Congenital Orotic Aciduria: Demonstration of an Enzyme Defect in Leukocytes and Comparison with Drug-induced Orotic Aciduria

By Harold J. Fallon, Myron Lotz and Lloyd H. Smith, Jr.

Congenital orotic aciduria, a disorder of pyrimidine metabolism described thus far in a single patient, is characterized by megaloblastic anemia unresponsive to conventional therapy, and by orotic acid crystalluria. A complete hematologic remission and marked reduction in orotic acid excretion followed the oral administration of yeast pyrimidine nucleotides and prednisone. The propositus died at the age of two of a severe varicella infection.

Recent studies have demonstrated reduced activities of orotidylic pyrophosphorylase and orotidylic decarboxylase in erythrocytes obtained from the parents and two of the three surviving siblings ("R" family) of the deceased child with congenital orotic aciduria (fig. 1). This evidence suggests that congenital orotic aciduria is a genetic disorder of pyrimidine metabolism transmitted as an autosomal trait, probably recessive in origin. A disorder of pyrimidine metabolism somewhat comparable to congenital orotic aciduria may be produced in man by the administration of the anti-neoplastic agent 6-azauridine. 6-Azauridine, following its enzymatic conversion to 6-azauridylic acid, is a specific competitive inhibitor of orotidylic decarboxylase.

This report describes enzymatic studies of leukocytes from the parents and two siblings of the reported case of congenital orotic aciduria. Defective metabolism of orotic acid was demonstrated in intact leukocytes and a specific deficiency of orotidylic decarboxylase in disrupted leukocytes. The absence of clinical manifestations of the disease and the relationship of the subjects to the deceased propositus suggest that the abnormalities detected reflect the heterozygous state of congenital orotic aciduria. Parallel studies of patients with chronic myelocytic leukemia treated with 6-azauridine, including enzyme studies in intact leukocytes and measurement of urinary orotic acid and orotidine, were obtained for comparison with the genetically-determined disorder.

Materials and Methods

Clinical Studies

The parents and two siblings of the propositus were hospitalized at the National Cancer Institute. Hematologic evaluation, liver function studies, BUN, urinalysis, and uric acid clearance were determined.* The patients remained on a diet low in purines and pyrimidines.
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Fig. 1.—Pathway of de novo synthesis of pyrimidine nucleotides, including the site of action of 6-azauridylic acid as an inhibitor of orotidylic decarboxylase. Abbreviations: CAP, carbamylphosphate; L-ASP, aspartic acid; CAA, carbamylaspartic acid; DHO, dihydroorotic acid; OA, orotic acid; O5P, orotidine-5'-phosphate; UMP, uridine-5'-phosphate; 6-AZUR, 6-azauridylic acid.

throughout their hospital stay. Patients with leukemia receiving 6-azauridine therapy were studied in a similar manner while on the same diet. Details of their treatment schedules are reported elsewhere.

Enzyme Studies

Leukocytes were isolated from venous blood by dextran sedimentation at 0 C. followed by hemolysis of residual contaminating erythrocytes by hypotonic saline. This method has been described in detail. Assays for orotidylic pyrophosphorylase and orotidylic decarboxylase activities were performed as previously described for erythrocytes, using leukocyte preparations disrupted by 3-minute sonication (Raytheon 10 Kc. Oscillator, Model DP101). In brief, the assay for orotidylic decarboxylase activity measured the release of C14O2 from biosynthetically prepared carboxyl-labeled orotidine-5'-phosphate. Orotidylic pyrophosphorylase activity was measured by the release of C14O2 from carboxyl-labeled orotic acid-C14 in the presence of Mg++, 5-phosphoribosylpyrophosphate, and excess partially purified orotidylic decarboxylase from yeast.

Intact leukocytes were incubated for 1 hour in Krebs-Ringer phosphate buffer with carboxyl-labeled orotic acid—C14 (2.5 X 10^-3 M) as previously described. This assay measures the overall conversion of orotic acid to uridine-5'-phosphate (fig. 1), dependent upon the activity of two enzymes. Activity for all assays is expressed as mU of orotic acid or orotidine-5'-phosphate decarboxylated per hour per 10^8 leukocytes.
Orotic Acid and Orotidine Excretion

The urinary excretion of these compounds was measured by previously described ion-exchange chromatographic technics. Concentrations of orotic acid and orotidine in excess of 5 mg. per cent in urine could be determined by this method, but the small amounts of these compounds found in the urine of normal persons could not be detected.

An infusion of orotic acid dissolved in 5 per cent dextrose and water at a concentration of 2 mg./ml. (sterilized by autoclaving) was administered to the father of the propositus and to two normal control subjects. The infusions were administered at rates of 1 Gm. and 2 Gm. of orotic acid over separate 12-hour periods and the recovery of orotic acid in the urine during the 24-hour period after the start of the infusion was determined by the method outlined above.

RESULTS

Clinical Studies

Study of the parents and two siblings of the propositus revealed no abnormalities on routine physical examination or by detailed hematologic and chemical determinations. The father presented a history of a renal stone but had no radiographic or urinary evidence of renal lithiasis on examination. One sibling gave a history of acute glomerulonephritis at age seven and had persistent microscopic hematuria.

Serum uric acid values varied from 2.5 to 5.4 mg. per cent and urate clearances from 5.7 to 9.1 ml., on repeated examinations, in the parents and two siblings of the propositus.

The patients with leukemia who received 6-azauridine had pronounced reduction in total leukocyte count, minimal alterations in hemoglobin determinations and a prompt uricosuric response as described in previous publications.

Enzyme Studies

The results of the assay of decarboxylation of orotic acid by intact leukocytes are noted in figure 2. This assay reflects the sequential activities of both orotidylic pyrophosphorylase and orotidylic decarboxylase. The mean value for a group of normal controls was $4.2 \pm 0.44$ S.D. $\mu$ mole of orotic acid decarboxylated per $10^8$ leukocytes per hour. The same value for eight separate determinations on the “R” family was $2.6 \pm 0.6$ S.D. The difference in the means of these two groups has a p value of less than .001. The reduction of leukocyte enzyme activity produced by the administration of 6-azauridine (AZUR) to patients with chronic myelocytic leukemia (CML) is also recorded in figure 2. Orotic acid metabolism was highly variable in chronic myelocytic leukemic cells, with levels in some patients as low as those found in leukocytes from the “R” family. An approximately parallel depression of enzyme activity as measured by this technic is noted in patients receiving AZUR and in the four members of the “R” family studied. The reduction of enzyme activity in one patient receiving 6-azauridine (AZUR) is illustrated in figure 3. Prompt and persistent reduction of enzyme activity accompanied a clinical remission of leukemia in this case.

The values for orotidylic decarboxylase activity in leukocytes disrupted by
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INTACT LEUKOCYTES

<table>
<thead>
<tr>
<th>CONTROLS</th>
<th>R. FAMILY</th>
<th>C.M.L.</th>
<th>C.M.L. ON AZUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>m \mu \text{mole} / 10^8 \text{WBC}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2.—Conversion of carboxyl-labeled orotic acid-C\textsuperscript{14} to C\textsuperscript{14}O\textsubscript{2} by intact leukocytes from control subjects, presumed heterozygotes of congenital orotic aciduria ("R" family), patients with chronic myelocytic leukemia (C.M.L.), and patients with leukemia under treatment with 6-azauridine.

Sonication are recorded for the “R” family and a group of normal controls in figure 4. Reduction of enzyme activity to approximately 35 per cent of normal controls is noted. The assay technic for orotidylic pyrophosphorylase in leukocytes proved to be too variable and nonreproducible for proper interpretation.

Excretion Studies

The presence of urinary orotic acid or orotidine in the “R” family or normal controls could not be determined by the technic utilized in this study. Small quantities of these compounds have been found in the urine of normal subjects and the “R” family by isotope dilution technics and will be described separately.\textsuperscript{8} Large quantities of orotic acid and orotidine appear in the urine of all patients receiving 6-azauridine (AZUR).\textsuperscript{3,7} This is illustrated for one patient in figure 3 and demonstrates the usual pattern of orotic aciduria and orotidinuria secondary to AZUR administration.

The infusion of orotic acid to two normal controls resulted in the urinary excretion of 21–42 per cent of this material in the urine. With increasing infusion rates in these subjects there appeared to be an increase in the amount of orotic acid which could be "metabolized" and therefore the urinary excretion never exceeded 42 per cent of the amount infused at rates as high as 2 Gm./12 hr. The amount "metabolized" or retained (equal to amount infused — amount excreted in the urine per unit time) increased from 40 mg./hr. to 140 mg./hr. in the normal controls. The father of the propositus did not increase the amount "metabolized" (50 mg./hr.) with increasing infusion rates and therefore excreted as much as 68 per cent of the administered orotic acid in the urine.
Fig. 3.—Changes induced by 6-azauridine on leukocyte count, intact leukocyte metabolism of orotic acid, and urinary orotidine and orotic acid in a patient with chronic myelocytic leukemia.

DISCUSSION

The only described patient with congenital orotic aciduria exhibited not only resistant megaloblastic anemia, but also leukopenia, diarrhea, and a retarded rate of growth and development. All of these features of the disease were remarkably improved following treatment with glucocorticoids and yeast nucleotides, suggesting that the enzymatic defect was not confined to the erythrocytic cell series. Previous studies revealed reduced activities of orotidylate pyrophosphorylase and orotidylate decarboxylase in erythrocytes from the parents and two of the three surviving siblings of the propositus of orotic aciduria. Another unrelated individual (J.S., fig. 4) consistently demonstrated low erythrocyte enzyme activities, levels comparable to those of the presumed heterozygotes of congenital orotic aciduria. In the current studies, intact leukocytes from the same subjects were found to exhibit a partial block in the metabolism of carboxyl-labeled orotic acid-C\(^{14}\) to carbon dioxide-C\(^{14}\) (a reduction in activity of approximately 40 per cent). Following disruption of the leukocytes, orotidylate decarboxylase activity was found to be reduced to approximately 35 per cent of that found in the control series. Initial attempts to measure orotidylate pyrophosphorylase activity in disrupted leukocytes have not been successful due to the low activities present and wide variations en-
Fig. 4.—Activity of orotidyl decarboxylase in disrupted leukocytes from control subjects, presumed heterozygotes of congenital orotic aciduria ("R" family), and an additional heterozygous subject previously described.

countered. The mature erythrocyte exhibits a complete block in de novo synthesis of both purine and pyrimidine nucleotides. The previous demonstration of a double enzyme defect in congenital orotic aciduria was based on measurements of vestigial enzyme activities in presumed heterozygotes. The present work, utilizing a cell with an intact pyrimidine nucleotide biosynthetic pathway as an enzyme source, confirms the deficiency of orotidyl decarboxylase. The presence of a double enzyme defect in such nucleated cells must await confirmation in other tissues, preferably from a homozygous patient.

Orotic aciduria may be acquired, notably during the use of 6-azauridine. Following its enzymatic conversion to 6-azauridyl acid, this chemotherapeutic agent serves as a potent, competitive, inhibitor of the decarboxylation of orotidine-5'-phosphate. It is instructive to compare the drug-induced derangement of pyrimidine biosynthesis with that found in the genetically-determined disease (table 1). The magnitude of orotic aciduria following 6-azauridine may greatly exceed that recorded in the congenital disorder, possibly reflecting differences in body size. Corrected to a body weight of 70 Kg., orotic acid excretion in the genetic disorder would have been approximately 12 Gm./24 hr. A striking difference is noted, however, in the excretion of large quantities of orotidine in the acquired disorder in amounts comparable to those of orotic acid. In congenital orotic aciduria no urinary orotidine was detected, its absence being consistent with a genetic defect of both orotidyl pyrophosphorylase and orotidyl decarboxylase (fig. 1). In the intact leukocyte assay depression of enzyme activity was comparable in acquired and congenital (heterozygous) orotic aciduria. No information is available on enzyme activities in hemic cells from a patient with homozygous congenital
Table 1.—A Comparison of Congenital and Drug-induced Orotic Aciduria

<table>
<thead>
<tr>
<th>Parameter Measured</th>
<th>Congenital Orotic Aciduria</th>
<th>Azauridine Administration (acquired orotic aciduria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>orotic aciduria 1.5 Gm./24 hr. up to 12 Gm./24 hr. 10 Gm./24 hr. present</td>
<td></td>
</tr>
<tr>
<td>uricosuria</td>
<td>none observed</td>
<td>present</td>
</tr>
<tr>
<td>orotidine</td>
<td>not found</td>
<td></td>
</tr>
<tr>
<td>Hematologic</td>
<td>anemia</td>
<td>anisoctytosis, hypochromic leukopenia, monocytosis megaloblastic</td>
</tr>
<tr>
<td>WBC</td>
<td></td>
<td>normocytic leukemia</td>
</tr>
<tr>
<td>bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to therapy</td>
<td>orotic aciduria</td>
<td>enzymatic inhibition WBC</td>
</tr>
<tr>
<td>oral nucleotides</td>
<td>↑ hemoglobin</td>
<td></td>
</tr>
<tr>
<td>uridine intravenously or nucleosides in vitro</td>
<td>not attempted</td>
<td></td>
</tr>
<tr>
<td>prednisone</td>
<td>↓ orotic aciduria</td>
<td>(no ↓ in orotic aciduria)</td>
</tr>
<tr>
<td>enzyme assay</td>
<td>intact cell assay</td>
<td>depressed 40% (presumed heterozygotes)</td>
</tr>
<tr>
<td>sonicates:</td>
<td>depressed 50-90%</td>
<td></td>
</tr>
<tr>
<td>05P pyrophosphorylase</td>
<td>no data</td>
<td>probably normal</td>
</tr>
<tr>
<td>05P decarboxylase</td>
<td>decreased 60-70%</td>
<td>(in vitro AZUR inhibits)</td>
</tr>
<tr>
<td>WBC enzyme assay</td>
<td>genetic deficiency:</td>
<td>competitive inhibition:</td>
</tr>
<tr>
<td>presumed defect</td>
<td>05P decarboxylase</td>
<td>05P pyrophosphorylase</td>
</tr>
<tr>
<td></td>
<td>705P pyrophosphorylase</td>
<td></td>
</tr>
</tbody>
</table>

Orotic aciduria. Absolute values for enzyme activity by the intact cell assay method will vary with substrate concentration since it is impossible to achieve zero order kinetics with this technic. This probably explains the differences in such values reported by Cardoso et al. who employed lower substrate concentrations than were used in this study. Levels of orotidyl pyrophosphorylase and orotidyl decarboxylase in disrupted leukocytes from patients receiving AZUR are difficult to interpret since AZUR is converted to a competitive inhibitor of decarboxylation, and cell disruption alters the substrate-inhibitor relationships which exist intracellularly. Preliminary measurements suggest that activities of these enzymes are not greatly altered when excess substrate is used during assay. Simultaneous administration of uridine reduces the effectiveness of AZUR as an inhibitor of orotidyl acid decarboxylase, probably by reducing the phosphorylation of AZUR to its active form, 6-azaauridylic acid. This effect seems quite distinct from the presumed feedback control of de novo pyrimidine metabolism exhibited by oral nucleotides in congenital orotic aciduria. The partial effectiveness of prednisone in the congenital disorder remains unexplained. There are certain interesting clinical and biochemical similarities in congenital and acquired orotic aciduria. Administration of the synthetic analog, 6-azauridine, simulates many of the features of the genetic disease by means of pharmacological inhibition of orotidyl decarboxylase activity. A major point of dissimilarity is the probable additional genetic defect in orotidyl pyrophosphorylase activity in congenital orotic aciduria, although this defect has been demonstrated so far only in erythrocytes from presumed heterozygotes.

The absence of hyperuricemia or any abnormality in urate clearance in members of the “R” family is of note. It was previously reported that two members of the “S” family exhibited clinical gout. The present findings
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Table 2.—Enzymatic Defects in Hemic Cells in Human Genetic Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme</th>
<th>Leukocyte</th>
<th>Erythrocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Galactosemia</td>
<td>galactose-1-phosphate</td>
<td>yes&lt;sup&gt;13&lt;/sup&gt;</td>
<td>yes&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Drug-induced hemolytic anemia</td>
<td>glucose-6-phosphate dehydrogenase</td>
<td>yes&lt;sup&gt;14*&lt;/sup&gt;</td>
<td>yes&lt;sup&gt;16&lt;/sup&gt;</td>
</tr>
<tr>
<td>3. Congenital erythropoietic porphyria</td>
<td>uroporphyrinogen isomerase</td>
<td>no&lt;sup&gt;15*&lt;/sup&gt;</td>
<td>yes&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>4. Hypophosphatasia</td>
<td>alkaline phosphatase</td>
<td>yes&lt;sup&gt;18&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5. Hereditary methemoglobinemia</td>
<td>diphosphopyridine nucleotide diaphorase</td>
<td>yes&lt;sup&gt;19&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>6. Acatalasemia</td>
<td>catalase</td>
<td></td>
<td>yes&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td>7. Maple-syrup-urine disease</td>
<td>&quot;branched-chain keto acid decarboxylase&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Glycogen storage disease (hepatic phosphorylase-deficient)</td>
<td>phosphorylase</td>
<td>yes&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>9. Congenital non-spherocytic hemolytic anemia</td>
<td>pyruvate kinase</td>
<td>no&lt;sup&gt;23&lt;/sup&gt;</td>
<td>yes&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td>10. Congenital orotic aciduria</td>
<td>orotidylic decarboxylase</td>
<td>yes (this report)</td>
<td>yes&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>orotidylic pyrophosphorylase</td>
<td></td>
<td>yes&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*These conflicting reports represent different population groups.

demonstrate that gout or asymptomatic hyperuricemia is not necessarily associated with congenital orotic aciduria. Uricosuric quantities of orotic acid were not excreted by heterozygous members of the “R” family.

Either of the two enzymatic assay technics described for leukocytes in this report, or the assay technics previously employed with erythrocytes<sup>2</sup> should permit a definitive diagnosis of homozygous or heterozygous congenital orotic aciduria. The frequency of the presumed heterozygous state in the general population may also be determined by any of these methods.

The present report furnishes another example of the use of the human leukocyte as a “tissue source” for the demonstration of an enzymatic abnormality in a genetic disorder. An increasing number of such genetic diseases have been detected in hemic cells. A summary of comparable previous studies is presented in table 2. The hemoglobinopathies have not been included in this list of enzymatic abnormalities.

**Summary**

1. Leukocytes from presumed heterozygotes of congenital orotic aciduria have been found to exhibit a partial defect in the metabolism of orotic acid. In disrupted cell preparations, orotidylic decarboxylase activity was reduced. Assay of orotidylic pyrophosphorylase activity proved to be unsatisfactory in leukocyte preparations.

2. Orotic aciduria and orotidinuria were produced by the administration of 6-azauridine to patients with chronic myelocytic leukemia. This drug-induced impairment of pyrimidine nucleotide biosynthesis has been compared to the genetically determined disorder.

3. A summary of enzymatic defects previously demonstrated in hemic cells in human genetic disorders has been presented.
SUMMARIO IN INTERLINGUA

1. Esseva trovate que le leucocytos de subjectos presumitemente heterozygotic pro congenite aciduria orotic exhibi un defecto partial in le metabolismo de acido orotic. In preparatos de disrumpite cellulas, le activitate de discarboxylase orotidylic esseva reducite. Le essayage del activitate de pyrophosphorylase orotidylic se provava non-satisfactori in preparatos de leucocytos.

2. Aciduria orotic e orotidinuria esseva producite in patientes con chronic leucemia myelocytic per medio del administration de 6-azauridina. Iste pharmacogene dysfunction del biosynthese de nucleotida pyrimidinic esseva comparate con le geneticamente determinate disordine.

3. Es presentate un summario del datos relative a previemente demonstrate defectos enzymatic in cellulas hemic in disordines genetic in le homine.

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Harold J. Fallon, M.D., Research Fellow, Department of Medicine, Yale University School of Medicine, New Haven, Conn.

Myron Lotz, M.D., Clinical Associate, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

Lloyd H. Smith, Jr., M.D., Assistant Professor of Medicine, Harvard Medical School; Chief of Endocrine Unit, Massachusetts General Hospital, Boston, Mass.
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