Pyruvic aciduria in the detection of thiamine responsive encephalopathy

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In 1951 Leigh[1] reported a case of encephalopathy that in some respects was similar to cases of thiamine deficiency. Since that time there has been interest in a group of diseases which may or may not be related.[2-4] Borit,[5] in a report of a case of Leigh's disease, described the clinical characteristics and reviewed some of the reported variations. We have studied four cases of different forms of encephalopathy that appear to have similar biochemical disturbances characterized by hyperpyruvicuria, hyperpyruvicemia, and hyperlactemia. Two of the patients excreted thiamine pyrophosphate (TPP) inhibitor substance in the urine.[6] One patient, a girl who suffered from self-mutilation of the lower lip similar to that seen in Lesch-Nyhan syndrome,[7] excreted abnormal concentrations of uric acid in the urine. A high serum uric acid was observed on one occasion. The result of the hypoxanthine-guanine phosphoribosyltransferase enzyme assay of this patient's red blood cells was normal. Hyperalaninemia, hyperalaninuria, or both were observed in three of the patients described in this report.

Case reports

Case 1. A white boy, now 11 years old, had episodes of cerebellar ataxia after infection, inoculation of vaccine, or head injury. His case was reported in 1969.[2] Since that time he has received 600 mg/day of thia-

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mine. Improvement in his general health has been measured by the fact that he has had only one brief episode of ataxia since beginning treatment. His ability in school and his coordination have improved greatly. His perception of Bender-Gestalt block designs, severely distorted during an ataxic episode, improved slowly during the first 6 months of treatment with thiamine. Fibroblast tissue culture was performed, and the cells were shown to have a defect in pyruvic decarboxylase activity.[8] Urine, blood, and cerebrospinal fluid specimens contained TPP inhibitor.

Case 2. A 15-month-old white girl was examined because of myoclonic seizures. The mother had had five spontaneous abortions. The family history was unrevealing and birth history was normal. Jerking of the extremities was first observed at the age of 6 weeks. Such episodes were worse in the presence of febrile infection. Mental retardation was severe and the head circumference was small. The electroencephalogram tracing differed only in detail from that described as hypsarrhythmia. Results of routine laboratory tests, including serum ammonium were normal. Fasting levels of serum lactic acid were 33.4 mg and 46.5 mg/100 ml on two separate tests (normal 26 mg/100 ml). The fasting serum pyruvate value was 4.8 mg/100 ml. High voltage electrophoresis combined with paper chromatography revealed hyperalaninuria, and urinary pyruvate ranged from 12 to 38 mg/10 hr (normal $<$ 5 mg/12 hr). A clinical trial of thiamine 600 mg/day produced no benefit. There was no TPP inhibitor substance in the urine.

Case 3. A 9-year-old white girl was examined because of severe choreoathetosis. Birth and family history were not remarkable, and she had been considered normal during the first year of life. At the age of 1 year she had a severe but brief illness of unknown cause during which she was in a semicoma. After the illness she regressed physically and mentally, and choreoathetosis and akinetic seizures developed.

On examination she exhibited typical movements of choreoathetosis, but there were no specific changes in reflex or muscular tone. Her speech was dysarthric. The optic fundi were normal. Several akinetic seizures were observed, some of which persisted several hours and, although she remained conscious, choreoathetotic movements ceased abruptly at the onset and recommenced just as abruptly on her recovery from the akinetic episode. Conventional anticonvulsant therapy failed to control either the seizures or the dyskinesia. The electroencephalogram was abnormal with multiple spike and wave activity. The I.Q. was 60 on the WISC Verbal Scale and 58 on the Peabody Scale. Routine laboratory studies were noncontributory.

In February 1971, during a prolonged akinetic seizure, a fasting serum pyruvate concentration was 3 mg/100 ml and the serum lactate concentration was 28.1 mg/100 ml. The electroencephalogram was abnormal, and chromatography revealed an unusual amount of alanine in the plasma, although it was not present in unusual amounts in the urine. Pyruvic acid in the urine varied from 7 to 19 mg/24 hr (normal $<$ 10 mg/24 hr). Urine contained a large amount of TPP inhibitor substance. From February to November 1971 she was treated with 2 g of thiamine orally. Her school work, coordination, and ambulation appeared to improve. The electroencephalogram showed clearing of the polyspike activity and improvement in alpha rhythm; seizures ceased. Fasting levels of both pyruvate and lactate decreased to the near normal range.

Case 4. A white girl was observed because of severe psychomotor retardation from the age of 2 months. The birth history was normal, but a low Apgar score, failure to suck from the bottle, cyanosis, repeated vomiting, and failure to thrive were early signs of a severe neurological disturbance. Rotary nystagmus and a persistent metabolic acidosis were observed throughout infancy, and hyperpyrexia occurred repeatedly. At the age of 9 months

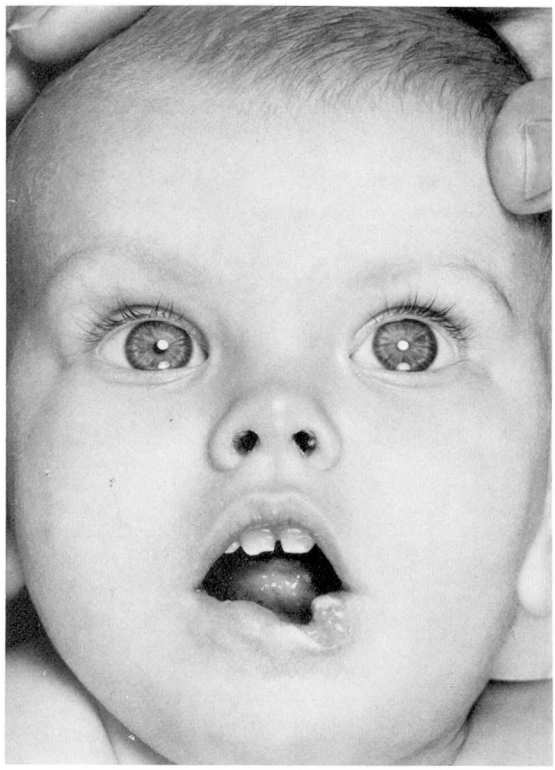

Fig. 1. Photograph of patient in case 4 shows self-multilation of lower lip.

she began self-mutilation of the lower lip (*Fig. 1*), eventually necessitating removal of the upper incisors. Repeated tests of uric acid levels in serum and cerebrospinal fluid were normal, although output of urine uric acid repeatedly exceeded the normal levels of 18 mg/kg/24 hr.[9]

In April 1971, when the patient was 5½ years old, the urine contained 20 mg/24 hr of pyruvic acid, and the serum pyruvate concentration was 2.7 mg/100 ml. Uric acid concentration in the serum was 7.7 mg/100 ml (normal = 3 to 6.5 mg/100 ml). Because of hyperpyruvicuria she received 600 mg of thiamine a day on an empirical trial, and 5 months later the urinary pyruvic acid concentration had decreased to 4 mg/24 hr. Increased activity, interest in her surroundings, and alertness suggested to the parents that there was some improvement.

Materials and methods

Serum lactates were measured by a commercial kit.* Serum pyruvic acid was measured by the method of Friedemann and Haugen.[10] To measure urinary pyruvic acid, a protein-free filtrate of urine was treated with 2,4-Dinitrophenylhydrazine and extracted with sodium hydroxide. The mixture of hydrazones thus formed was spotted onto paper and a two dimensional paper chromatogram was performed.[11] The spot of pyruvic acid hydrazone was eluted from the paper and measured spectrophotometrically.

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* *Sigma Chemical Company, St. Louis, Missouri.*

Results

Urine specimens from all four patients were collected on numerous occasions and analyzed for pyruvic acid content (*Table*). Intravenous glucose tolerances were performed using 0.5 g/kg of body weight, and concomitant concentrations of glucose, lactic acid, and pyruvic acid were measured in the blood in the fasting state, on the half hour, 1 hour, 2 hours, and 3 hours after administration of glucose (*Figs. 2 through 7*). Glucose tolerances in all four patients were not significantly different from that of a normal child. Lactate levels increased in cases 3 and 4 (*Fig. 3*). Data presented in *Figures 4 through 7* show the pyruvate levels for each patient during the glucose tolerance tests. The difference between "calculated" and "observed" pyruvate is based on a formula which relies upon the observation that pyruvate is directly proportional to lactate.[12] The

Table. Urinary pyruvic acid

	Normal range*	Mean	Case 1	Case 2	Case 3	Case 4
Day 09.00–18.00 hours (mg/12 hours)	0.7–5.5	3.4	2.6–2.3	12.6–38	3.9–14.0	2.0–15.5
Night 18.00–09.00 hours (mg/12 hours)	1.0–4.8	2.5	1.5–41.6	1.8–11.2	0.4–4.7	1.9–4.3
24 hours	2.5–9.4	6.1	—	23.8–40	6.4–18.7	3.9–19.8

* Normal range of urinary pyruvic acid from 18 normal children.

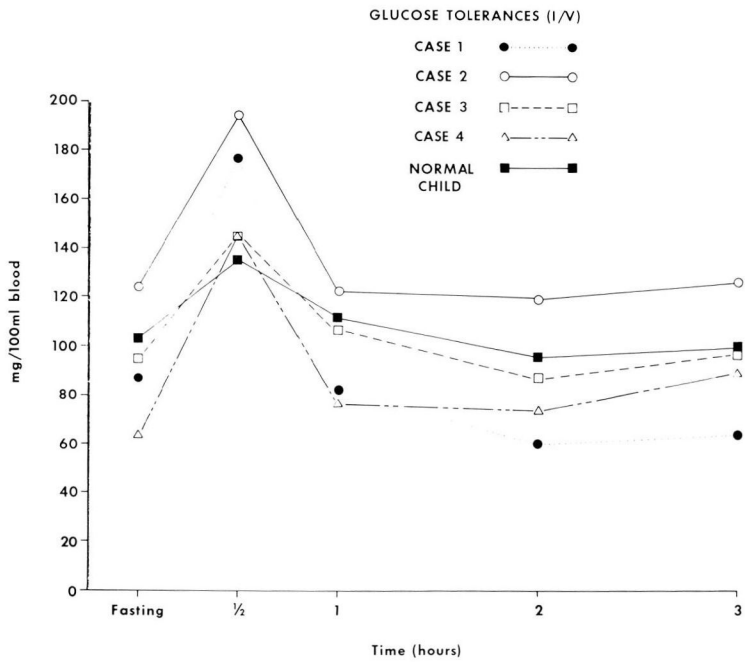

Fig. 2. Serum glucose concentrations after intravenous administration of glucose.

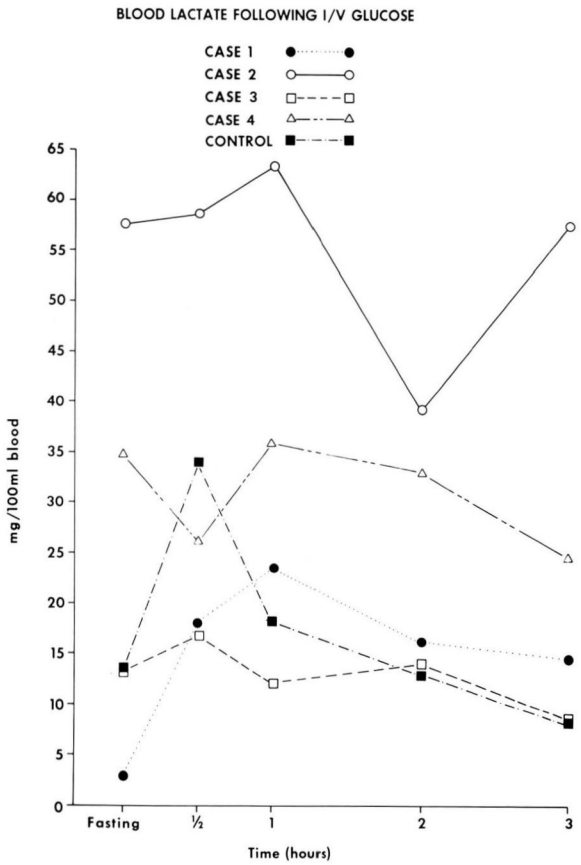

Fig. 3. Serum lactic acid concentrations after intravenous administration of glucose.

pyruvate is then calculated from the observed lactate level; a wide discrepancy between the observed and the calculated pyruvate concentration occurs in thiamine deficiency. The discrepancy shown in these patients reveals an altered lactate to pyruvate ratio suggesting that there was an obstruction in pyruvate metabolism, perhaps similar to the metabolic situation in thiamine deficiency.

Discussion

These four patients differ considerably clinically, but have similarities from a biochemical standpoint. All four patients excreted abnormal concentrations of pyruvic acid in the urine and had increased concentrations of pyruvic and lactic acids in serum. The clinical improvement in three of the four, after the administration of large doses of thiamine, is difficult to document; and the somewhat subjective reports of hopeful parents are too anecdotal. The decrease in concentration of urinary metabolites is, however, evidence that some effect was produced and suggests that these patients had some defect in common at the level of oxidative decarboxylation of pyruvic acid. There is still much argument about the nature of Leigh's encephalopathy and the location of the meta-

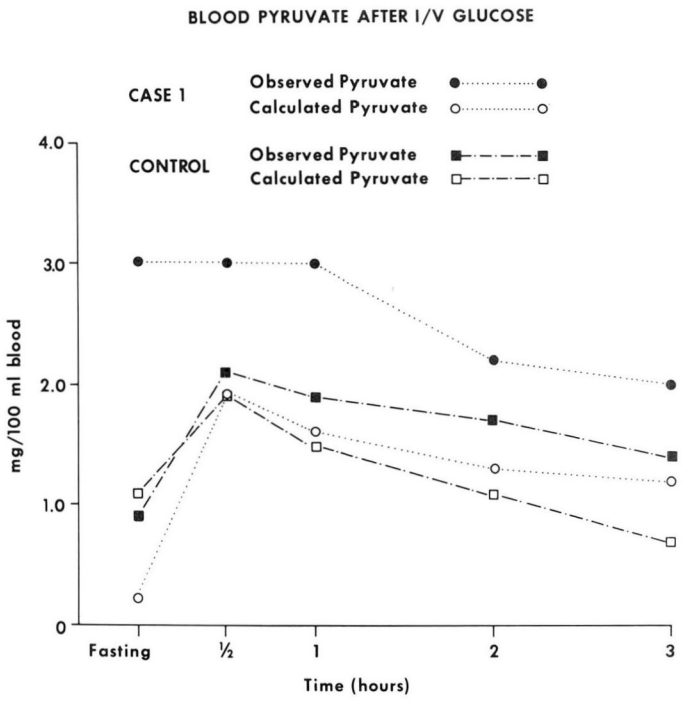

Fig. 4. Serum pyruvic acid concentrations in case 1 after intravenous administration of glucose.

bolic defect. Many investigators contend that this is a generic group of diseases with various enzyme defects. Thiamine,[13] lipoic acid,[14] pyridoxine,[15] and glutamine[16] have been prescribed to treat the disease. Diagnosis of the clinical entity is difficult unless an autopsy has proved death from the disease has occurred in an older sibling. Cooper et al[4] have emphasized the importance of recognition of the disease by detecting TPP inhibitor in urine, and believe that it is a diagnostic test, although this is not yet proved. However, they have shown that children dying from the disease have a deficiency of thiamine triphosphate in brain tissue, and the inhibitor is believed to act by interference of the enzymatic conversion of thiamine pyrophosphate to the triphosphate active principle. Whatever the role of thiamine is in neural tissue, it has a cellular function as a coenzyme, and its deficiency from dietary causes affects the thiamine-dependent pyruvic dehydrogenase complex to produce the well-known but protean manifestations seen in beriberi. Wortis et al[17] discovered its deficiency in explaining Wernicke encephalopathy, and it has been studied as an important deficiency in disease in animals.[18, 19] It is extremely doubtful that our four patients have dietary deficiency; they may represent only phenotypes of a generic group of diseases in which abnormal pyruvate metabolism is but the common denominator. There are two basic enzyme pathways which may be damaged by one of several mechanisms, including coenzyme deficiency, coenzyme dependency, enzyme inhibition, or marginal apoenzyme insuffi-

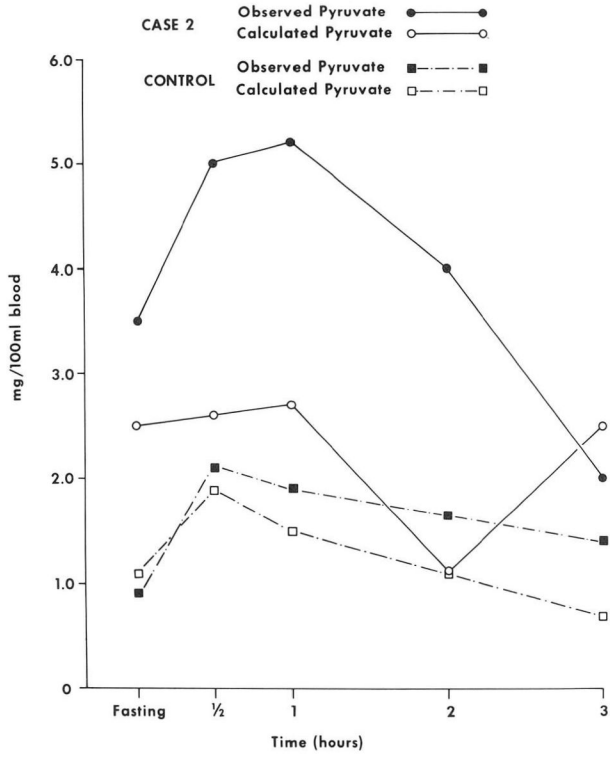

Fig. 5. Serum pyruvic acid concentrations in case 2 after intravenous administration of glucose.

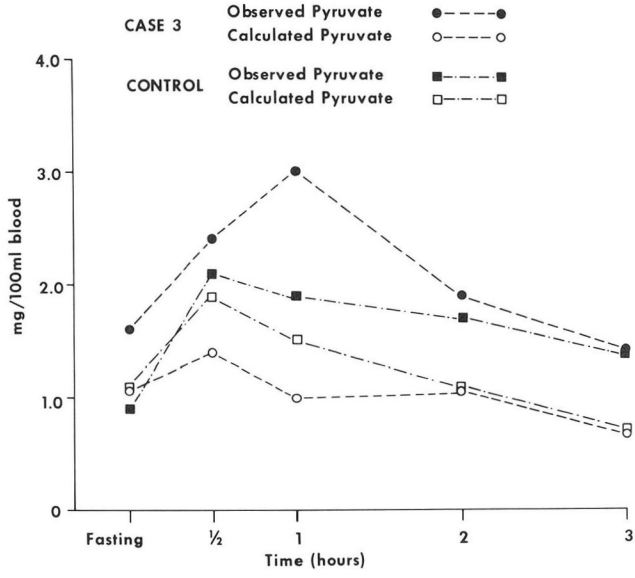

Fig. 6. Serum pyruvic acid concentrations in case 3 after intravenous administration of glucose.

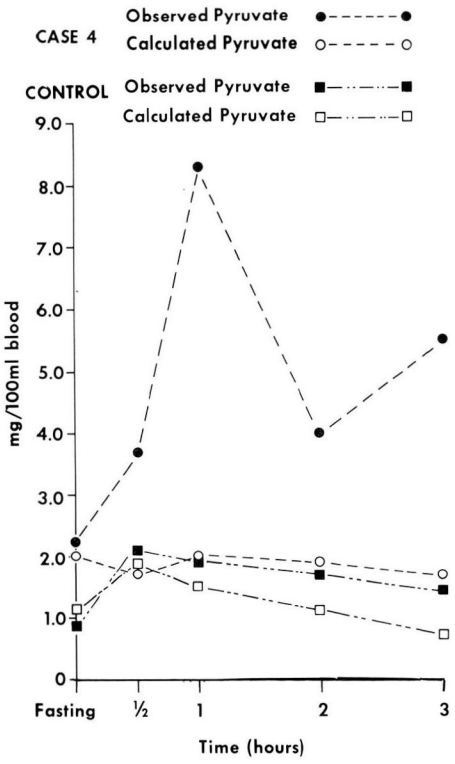

Fig. 7. Serum pyruvic acid concentrations in case 4 after intravenous administration of glucose.

ciency. Brunette et al[20] have recently postulated that thiamine may accelerate the action of decarboxylation within the dehydrogenase complex, thus increasing the concentration of acetyl CoA (*Fig. 8*). By a positive feedback mechanism, the concentration of this compound stimulates one of the two biotin dependent carboxylases and thus accelerates the production of oxaloacetate. The condensation of oxaloacetate and acetyl CoA to form citrate is the first step in the citric acid cycle. If thiamine works this way in vivo it may be clinically helpful, whether the primary defect is in the carboxylase or the dehydrogenase.

It seems necessary to discuss briefly the mechanism of excess uric acid production, a biochemical phenomenon that is related to abnormal carbohydrate metabolism,[21] and also to purine biosynthesis.[7] The patient in case 4 excreted excessive amounts of pyruvic acid and uric acid in the urine, and was known to have had persistent metabolic acidosis of unknown cause for some years. Her response to thiamine suggested that the primary disturbance was in carbohydrate metabolism. Fox and Kelley[22] pointed out that the intracellular concentration of phosphoribosylpyrophosphate (PRPP) has a critical role in the regulation of purine metabolism in man, and that PRPP synthesis is regulated by the availability of ribose-5-phosphate, itself increased in concentration by accelerating the rate of the hexose monophosphate shunt. Ribose-5-phosphate is closely related to the presence of glycolytic intermediates in its regulation. Therefore, it can be postulated that a primary defect in carbohydrate metabolism might produce excessive uric acid by this mechanism. The cause of diurnal variation in concentration of pyruvic acid is not clear, but it is assumed to be related to diet because of its greater concentration during daylight hours. For this reason candy and sugar have been partially restricted in the diet of our patient in case 1. Normal children showed little variation between night and day concentrations, and this difference in the concentration of pyruvic acid appears to become more marked in pathologic states. Results of glucose tolerance tests are puzzling. It might be expected that a defect in pyruvate metabolism would result in a glucose tolerance similar to

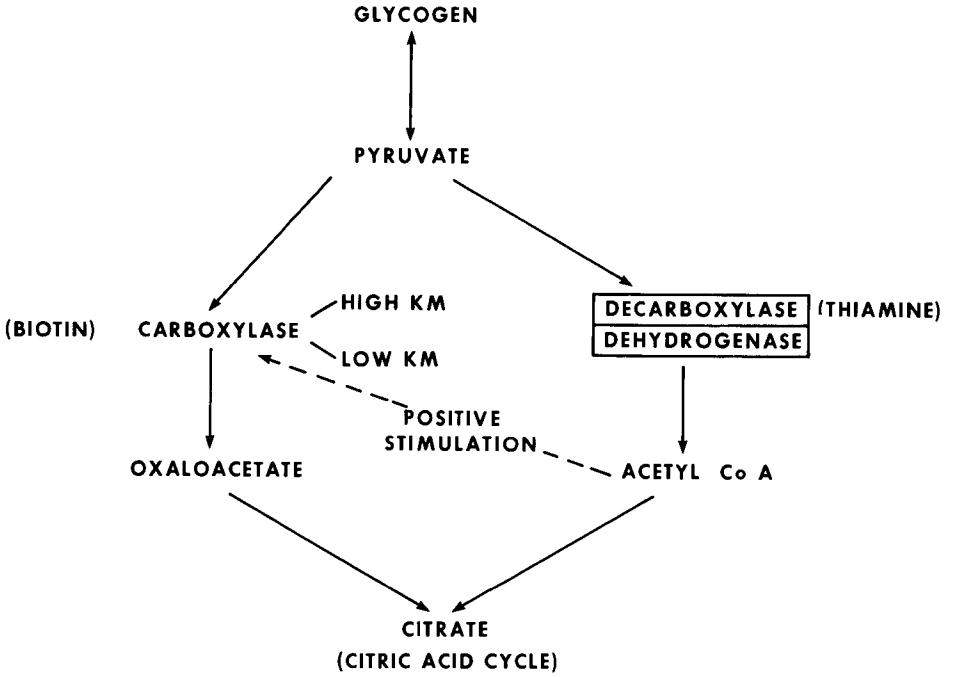

Fig. 8. Diagram shows metabolic pathway for entry of pyruvate to Krebs cycle.

that in diabetes mellitus. Blass et al[3] observed a similar condition in a patient whom they found to be deficient in pyruvate decarboxylase.

Summary

Four cases of different forms of encephalopathy are reported. All patients had significant hyperpyruvicuria with or without changes in alanine concentration in the blood or urine. Proof of decarboxylase deficiency in one patient and biochemical similarity in all led to the deduction that their diseases might have a common defect in carbohydrate metabolism at the level of oxidative decarboxylation of pyruvate. Evidence of a response to large doses of thiamine in three of the patients and the finding of TPP inhibitor in the urine of two of them supports this hypothesis.

Acknowledgment

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