adult subjects. The control value of ammonia after loading was calculated by averaging the highest value obtained in the three samples from each control subject. The differences between each control subject's maximum and fasting values were also averaged. Control values are given as the mean \pm sd.

the blood urea nitrogen rose to a peak of 11 mg/100 ml blood at 1 week of age, the concentration soon fell and remained below 5 mg/100 ml thereafter.

Peritoneal dialysis was effective in lowering the concentration of ammonia in blood and also alleviated some of the infant's symptoms. Death, although forestalled, could not be prevented.

Family of *III-8*

The ammonia loading test (Table II) resulted in a mild but significant increase in the concentration of ammonia in blood of the proband's mother, *II-6*, at 2 hr, and a marked increase at 1 hr in a maternal aunt, *II-1* (who became acutely ill with symptoms of headache, nausea, and abdominal pain and was debilitated for a 2-week period). None of the other subjects developed significant hyperammonemia during the 4 hr of the test. The concentration of orotic acid in urine increased to 3–4 times those of control subjects in the aunt, *II-1*; and both of her daughters, *III-1*, and *III-2*, had 2 times the concentration of orotic acid in their urine after the load as did the control subjects. The grandmother, *I-1*, had increased concentrations of orotic acid in urine samples obtained before and after the administration of ammonium chloride.

Protein loading tests (Table III) resulted in rapid elevations of orotic acid in urine in the mother, *II-6*, and the cousin, *III-1*. The aunt, *II-1*, who again became severely ill, had a slower but consistent rise in orotic acid. Protein loading also resulted in a significant elevation of urine orotic acid in the cousin, *III-2*. The rise in orotic acid in the grandmother, *I-1*, was slower but still significant. Similar protein loads were given to male members of the family (*II-4*, *III-3*, and *III-5*), but there were no elevations in the concentration of orotic acid in any of the urine specimens.

Table III. Protein loading test¹

	Orotic acid in urine, $\mu\text{g}/\text{mg}$ creatinine		
	Fasting	Protein load	Protein load minus fasting
Control subjects	5.2 ± 4.42	6.8 ± 5.54	1.6 ± 3.0
<i>II-6</i> (mother)	6.2	86.0	79.8
<i>I-1</i> (maternal grandmother)	9.5	20.0	10.5
<i>II-1</i> (maternal aunt)	8.0	46.0	38.0
<i>II-4</i> (maternal uncle)	4.8	6.3	1.5
<i>III-1</i> (daughter of <i>II-1</i>)	14.3	73.0	58.7
<i>III-2</i> (daughter of <i>II-1</i>)	9.0	33.0	24.0
<i>III-3</i> (half-brother)	8.9	6.3	2.6
<i>III-5</i> (brother)	4.9	7.2	2.3

¹ The orotic acid content of urine was the highest in the samples collected within 12 hr of ingesting the high protein meal. Control values were obtained from eight unrelated adult subjects, three males and five females. Control values are given as the mean \pm sd.

Discussion

We have described a patient with OTC deficiency. The clinical course and pedigree of this patient are similar to that of other male patients with this genetic lesion [5, 12, 18, 24, 26], and substantiate the X-linked dominant transmission of the defect with varying degrees of illness in females. The pathogenesis of the disorder has been discussed extensively in previous reviews [5, 6, 15, 25, 29].

Because OTC catalyzes the carbamylation of ornithine into citrulline, a deficiency of this enzyme could result in elevated levels of carbamyl phosphate and ornithine. However, these metabolites are not elevated

in either blood or urine of these patients [13, 25, 29]. Ornithine may be converted to proline or glutamine through the glutamic acid-proline shunt. The concentration of proline in urine has been elevated in some of the patients with OTC deficiency [5, 29], including the patient described in this study, but proline excretion has been inconsistent both in patients with this disease and in control subjects, presumably because of the variable content of this amino acid in the ingested proteins.

Glutamine may also become elevated as the result of increased combination of ammonia with glutamic acid in some patients with OTC deficiency [5-7, 13, 15, 25]. However, the concentration of this amino acid in the plasma of carriers has not been elevated consistently enough to be used as a marker to identify the heterozygote [6, 15].

Carbamyl phosphate is an intermediate not only in urea biosynthesis, but in *de novo* pyrimidine biosynthesis as well. Two separate enzymes catalyze the synthesis of carbamyl phosphate: an intramitochondrial carbamyl phosphate synthetase which is found predominantly in liver and subserves urea biosynthesis; and a cytoplasmic carbamyl phosphate synthetase which has more ubiquitous tissue distribution and is presumed to be associated with pyrimidine biosynthesis [11, 27]. Recent data indicate that carbamyl phosphate may leak from mitochondria and be utilized for pyrimidine biosynthesis [30]. Pyrimidine nucleotide biosynthesis is normally controlled by feedback inhibition of the carbamyl phosphate synthetase which subserves the *de novo* pyrimidine pathway [16, 30]. However, this control mechanism does not significantly limit the entry of carbamyl phosphate into the pyrimidine pathway in patients with OTC deficiency. The block in OTC may therefore be expected to result in an excess synthesis of pyrimidines, as was found in our patient. The elevation of orotic acid in urine closely followed the increased levels of ammonia in blood. Although it is not clear why orotic acid accumulates rather than other products of the *de novo* pyrimidine pathway, two explanations are possible: (1) work with rat hepatoma cells has shown that addition of uridine to the culture medium rapidly represses the levels of orotidine pyrophosphorylase in these cells [9]; (2) rat liver orotidine decarboxylase is inhibited by uridine monophosphate [3]. Because the decarboxylase has greater activity than the pyrophosphorylase, and because the K_i for uridine monophosphate is very high (7 mM), it is more likely that orotic acid accumulates

because of a feedback effect on pyrophosphorylase than on decarboxylase. Asymptomatic orotic aciduria has also been found in heterozygotes of congenital orotic aciduria [17], patients receiving 6-azauridine [17, 31] and allopurinol [31], normal pregnant women [31], and in some patients receiving parenteral nutrition [28].

Because orotic aciduria accompanied hyperammonemia in the patient with OTC deficiency, orotic acid was measured as an indication of a partial OTC deficiency after ammonia and protein loading in members of the patient's family. Both the mother, an obligate carrier, and an aunt, the only symptomatic female in this pedigree, had hyperammonemia after the ingestion of ammonium chloride (Table II). They also had significant orotic aciduria after a protein load as did the two female cousins whose ammonium tolerance was normal (Table III).

The responses of the males in this pedigree to both loading tests were similar to those of control subjects. The maternal grandmother excreted a large amount of orotic acid in her urine relatively consistently and not necessarily in response to ammonia or protein loading. These data suggest that the two female cousins and the maternal grandmother are asymptomatic female heterozygotes despite their normal ammonium chloride loading tests.

Protein loading in the form of a relatively palatable and innocuous meal followed by determination of orotic acid content in urine may prove to be a sensitive, noninvasive, and easy means to identify female carriers of ornithine transcarbamylase deficiency.

Summary

We have described and discussed the genetic, clinical, and biochemical aspects of ornithine transcarbamylase (OTC) deficiency. This disorder is probably X-linked, fatal in males, and with a variable degree of illness in female heterozygotes. Although the patient in this study was kept alive for 25 days, and several modes of therapy were employed, there was no indication of OTC induction or any other reversal of the fatal nature of this illness. Orotic acid concentration in the urine of the patient very closely paralleled the concentration of ammonia in his blood. Concentration of orotic acid in urine of the family members was determined in an attempt to accurately and easily identify female heterozygotes of OTC deficiency. Our results indicate that a high protein meal followed by determinations of orotic acid in urine specimens collected for

12 hr thereafter is a sensitive and noninvasive means of diagnosis. It is possible that three asymptomatic females in this pedigree who had normal tolerance to ammonium chloride are carriers of OTC deficiency as demonstrated by this method.

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