Hyperalaninemia with Pyruvicemia due to Pyruvate Carboxylase Deficiency of the Liver

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A 10-year-old girl with mental retardation, hyperalaninemia and pyruvicemia was described. Studies on liver biopsy specimens from this patient revealed that pyruvate carboxylase activity was markedly decreased, whereas both pyruvate decarboxylation and glutamic pyruvic transaminase were normal in activity. Furthermore, it was found that an incorporation of pyruvate-2-¹⁴C into glycogen in the liver was markedly diminished in the patient as compared with that in a control.

These findings suggest that the metabolic lesion in the patient with hyperalaninemia and pyruvicemia is a defective activity of pyruvate carboxylase of the liver, leading to an impaired gluconeogenesis.

In a preliminary report of ours,¹ a mentally retarded girl with hyperalaninemia and pyruvicemia was presented and a defect of pyruvate carboxylase activity of the liver was demonstrated as a primary lesion of this illness.

The present paper deals with details of clinical and biochemical findings, including results of an investigation on gluconeogenesis, of this patient.

CASE REPORT

M.O., a 10-year-old girl was referred to the University Hospital on June 13, 1968, because of detailed examination for mental retardation. Her parents were not consanguineous. There were two siblings; one elder sister was also retarded mentally and died when 5 years old, another elder sister being healthy and of normal intelligence.

The patient was born after an uneventful pregnancy and full-term delivery. There was no abnormality in her neonatal history. Her mental and physical development was retarded; she smiled at the age of 18 months, held the head steadily at 1 year and was able to sit up alone at 4 years. Since 8 years of age, she had been admitted to an Institution for Retarded Children, Nishitaga, Sendai, then referred to the University Hospital for further investigation.

On admission when 10 years old, physical examination revealed a well nourished girl with apathetic countenance. She could not walk alone and could

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speak nothing. Body weight was 20 kg (normal, 19.4 kg) and circumference of the head was 48 cm (normal, 51.9 cm). The lungs and heart were clear on auscultation. The liver and spleen were not palpable. Extremities were somewhat rigid and deep tendon reflexes were decreased on both sides. Pathological reflexes were not elicited.

Laboratory findings: Routine urinalysis revealed no abnormality. Serum electrolytes, lipids and protein were normal. Hematologic study showed that red cell count was $364 \times 10^4/\text{cmm}$; hemoglobin, 12.1 g/100 ml; hematocrit, 37.0%; and white blood cell count, 9,450/cmm. Bone marrow findings were not contributory. Liver function tests revealed no abnormality. Fasting blood glucose was 80 mg/100 ml.

Serum urea-nitrogen was 14.5 mg/100 ml, and uric acid was 3.8 mg/100 ml. Serum folic acid (*L. casei*) was 4.8 m γ /ml. Nonesterified fatty acid in serum was 3.09 mg/100 ml.

Blood gas analysis showed that; pH was 7.39; pO₂, 90.1 mm Hg; pCO₂, 20.7 mm Hg; base excess, -9.5 mEq/L; standard bicarbonate, 17.2 mEq/L; and buffer base, 40.9 mEq/L.

Tryptophan loading test, (100 mg/kg of L-tryptophan), showed normal values in the urinary excretion of xanthurenic acid and pyridon; 0.4 μ moles/kg of bodyweight per 8 hours and 2.2 μ moles/kg of bodyweight per 8 hours, respectively.

Paperchromatography for urinary organic acids showed an excessive excretion of pyruvic acid, but the absence of a spot for methylmalonic acid.

Electroencephalography revealed a typical pattern of hypsarhythmia. Pneumoencephalography showed a dilatation of cerebral ventricles with the Evans' ratio of 0.52. Ophthalmological examination revealed bilateral myopia.

Histological findings of the liver showed no abnormality.

TABLE 1. Serum amino acid levels (μ moles/l) of our patient (M.O.)

Amino acids	Patient (M. O.)	Normal range ^a
Threonine	246	48-134
Serine	515	216-699
Glutamine		
Proline	133	116-181
Glutamic acid	127	62-289
Glycine	245	164-400
Alanine	827	168-483
Valine	143	80-310
Methionine	38	8-25
Isoleucine	46	29-90
Leucine	79	73-164
Tyrosine	54	37-97
Phenylalanine	43	28-125
Lysine	67	50-148
Histidine	46	38-70
Arginine	30	Trace -89

Analysis for amino acids in serum and urine: Thin layer chromatography of urinary amino acids revealed a slight increase in alanine. Serum amino acid patterns, analyzed by an automatic amino acid analyzer, showed a definite increase in alanine (cf. Table 1). Serum alanine levels were found to be ranging from 562 to 899 μ moles/L during the observation period of 10 months.

Serum alanine levels following an oral alanine loading (100 mg of alanine/kg) showed an almost normal clearance curve except for an elevated initial values (cf. Fig. 1).

Serum alanine levels of her mother and father were 259 and 432 μ moles/L, respectively.

Fig. 1. Alanine tolerance curve in our patient (M.O.) and controls. Alanine (100 mg per kg of body weight) was given orally.

TABLE 2. Free amino acid pattern in the liver of our patient (M.O.) (γ /g)

Amino acids	Patient (M. O.)	Normal range ³
Aspartic acid	75.0	25.1-113.0
Threonine	86.0	20.6-89.0
Serine	214.5	31.5-268.0
Glutamine		
Glutamic acid	252.0	57.4-454.0
Glycine	82.3	22.8-118.0
Alanine	259.0	23.3-184.0
Valine	17.6	6.8-63.6
Methionine	16.1	3.7-22.2
Isoleucine	20.7	6.8-28.3
Leucine	35.0	7.8-40.4
Tyrosine	14.3	2.8-21.4
Phenylalanine	23.6	4.4-25.6

TABLE 3. *The glutamic pyruvic transaminase (GPT) activity of the liver and leucocytes of our patient (M.O.)*

Case		Patient (M. O.)	Control
Material			
Liver	GPTs*	327,000	443,000
	GPTm*	241	446
Leucocytes		19.1	29.6

Unit is expressed as Karmen unit/g of wet weight of liver and Karmen unit/ 10^8 of leucocytes.

* GPTs stands for GPT in soluble fraction, GPTm for GPT in mitochondria.

TABLE 4. *Blood pyruvate and lactate levels of our patient (M.O.)*

	Pyruvate (mg/100ml)	Lactate (mg/100ml)
1968, July 15	2.9	16.6
Aug. 19	4.1	16.9
Oct. 9	3.2	14.3
Dec. 5	3.9	8.3
1969, Feb. 26	2.7	23.5
Mar. 28	3.8	36.4
Normal range	0.5-1.5	5-20

TABLE 5. *Lactic acid formation from glucose by erythrocytes (γ per 100 mg of hemoglobin)*

Patient (M. O.)	143
Controls 1	147
2	115

Note: 1.0 ml of washed erythrocytes was incubated with 1.0 ml of isotonic saline phosphate buffer (pH 7.4) containing 300 mg/100 ml of glucose and 500 units per ml of water soluble penicillin at 37°C for 2 hours. Before and after the incubation, the content of lactic acid in the mixture was determined by Barker-Summerson's method.⁴

Free amino acid pattern in the liver specimen obtained by a percutaneous biopsy showed a marked increase in alanine (cf. Table 2).

The glutamic pyruvic transaminase activity of the liver from the patient was found to be normal (cf. Table 3).

Blood pyruvate and lactate were estimated by Friedeman's method³ and Barker-Summerson's method,⁴ respectively. The blood pyruvate was found to be constantly elevated with a range from 2.9 to 4.1 mg per 100 ml, and the blood lactate also tended to remain in a high level, ranging from 16.6 to 36.4 mg per 100 ml (cf. Table 4).

The lactic acid formation from glucose was investigated by using erythrocytes. There was no difference in the *in vitro* lactate production from glucose by erythrocytes between the patient and controls (cf. Table 5).

Decarboxylation of pyruvate and pyruvate carboxylase activity in the liver: Decarboxylation of pyruvate was assayed by determining the $^{14}\text{CO}_2$ formation from pyruvate-1- ^{14}C , and the result showed no difference in decarboxylation of pyruvate between the patient and controls (cf. Table 6).

Pyruvate carboxylase activity was assayed by determining the incorporation of $^{14}\text{CO}_2$ to oxalacetate according to the method of Utter and Keech,⁵ and the results revealed that the pyruvate carboxylase activity was markedly decreased in the patient's liver (cf. Table 7).

Investigation on gluconeogenesis in the liver: After a 24-hour-fast liver specimens were obtained by the needle biopsy from this patient and a control subject without hepatic involvement. The assay for glycogen synthesis was carried out according to Hiatt *et al.*'s method⁶ with a slight modification: One ml of the Krebs-Ringer bicarbonate buffer containing 0.022 M of lactate, 0.016 M of pyruvate, $2\mu\text{C}$ of pyruvate-2- ^{14}C and liver slices (approximately 25 mg in wet weight), was incubated at 37°C in an atmosphere consisting of 95 per cent O_2 and 6 per cent CO_2 . After incubated for 15 minutes and 30 minutes, glycogen was separated by Bergstrom *et al.*'s method.⁷ The results showed a markedly diminished rate of incorporation of pyruvate-2- ^{14}C into glycogen in the patient's liver than in a control's liver (cf. Table 8).

TABLE 6. *Activities of pyruvate decarboxylation in the liver from our patient (M.O.) and controls (10^3 cpm per g of wet weight)*

Patient (M. O.)		149
Controls	1	234
	2	147

Liver slices were incubated with pyruvate-1- ^{14}C ($0.1\mu\text{C}/\mu$ mole) and Krebs-Ringer phosphate buffer for 60 min at 37°C and $^{14}\text{CO}_2$ liberated was determined.

TABLE 7. *Activities of pyruvate carboxylase of the liver from our patient (M.O.) and controls*

		μ moles per g of wet weight	μ mole per mg of protein
Patient (M.O.)	(10 y.)	0.19	0.0042
Controls	1 (5 y.)	1.16	0.0368
	2 (14 y.)	0.87	0.0264
	3 (7 y.)	0.69	0.0214

TABLE 8. *An incorporation of pyruvate-2- ^{14}C into glycogen of the liver (10^3 cpm per g of wet weight)*

Incubation time	Patient (M.O.)	Control
15 min	7.9	24.9
30 min	10.2	189.2

The rate of gluconeogenesis was expressed as cpm of ^{14}C incorporated to glycogen from pyruvate-2- ^{14}C .

COMMENT

Hyperalaninemia with pyruvicemia was first reported by Lonsdale⁸ in 1968, who described a six-year-old boy with recurrent episodes of cerebellar ataxia and high serum levels of both alanine and pyruvate. However, the site of biochemical lesion of this disorder had remained unsolved at that time.

The present study of ours revealed that hyperalaninemia with pyruvicemia in a mentally retarded girl was due to a defect in the pyruvate carboxylase activity of the liver and that the defective activity of pyruvate carboxylase in the liver was also supported by a demonstration of a decreased rate of incorporation of pyruvate-2-C¹⁴ into glycogen in the liver from this patient (cf. Fig. 2).

Fig. 2. The glycolytic and gluconeogenic pathways.

- A: Pyruvate decarboxylation   B: Pyruvate carboxylase
 C: Phosphoenol pyruvate carboxykinase   D: GPT (Glutamic pyruvic transaminase)
 E: GOT (Glutamic oxalacetic transaminase)   F: LDH (Lactic dehydrogenase)
 = Biochemical lesion in our patient

In 1962 Hartman *et al.*⁹ reported a case with lactic acidosis of unknown cause. Since then about 10 patients with idiopathic hyperlacticacidemia have been reported and these patients showed a similarity in clinical features such as attacks of tachypnea, convulsion, lethargy, muscular hypotonia, psychomotor retardation and obesity, and in all the patients there were signs of recurrent or chronic acidosis with an increase in both lactate and pyruvate in blood.

As for the biochemical mechanism responsible for lactic acidosis in these cases, no clearcut explanation has been described except that Schärer *et al.*¹⁰ suggested that the biochemical lesion might be at the level of pyruvate oxidation.

In 1968 Hommes *et al.*¹¹ made a biochemical study upon a patient with Leigh's

encephalomyelopathy (subacute necrotizing encephalomyelopathy) and found a defective activity of pyruvate carboxylase in the liver of this patient. Furthermore, according to Hommes *et al.*, blood lactate and pyruvate levels of this patient were 30.4 mg and 4.4 mg per 100 ml, respectively, with a somewhat low fasting levels of blood glucose, but there was no description on serum alanine levels.

The elevation of pyruvate and lactate in blood was reported in some cases of methylmalonic aciduria.¹³ Oberholzer *et al.*¹⁴ postulated that the elevation of blood pyruvate in cases with methylmalonic aciduria might be due to an inhibiting effect of methylmalonyl-CoA upon pyruvate carboxylase. In our patient with hyperalaninemia and pyruvicemia an excessive amount of methylmalonic acid was not detected in urine.

It may be, therefore, said that there is a possibility that among cases of chronic lactic acidosis reported so far, some may be identical with hyperalaninemia with pyruvicemia⁸ or with methylmalonic acidemia.¹⁴

Furthermore it remains unsolved whether or not hyperalaninemia with pyruvicemia and Leigh's encephalomyelopathy may be included into a same entity characterized by a defective activity of the pyruvate carboxylase in the liver.

References

- 1) Tada, K., Yoshida, T., Konno, T., Wada, Y., Yokoyama, Y. & Arakawa, Ts. Hyperalaninemia with pyruvicemia (Preliminary report). *Tohoku J. exp. Med.*, 1969, **97**, 99-100.
- 2) Tada, K. Serum amino acid pattern in normal children, assayed by automatic amino acid analyzer. *Rinshoshoniigaku* (Jap.), 1964, **12**, 129-132.
- 3) Friedemann, T.E. & Haugen, G.E. Pyruvic acid. I. Collection of blood for the determination of pyruvic and lactic acids. *J. biol. Chem.*, 1942, **144**, 67-77.
- 4) Barker, S.B. & Summerson, W.H. The colorimetric determination of lactic acid in biological material. *J. biol. Chem.*, 1941, **138**, 535-554.
- 5) Utter, M.F. & Keech, D.B. Pyruvate carboxylase. I. Nature of the reaction. *J. biol. Chem.*, 1963, **238**, 2603-2608.
- 6) Hiatt, H.H., Goldstein, M., Lareau, J. & Horecker, B.L. The pathway of hexose synthesis from pyruvate in muscle. *J. biol. Chem.*, 1958, **231**, 303-307.
- 7) Bergstrom, J., Findor, J. & Hultman, E. The contents of glycogen and potassium in human liver tissue obtained by needle biopsy. *Scand. J. clin. Lab. Invest.*, 1961, **12**, 353-354.
- 8) Lonsdale, D. Hyperalaninemia with pyruvicemia. *New Eng. J. Med.*, 1968, **278**, 1235.
- 9) Hartmann, A.F., Wohltmann, H.J., Purkerson, M.L. & Wesley, M.E. Lactate metabolism. Studies of a child with a serious congenital deviation. *J. Pediat.*, 1962, **61**, 165-180.
- 10) Schärer, K., Marty, A. & Mühlethaler, J.P. Chronic congenital lactic acidosis. A fatal case with hyperphosphatemia and hyperlipemia. *Helv. paediat. Acta*, 1968, **23**, 107-127.
- 11) Hommes, F.A., Polman, H.A. & Reerink, J.D. Leigh's encephalomyelopathy: An inborn error of gluconeogenesis. *Arch. Dis. Childh.*, 1968, **43**, 423-426.
- 12) Leight, D. Subacute necrotizing encephalomyelopathy in an infant. *J. Neurol. Neurosurg. Psychiat.*, 1951, **14**, 216-221.
- 13) Lindblad, B., Lindblad, B.S., Olin, P., Svanberg, B. & Zetterström, R. Methylmalonic acidemia. A disorder associated with acidosis, hyperglycinemia, and hyperlactatemia. *Acta paediat. scand.*, 1968, **57**, 417-424.

- 14) Oberholzer, V.G. Burgess, E.A. & Young, W.F. Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. *Arch. Dis. Childh.*, 1967, **42**, 492-504.
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