Systemic Deficiency of the First Component of the Pyruvate Dehydrogenase Complex

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ABSTRACT. An infant with lactic acidosis and developmental delay had neuropathological changes consistent with Leigh's necrotizing encephalomyelopathy. Total pyruvate dehydrogenase complex (PDC) activity was low relative to controls in lymphocytes (0.2 *versus* 1.9 ± 0.6 SD nmol/min/mg protein) and cultured skin fibroblasts (0.9 *versus* 2.7 ± 1.0). Liver, muscle, heart, and kidney mitochondria oxidized several substrates normally, but did not oxidize pyruvate. PDC activity was absent in these mitochondria (0.1 *versus* 9.8 ± 4.2 in liver and 0.7 *versus* 75 ± 26 in muscle) and was very low in all tissue homogenates. Activity of the first component was low in liver mitochondria, whereas activities of the second and third components were normal. Western blot analysis of tissue proteins showed normal amounts of second and third component of PDC but undetectable to trace amounts of both α and β subunits of the first component of PDC in liver, brain, kidney, heart, and skin fibroblasts. Thus, profound systemic deficiency of PDC was due to lack of both subunit proteins of the first component of PDC. (*Pediatr Res* 22: 312–318, 1987)

Abbreviations

PDC, pyruvate dehydrogenase complex
E_1, first component of PDC (pyruvate dehydrogenase)
E_2, second component of PDC (dihydrolipoyl transacetylase)
E_3, third component of PDC (dihydrolipoamide dehydrogenase)
MOPS, morpholinopropane sulfonic acid
SDS, sodium dodecyl sulfate

PDC catalyses a key regulatory step in energy metabolism, the irreversible oxidation of pyruvate to acetyl-CoA, which permits energy derived from carbohydrate to enter the Krebs cycle. Defects of PDC have been described in association with a variety of clinical syndromes. Common features of these disorders include lactic acidosis and neurological dysfunction, but there is great variation of the severity and systemic expression of reported defects. This heterogeneity may not seem surprising, considering the many possibilities for genetic variation affecting this multienzyme complex containing at least six gene products which, if defective, could result in loss of activity. To date, some 90 individuals with defects of PDC have been described (1, 2), but relatively few of these cases have been investigated in detail.

Approximately 28 cases of defects involving E_1 have been reported (1–11). Most of these defects have been characterized by decreased activity of PDC in skin fibroblasts and by decreased activity of the E_1 component assayed by a partial reaction in which ferricyanide was used as an artificial electron acceptor (3, 12). The activities of PDC and E_1 were measured in white blood cells in one case and in homogenates of other tissues in two patients (3, 4, 7). Immunological techniques have been applied to the analysis of PDC mutants (9–11), and decreased enzyme activity has been described in association with a decrease of immunoreactive protein in two cases of E_1 deficiency (10, 11) and in one case which involved the E_3 component (13).

We have investigated a case of severe PDC deficiency in some detail utilizing the combined resources of several laboratories to determine the systemic physiological consequences and mechanisms responsible for profound deficiency of this key enzyme of energy metabolism.

METHODS

Human subjects. Collections of blood and tissue specimens from the patient and controls were carried out with informed consent according to protocols approved by the Institutional Review Board of University Hospitals of Cleveland. Blood samples for preparation of lymphocyte controls were obtained from adult volunteers. Skin fibroblast controls were obtained from established cell lines derived from infants and children and forwarded to us from a number of laboratories along with cells sent for analysis of possible defects of pyruvate metabolism. Samples of liver and rectus abdominus muscle used for control data were obtained from 12 children who underwent elective biopsies for diagnosis of a variety of other defects associated with lactic acidosis or abnormalities of fatty acid oxidation. Rectus abdominus muscle biopsies for preparation of mitochondria were also obtained from six adults undergoing elective abdominal surgery.

Preparation of lymphocytes and fibroblasts. Lymphocytes were obtained from whole blood collected under sterile conditions, anticoagulated with citrate-phosphate-dextrose-adenine solution (CPDA-1, 0.15 ml/ml blood; Fenwal Laboratories, Deerfield,

[1] Subsequent to investigation of the present case, we have assayed cells and tissues from two brothers who have normal PDC activity in skin fibroblasts but lack PDC activity in lymphocytes. PDC activity was present in kidney, but not in liver, muscle, and heart (41).

IL), shipped as whole blood along with control blood samples from two adult volunteers, and separated for assay after two days. Lymphocytes were isolated by the Ficoll-Paque method (Pharmacia Inc., Piscataway, NJ) (14), and the final pellet was resuspended in phosphate-buffered saline. Skin fibroblasts were cultured in F12 medium (Gibco Laboratories, Chagrin Falls, OH) with 16% fetal calf serum, fortified with penicillin and streptomycin, and subcultured until sufficient cells reached confluence (3 T75 flasks required to provide sufficient protein for assay of PDC). The cells were harvested with 0.5% trypsin, scraped off with a rubber policeman, and washed in phosphate-buffered saline prior to preparation for assay.

Enzyme assays. PDC activity was measured as thiamine pyrophosphate and coenzyme A-dependent decarboxylation of [1-^{14}C]pyruvate by the method of Sheu *et al.* (15), with the following minor modifications. Cells or mitochondria were preincubated for 15 min in the presence of either dichloroacetate, 5 mM, or fluoride, 50 mM, frozen and thawed twice, and incubated in 0.1 ml final volume of the assay mixture as previously described (15), with the addition of fetal calf serum, 2.5%. The incubation tube was sealed with a serum stopper from which a plastic cup was suspended, containing filter paper impregnated with hyamine hydroxide. Incubation times varied from 4 to 20 min for fibroblasts, lymphocytes, and mitochondria. After trapping the evolved $^{14}CO_2$, the entire cup and hyamine saturated filter paper was transferred to a vial for liquid scintillation counting. Thiamine pyrophosphate and coenzyme A were omitted from blank tubes. Each assay included quadruplicate reaction tubes and quadruplicate blanks for each of the following conditions: untreated, dichloroacetate activated, and fluoride inactivated cells or mitochondria. Control cells or mitochondria were prepared simultaneously with samples from the patient and included in each assay.

E_1 was assayed in the same way except that coenzyme A and NAD were omitted and ferricyanide, 67 mM, was added. It was found that ferricyanide is not required for this reaction with crude homogenates or even with purified PDC, but the reaction is dependent on added thiamine pyrophosphate, which was omitted from the blank. Although phosphorylation-regulated activity of E_1 is considered rate limiting for PDC, the rate of this nonphysiological partial E_1 reaction is less than 10% of the rate of the overall PDC reaction, since continuing transfer of the acetyl group from thiamine pyrophosphate to the lipoyl moiety of E_2 is not possible in the absence of coenzyme A and NAD. E_2 was assayed by acetylation of reduced lipoamide with [1-^{14}C] acetyl-CoA (14). To correct for a significant nonenzymatic rate for this reaction, extracts were omitted from blanks which were incubated for the same time. E_3 was assayed spectrophotometrically by reduction of NAD in the presence of reduced lipoamide and/or by oxidation of NADH in the presence of lipoamide (16). Pyruvate carboxylase and phosphoenolpyruvate carboxykinase were measured by fixation of $^{14}CO_2$ and conversion of the oxalacetate formed to citrate or malate, as previously described (15). Protein was measured by the method of Lowry using bovine serum albumin as a standard (18).

Preparation of mitochondria. Samples of liver, skeletal muscle, heart, and kidney obtained within an hour of death were placed in ice cold media (A) containing: mannitol, 225 mM; sucrose, 75 mM; MOPS, 5 mM, pH 7.0; and EDTA, 50 μM. All tissues were minced and rinsed free of blood in media A. Liver and kidney tissues were homogenized in 50 ml per g wet weight of media A by brief treatment (10 s) with a Polytron tissue disrupter set at low speed, and the mitochondria were isolated by standard techniques of differential centrifugation (19). The cardiac and skeletal muscle minced specimens were transferred to a different media (B), 10 ml/g wet weight, which contained: KCl, 140 mM; MgCl$_2$, 2.5 mM; ATP, 2.5 mM; EDTA, 50 μM; MOPS, 5 mM, pH 7.0; and Nagarse protease, 0.4 mg/ml. The minces were kept on ice with the protease for 5 min and then diluted 10-fold with media B and homogenized with a polytron tissue disrupter at

low speed for 10 s. The homogenates were centrifuged at 2,000 × g for 5 min and the pellets discarded. The supernatants were centrifuged at 8000 × g for 10 min and the pellets saved. The pellets were resuspended and washed twice in media A. Liver mitochondria from control subjects were prepared in a similar manner, as previously described (20). Skeletal muscle mitochondria from control subjects were prepared with minor variations from the method above, as previously described (21) with addition of ATP, 1 mM, and MgCl$_2$, 5 mM (22, 23).

Oxygen uptake studies. Oxidation of substrates by freshly isolated mitochondria from the patient was measured polarographically with a Clark electrode at 28°. The incubation buffer contained KCl, 150 mM; MOPS, 20 mM; KH$_2$PO$_4$, 5 mM; and EDTA, 50 μM; at pH 7.2. Endogenous substrates were depleted by addition of ADP, followed by addition of specific substrates at the concentrations shown in Table 2. Rates of oxygen uptake were determined in the presence and absence of ADP, 5 mM (corresponding to states 3 and 4 respectively), and the respiratory control ratio (state 3/state 4) determined (24). Data from control liver and skeletal muscle mitochondria were obtained at 30° by similar techniques (see Table 2) (23).

Immunoassays. PDC components were analyzed by the Western blot method (10). Frozen tissues or mitochondria were suspended in potassium phosphate, 20 mM, pH 7.0, and Triton X-100, 1%. The samples were sonicated for two 10-s continuous pulses with a microtip, followed by centrifugation (12,000 × g, 15 min, 4°). Aliquots of the supernatant were dissociated by boiling for 10 min in dithiothreitol, 17 mM, and SDS, 2%. Protein extracts were separated by electrophoresis in discontinuous SDS-polyacrylamide gels (6% polyacrylamide stacking; 9% polyacrylamide separation) (25). Proteins were electroeluted from the gel onto a nylon-based membrane (Zeta-probe, BioRad Laboratories, Richmond, CA) with a Bio-Rad transblot apparatus at room temperature for 8 h at 75 mamp, essentially as described in Bio-Rad instruction manual no. 85-0176-485. The membranes were then incubated with specific antisera followed by [^{125}I]protein A (0.1 μg, 36 μC/μg; ICN Biochemicals, Irvine, CA). Immunoreactive proteins were visualized by exposing the membrane to X-Omat AR film (Eastman Kodak Co., Rochester, NY). Where indicated, a goat antirabbit IgG conjugated with horseradish peroxidase was used instead of [^{125}I]protein A and immunoreactive proteins visualized by stain deposition from the oxidation of 4-chloro-1-napthol, following the procedure described in Bio-Rad instruction manual no. 83-0007-0283.

Antisera against individual components E_1, E_2, and E_3 were raised in rabbits using purified enzymes and standard immunization procedures (26, 27). Antigens for immunization of rabbits were obtained as follows: purified bovine kidney E_1 was a gift from Dr. Tracy Linn to the late Merton Utter, bovine heart E_2 was a gift from Dr. Lester Reed, and porcine heart E_3 was obtained from a commercial source (Sigma Chemical Co., St. Louis, MO). Bovine E_1 and E_3 used as standards were also generously provided by Dr. L. Reed. The anti-E_1 and anti-E_2 sera are specific for the E_1 and E_2 components of PDC, respectively. In contrast, the anti-E_3 serum reacts with the E_3 components of pyruvate, α-ketoglutarate, and branched-chain α-ketoacid dehydrogenase complexes (Carothers DJ, Patel MS, unpublished data). For immunoblot analysis, antisera were diluted with TrisCl, 20 mM, pH 7.5; NaCl, 500 mM; and bovine serum albumin, 5%. Antisera for E_1, E_2, and E_3 were diluted 20-, 50-, and 50-fold, respectively. Deoxyribonucleic acid was assayed fluorometrically using salmon sperm DNA as a standard (28).

CASE REPORT

The patient was the product of a 37-wk pregnancy in a 19-yr-old primiparous mother. The pregnancy was complicated by preeclampsia treated with hydralazine and MgSO$_4$. The infant was small for gestational age with a birth weight of 2325 g. Apgar scores were 6 (1 min) and 9 (5 min). Shortly after birth the baby

was noted to be flaccid and required oxygen for a brief period. Blood gases revealed metabolic acidosis (pH 7.29; bicarbonate, 8 mEq/liter). The patient was transferred to Hershey Medical Center. The acidosis was corrected with sodium bicarbonate. The patient initially was moderately hypotonic but gradually improved. Responsiveness to auditory stimuli was diminished and an intermittent strabismus was present. An ultrasound examination of the brain was normal. There was no evidence of cardiac disease. Blood ammonia was 71 and 45 μM when repeated. Metabolic screening tests showed increased alanine in serum and urine. Organic acid analysis of the urine by gas chromatography showed greatly increased lactic acid (3065 mg/mg creatinine). Blood lactate was 5.8 mM (normal 0.5–2.2).

Following the first hospitalization the patient was given a standard infant formula with addition of sodium bicarbonate, 2 mEq/4 oz feeding, later increased to 5 mEq. He gained weight slowly (25th percentile). At age 3 months he was hospitalized following a brief tonic clonic seizure and an apneic episode. On admission he was noted to have poor head control, a weak cry, and intermittent strabismus. Blood lactate was 8.0 mM. Treatment with phenobarbital was started, but in the hospital he continued to have brief seizures until phenytoin was added. There were no further seizures for the duration of his life. On two occasions his EEG was normal. His opthalmological examination was normal, with no evidence for optic atrophy. An NMR imaging scan of the head was interpreted as possibly showing increased density lateral to the midportion and posterior to both lateral ventricles, perhaps due to a demyelinating process. His cardiovascular examination and an electrocardiogram were normal. Plasma total carnitine was 25 nmol/ml (low normal). A chromosome analysis was normal. Prior to discharge the dose of bicarbonate was increased to 7 mEq per feed. He was treated briefly with biotin, 10 mg/day, without apparent benefit.

When a metabolic diagnosis was made, the formula was modified by addition of vegetable oil to provide 60% of energy as fat, 110 kcal/kg/day, and approximately 2.0 g protein/kg/day. The bicarbonate dose was gradually increased to 15 mEq every 4 h. However, during the second 6 months of life, the patient gained weight slowly, had increased lethargy, poor feeding, and occasional fevers of undetermined etiology. Feeding became progressively more difficult, eventually requiring that he be fed by nasogastric tube. At age 9 months blood lactate was 11 mM and pyruvate was 0.95 mM, giving a ratio of 12 (normal 10–20). After 10 months age, he had five more hospital admissions for bronchiolitis, apnea, or metabolic acidosis. The final hospitalization at age 12 months was for marked lethargy and fever. In the hospital, 2 days before death, he had a respiratory arrest with severe metabolic acidosis (pH 7.03). He was rapidly resuscitated but required large amounts of sodium bicarbonate and remained ventilator dependent and hypotensive. He developed fixed dilated pupils, an EEG showed flat brain wave function, and he was pronounced dead. Permission was given for an immediate autopsy.

Autopsy showed bronchopneumonia, septicemia, and acute focal septic hepatitis. There was no evidence of cardiac or skeletal muscle abnormalities by gross examination, or light or electron microscopy. The remaining abnormalities were confined to the central nervous system. There was symmetrical focal cystic necrosis of much of the putamen and less severely of the caudate nuclei, characterized by neuronal loss and astrocytosis without remarkable vascular proliferation. There was mild hypomyelination of the white matter of the cerebral hemispheres and loss of myelinated axons from the central portions of the optic nerves and posterior columns of the spinal cord. The latter also showed vacuolation. The hypothalamus, mammilary bodies, midbrain, and pons appeared normal. In addition, there were changes consistent with diffuse acute anoxic-ischemic damage involving cerebral and cerebellar cortices, basal ganglia, thalami, and reticular nuclei of the medulla. These changes included eosinophilia of neurons and varying degrees of vacuolization of neuropile.

There was also evidence of recent necrosis of the posterior horns and chromatolysis of the anterior horns in the lumbo-sacral spinal cord. The spinal roots showed a moderate degree of axonal degeneration.

RESULTS

PDC activity in fibroblasts and lymphocytes. Deficiency of PDC activity was initially detected in lymphocytes isolated from whole blood. Lymphocyte PDC activity is normally similar to that in fibroblasts, and can be activated by preincubation with dichloroacetate or inactivated by preincubation with fluoride (Table 1) (29). Dichloroacetate-treated lymphocytes from the patient had less than 10% as much activity as a concurrent control or than the average activity found in other controls; untreated lymphocytes had 6% as much activity as controls. The activity in cultured skin fibroblasts, assayed on two separate occasions, was somewhat higher, corresponding to about a third of the average activity found in dichloroacetate-activated or untreated control fibroblasts. The range of activity found in control lymphocytes and fibroblasts is quite large, but the activity in both types of cells from the patient was less than the lower range of controls.

Lymphocytes from the patient's mother were obtained for measurement of PDC activity (the father was not available). The activity after dichloroacetate treatment was 1.4 nmol/min/mg protein, which is within 1 SD of the mean of controls.

Oxidation of substrates by intact mitochondria. Within 1 h of the patient's death, samples of liver, skeletal muscle, heart, and kidney were obtained for preparation of mitochondria. Mitochondria prepared from rat liver served as a concurrent control. The freshly isolated mitochondria were incubated with a number of substrates and oxygen uptake was measured in the presence and absence of ADP. The mitochondria oxidized most of the substrates tested very actively and showed appropriately high respiratory control ratios, in the range of 4 to 10 with glutamate. Therefore, these were relatively intact mitochondria despite having been obtained postmortem. The ADP stimulated rates of oxidation of palmitoyl-carnitine and succinate are equal to those we found with human liver and skeletal muscle mitochondria prepared from biopsies obtained from control child and adult subjects (Table 2). The rates of oxidation of substrates other than pyruvate by the patient's heart and kidney mitochondria appear normal compared to rates previously reported for heart and kidney mitochondria from rats or rabbits (30, 31) and a human subject (32).

Addition of pyruvate did not result in any measurable increase in the rate of oxygen uptake by any of these four types of mitochondria. They appeared to be completely unable to oxidize pyruvate. Oxidation of pyruvate by intact mitochondria normally requires catalytic amounts of malate as a source of oxalacetate in order to remove acetyl-CoA, the product of pyruvate oxidation, by formation of citrate. In the presence of malate alone, there was a slow basal rate of oxidation, at least by liver,

Table 1. *Pyruvate dehydrogenase complex activity in lymphocytes and fibroblasts (nmol/min/mg protein)*

Cell type	Subject	Untreated	Dichloroacetate activated	Fluoride inactivated
Lymphocytes	Patient	0.05	0.16	0.02
	Controls*	0.83 ± 0.39	1.89 ± 0.65	0.51 ± 0.38
	(Range)	(0.25–1.92)	(0.92–3.33)	(0.13–1.84)
Fibroblasts	Patient	0.52, 0.34	0.83, 0.88	0.18, 0.07
	Controls†	1.70 ± 0.84	2.69 ± 0.98	1.21 ± 0.72
	(Range)	(0.47–3.92)	(1.13–6.67)	(0.07–3.30)

* Control values for lymphocytes from 45 normal adults, mean ± SD.
† Control values from 39 skin fibroblast lines from children, mean ± SD.

heart, and kidney mitochondria. When pyruvate and ADP were added, there was no increase in oxygen uptake with any of the patient's mitochondria, in contrast to the large increase observed with liver and muscle mitochondria from controls or that reported for kidney and heart mitochondria from animal and human sources (30–32).

PDC activity in mitochondria and tissue homogenates. Aliquots of fresh intact mitochondria from the patient and controls were preincubated with either dichloroacetate or fluoride before assay of PDC. There was virtually no detectable PDC activity in any of the patient's mitochondria whether untreated, activated with dichloroacetate, or inactivated with fluoride (Table 3). The activity in liver and muscle mitochondria was less than 2% of the average of controls. The activity in kidney or heart mitochondria was much less than a single control; the relative activity of PDC in rat kidney or heart is higher than in liver or skeletal muscle, respectively (33).

PDC activity was also measured in frozen whole tissues obtained from the patient at autopsy and from controls. The activities of PDC in the patient's brain and kidney were about 1 to 4% of the average of controls and the activities in heart and liver were about 6 to 12% of controls, respectively. Therefore, PDC activity was extremely low in all tissues tested from this patient as well as in isolated mitochondria. Since PDC is a mitochondrial enzyme, the apparent difference between the patient and controls was not as striking in comparing the frozen tissues.

Activity of the components of PDC. Activities of the three catalytic components of the PDC were assayed in skin fibroblasts and in liver and muscle mitochondria (Table 4). Activity of E_1, which is a partial reaction, is normally very slow, being only about 5% of the overall reaction rate. The activity of E_1 in liver mitochondria from the patient was barely detectable, about 10% of the average of controls. It has been generally assumed that ferricyanide acts as an artificial electron acceptor in this reaction, but we found that ferricyanide is not required. The rate of E_1 in fibroblasts was relatively higher, about half that of controls. On the other hand, the activities of E_2 and E_3 in fibroblasts and liver and muscle mitochondria were normal relative to controls (the activity of E_3 was lower in muscle mitochondria from the patient than controls, but it is suspected that this was an artifact due to a technical problem in preparation of these mitochondria resulting in a low yield). In addition, the activities of two other enzymes of pyruvate metabolism, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase were normal in lymphocytes, fibroblasts, and liver mitochondria (Table 5).

Immunological analysis of the components of PDC. Western blot analysis was used to compare extracts of liver mitochondria and whole liver (Fig. 1), kidney, heart, and brain (Fig. 2) from the patient and from the control infant subject with normal PDC activity described above. Equal quantities of protein from the patient and control showed similar amounts of an immunoreactive protein corresponding to purified bovine heart E_2 and E_3 (Figs. 1 and 2). Bovine heart E_2 migrates more slowly than E_2 from human (10) and rat (34) liver. Using the same technique with anti-E_1 antibody, both the α and β subunits of E_1 were detected in extracts of all the frozen tissues from the control (Figs. 1 and 2). In contrast, no immunoreactive protein was detected in positions corresponding to either $E_{1\alpha}$ or $E_{1\beta}$ in extracts from the patient's liver or brain, and only barely detectable trace amounts were present in the heart and kidney homogenates. Extracts of cultured skin fibroblasts were analyzed in the same way and were found to contain greatly reduced, but detectable, amounts of $E_{1\alpha}$ and $E_{1\beta}$ compared with control fibroblasts, whereas the content of E_2 and E_3 antigens was normal (results not shown). There was no evidence for accumulation of aberrant immunoreactive precursor-peptides in any of the pa-

Table 2. *Rates of oxygen uptake by intact mitochondria**

Substrates	Subject	Liver†	Muscle‡	Heart§	Kidney§
Malate	Patient	8	0	35	23
	Controls	13 ± 3	8 ± 7		
Pyruvate + malate	Patient	8	0	34	24
	Controls	21 ± 4	107 ± 27		
Glutamate + malate	Patient	44	36	311	76
	Controls	32 ± 6	113 ± 14		
Palmitoyl-carnitine + malate	Patient	27		276	58
	Controls	30 ± 8	73 ± 6		
Succinate + rotenone	Patient	66	65	145	88
	Controls	50 ± 27	145 ± 18		

* State 3 rates (in the presence of ADP, 5 mM), natoms 0/min/mg protein. Substrate concentrations used with the patient's mitochondria were as follows: malate, 1 mM; pyruvate, 5 mM; glutamate, 10 mM for liver and kidney and 20 mM for heart and skeletal muscle; palmitoylcarnitine, 50 μM; succinate, 10 mM; and rotenone, 5 μM. Substrate concentrations used for controls were similar, except for malate, 5 mM; and pyruvate, 10 mM; glutamate was assayed in the livers from controls without added malate.

† Liver controls from five infants with other disorders, mean ± SD.
‡ Muscle controls from five children and adults, mean ± SD (23).
§ See text for references for control data for kidney and heart.

Table 3. *Pyruvate dehydrogenase complex activity in mitochondria and tissue homogenates (nmol/min/mg protein)*

Tissue	Fraction	Untreated		Dichloroacetate activated		Fluoride inactivated	
		Patient	Controls*	Patient	Controls*	Patient	Controls*
Liver	Mitochondria	0.25	8.1 (1.5–20)	0.1	9.8 (4.2–18)	0.1	5.0 (0.5–14)
	Homogenate	0.20	1.6 (0.2–4.6)				
Muscle	Mitochondria	0.5	67 (34–127)	0.7	75 (36–127)	0.1	53 (32–75)
	Homogenate		2.7 (0.9–6.7)				
Kidney	Mitochondria	0.6	22	0.8	24	0.5	8.6
	Homogenate	0.2	5.3 (2.7–9.5)				
Heart	Mitochondria	3.6	77	3.9	62	2.0	57
	Homogenate	0.4	7.2 (1.9–16)				
Brain	Homogenate	0.1	6.4 (3.9–9.0)				

* Control values are means with the range shown in parentheses. Mitochondria from controls were obtained from biopsies ($n = 11$ for liver and 8 for muscle) or from autopsy ($n = 1$ for kidney and heart). Whole tissue samples for homogenates were obtained by biopsy or autopsy ($n = 20$ for liver, 12 for muscle, 4 for kidney, 6 for heart, and 2 for brain).

Table 4. *Activity of components of pyruvate dehydrogenase complex in skin fibroblasts and mitochondria (nmol/min/mg protein)*

Cell or tissue	Subject	E_1	E_2	E_3*
Fibroblasts	Patient	0.04	2.9	61
	Controls†	0.09 ± 0.01 (7)	2.9 ± 1.2 (10)	39 ± 17 (13)
	(Range)	(0.06–0.11)	(1.3–5.3)	(19–67)
Liver mitochondria	Patient	0.02	63	824
	Controls†	0.23 (3)	69 ± 23 (5)	510 ± 118 (4)
	(Range)	(0.19–0.30)	(30–93)	(351–626)
Muscle mitochondria	Patient		77	102
	Controls†	0.79 (3)	94 ± 44 (5)	313 (3)
	(Range)	(0.34–1.36)	(46–160)	(226–398)

* The activity of dihydrolipoamide dehydrogenase was measured in the direction of reduction of NAD, except muscle mitochondria were assayed in the direction of NADH oxidation.

† Mean ± SD for controls, with the number of subjects shown in parentheses. Liver and muscle mitochondria controls from biopsies.

Table 5. *Activity of other mitochondrial enzymes related to pyruvate metabolism*

Cell or tissue	Subject	Pyruvate carboxylase	Phosphoenolpyruvate carboxykinase
Lymphocytes	Patient	0.15	1.76
	Controls*	0.08 ± 0.04	1.41 ± 0.52
	(Range)	(0.04–0.21)	(0.80–2.81)
Fibroblasts	Patient	2.00	6.6
	Controls†	1.36 ± 0.76	5.0 ± 3.4
	(Range)	(0.45–3.82)	(0.74–13.8)
Liver mitochondria	Patient	287	436
	Controls‡	310 ± 173	322 ± 203
	(Range)	(96–692)	(109–789)

* Control values for lymphocytes from 26 normal adults, mean ± SD.

† Control values from 37 skin fibroblast lines from children, mean ± SD.

‡ Liver mitochondria control values from 10 biopsies, mean ± SD.

tient's tissues. In a separate experiment, the amounts of both E_1 peptides were greatly diminished in extracts of liver and kidney mitochondria from the patient relative to a different control. When purified bovine heart E_1 was mixed with an extract of liver mitochondrial protein from the patient, both $E_1\alpha$ and $E_1\beta$ were present in the expected amounts (results not shown), indicating that failure to detect E_1 peptides in the patient's tissues was not due to interference with the detection method used.

DISCUSSION

The findings in this case provide unequivocal evidence for profound systemic deficiency of PDC due to lack of the first component, E_1. These findings include: 1) persistent lactic acidosis from birth in a neurologically deteriorating infant, with a normal lactate to pyruvate ratio and without other metabolic abnormalities in blood and urine except for increased alanine; 2) inability of isolated mitochondria from liver, skeletal muscle, kidney, or heart to oxidize pyruvate; 3) markedly decreased activity of PDC in isolated lymphocytes, skin fibroblasts, liver and muscle mitochondria, and frozen liver, kidney, heart, and brain; 4) decreased activity of the E_1 component and normal activity of E_2 and E_3 in fibroblasts and liver and muscle mitochondria; and 5) undetectable or greatly diminished amounts of immunoreactive proteins corresponding to the α and β subunits of E_1 with normal content of the E_2 and E_3 proteins in liver, brain, kidney, heart, and skin fibroblasts. No other case of PDC deficiency has been so thoroughly characterized.

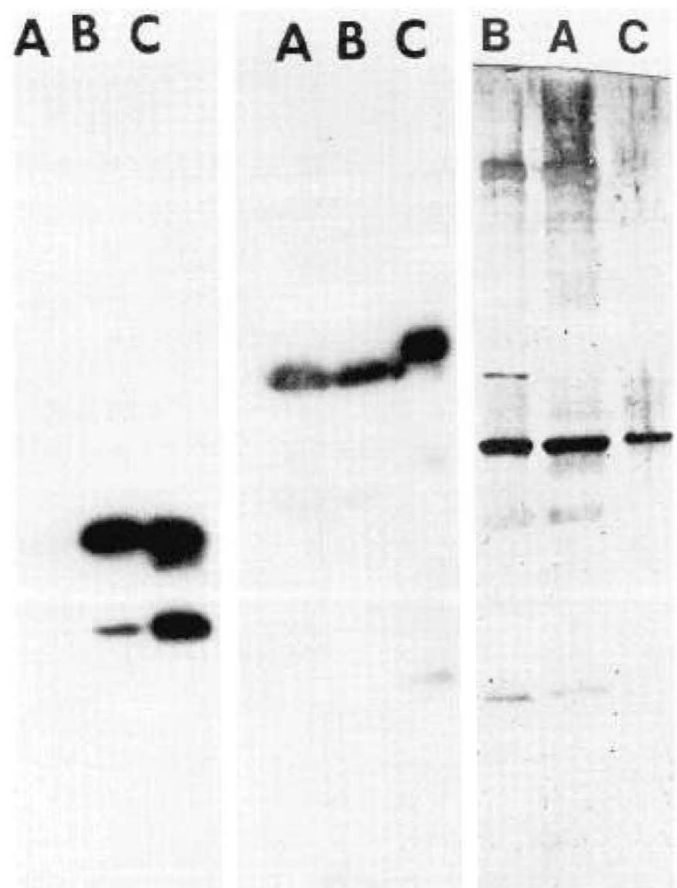It is difficult to comprehend how this infant was able to oxidize

Fig. 1. Immunoblot analysis of PDC components in liver from the patient and a control. *Left,* E_1 immunoblot of liver homogenates. Extracts of homogenized frozen liver were used for analysis (aliquots are expressed in relation to their content of DNA). The membrane was treated with anti-E_1 serum followed by [125I]-protein A. *Lane A,* patient (28 μg of DNA); *lane B,* control (15 μg DNA); *lane C,* purified bovine kidney E_1 (*upper band*: $E_1\alpha$; *lower band*: $E_1\beta$). *Middle,* E_2 immunoblot of liver homogenates. Extracts of liver homogenates containing similar quantities of DNA were analyzed. The membrane was treated with anti-E_2 followed by [125I]-protein A. *Lane A,* patient (7.7 μg DNA); *lane B,* control (7.6 μg DNA); *lane C,* purified bovine heart E_2. *Right,* E_3 immunoblot of liver mitochondria. Mitochondrial extracts containing equal amounts of protein were analyzed. The membrane was treated with anti-E_3 serum followed by goat antirabbit IgG conjugated with horse radish peroxidase. *Lane A,* patient (50 μg protein); *lane B,* control (50 μg protein); *lane C,* purified bovine heart E_3 (0.20 μg protein).
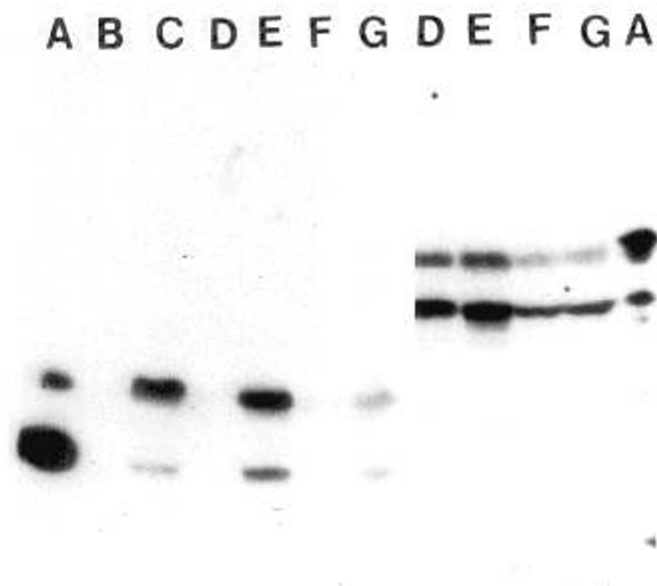
Fig. 2. Immunoblot of PDC components in kidney, heart, and brain homogenates from the patient and a control. *Left*, E_1 immunoblot. Extracts of homogenates of frozen tissues containing 0.4 mg of protein were analyzed. The membrane was treated with anti-E_1 serum followed by [^{125}I]-protein A. *Upper band*, $E_1\alpha$; *lower band*, $E_1\beta$. *Right*, simultaneous immunoblot of E_2 and E_3. Extracts of homogenates containing 0.2 mg of protein were analyzed. The membrane was treated simultaneously with anti-E_2 and anti-E_3 sera followed by [^{125}I]-protein A. *Upper band*, E_2; *lower band*, E_3. Both panels, *lane A*, bovine heart PDC; *lane B*, patient brain; *lane C*, control brain; *lane D*, patient heart; *lane E*, control heart; *lane F*, patient kidney; *lane G*, control kidney.

pyruvate at all or survive for even as long as he did with so little residual PDC activity. Remarkably, there was no evidence of cardiomyopathy or hepatic or renal dysfunction. It is conceivable that the E_1 subunits were present in greater amounts *in vivo* but were uniquely labile and hence disappeared during sample processing, or, alternatively, the E_1 was present at a higher level earlier in development and gradually disappeared before death. However, there are no data to support these possibilities, and the lack of PDC activity in freshly isolated lymphocytes and the specific failure of otherwise metabolically active intact mitochondria to oxidize pyruvate cannot be explained as postmortem artifacts. Glucose oxidation is largely dependent on flux through PDC, and flux through PDC is regulated in part by the state of phosphorylation of E_1; since inactivation of normal PDC by phosphorylation results in less loss of activity than was observed in this defect (as shown Table 1), it is difficult to imagine that the very small amounts of residual PDC activity or E_1 found, for example, in this infant's heart and kidney would be sufficient to account for more than minimal energy production from glucose oxidation. One must presume, therefore, that most of the energy required by liver, skeletal muscle, heart, and kidney was derived from oxidation of fatty acids, at least postnatally.

As has been described in other cases of PDC deficiency, brain function and development were severely impaired and pathological abnormalities were confined to the central nervous system. This suggests that dependence on pyruvate oxidation as an energy source is relatively unique to certain areas within the central nervous system, consistent with the well known effects of glucose deprivation on brain functioning. Why the affected areas are particularly vulnerable to lack of PDC is not known. Fetal

development was apparently normal, and this is difficult to reconcile with the more general dependence of the fetus on glucose oxidation as an energy source. Conceivably, chronic adaptation of the fetus to nonoxidative glycolysis (which provides less than 10% as much energy as glucose oxidation) and of the mother to gluconeogenesis from lactate (the Cori cycle) could have provided sufficient energy for the fetus, although there is no evidence that this type of accelerated recycling actually occurs.

The significant neuropathological findings included bilateral necrosis of the putamen, caudate, and spinal cord with demyelination and axonal loss in the optic nerve and spinal cord tracts. These changes are consistent with the necrotizing encephalomyelopathy described by Leigh (35). Similar neuropathological findings have been described in several previously reported cases of PDC deficiency (7, 36, 37), including a recent case which was also shown to lack both subunits of the E_1 component in skin fibroblasts (10, 38). This latter patient lived for 7 yr, had optic atrophy, retinal changes, episodic ataxia, and a central hypoventilation syndrome. The neuropathological findings in the present case and this latter case are similar, with evidence of involvement of the basal ganglia and optic nerves. However, the latter case had more severe involvement of these areas as well as involvement of the cerebellum and brain stem. This degree of variation has been described within the spectrum of the neuropathology associated with Leigh's disease, and in these two biochemically similar cases could be due to different ages at death and differences in the amount of residual PDC activity. PDC deficiency should be included as one of the possible contributing defects. Earlier reports had suggested an association of Leigh's disease with other defects of pyruvate metabolism, including pyruvate carboxylase deficiency, but careful investigation of a series of cases of well-established Leigh's disease failed to provide evidence for pyruvate carboxylase deficiency (39, 40).

Skin fibroblasts from this patient did not accurately reflect the severity of PDC deficiency which was evident in all other cells and tissues. Fibroblasts have been used most commonly for detection of PDC deficiency. Lymphocytes in this case reflected the severity of systemic PDC deficiency more accurately, and lymphocytes provide a practical alternative to fibroblasts for detection purposes (29).[1] Reduced PDC activity has been detected in skin fibroblasts from parents of children with PDC deficiency, consistent with the obligatory heterozygous state of an autosomal recessively transmitted genetic disorder (15). We were not able to demonstrate significantly reduced PDC activity in lymphocytes from the mother, possibly because of the large variation of controls. Further family studies were not possible because the parents were not available and had no other children in common.

The nearly complete absence of the α and α subunits of E_1 in tissues from this case accounts for the great reduction of PDC activity, since E_1 is the rate-limiting enzyme in the overall reaction. The activity of the partial reaction catalyzed by E_1 was also very low. Since this activity is normally very low, it is of limited significance as a measure of E_1 function. Other investigators have also found rates for E_1 which are not more than about 10% of the nonactivated rate. For these reasons, definition of E_1 deficiency should at least include evidence of normal E_2 and E_3 activity, and is greatly strengthened, as in the present case, by the additional finding of altered immunoreactivity or electrophoretic mobility of one of the E_1 proteins. However, other E_1 mutants may not show any abnormality in this type of immunological analysis.

The marked reduction in amounts of both α and β subunits of E_1 is intriguing, since it would not be expected that a single mutation would affect two separate proteins. A similar reduction of both subunits was detected in skin fibroblasts from one other case of E_1 deficiency (10). In another case, the α subunit was found to be missing (11), and in four additional reported cases of apparent E_1 deficiency both subunits were found to be present

by immunological analysis (9, 11). Some possible explanations for absence of these two gene products are : 1) mutation of a single regulatory genetic locus could prevent expression of two separate genes; and 2) one subunit could be synthesized and the other not and in the absence of the second subunit the first might be rapidly degraded. Recently, distinct cDNAs for $E_1\alpha$ and $E_1\beta$ have been isolated in our laboratory (42). As there is no immunological cross-reactivity between proteins produced from these cDNAs or cross-hybridization of specific oligodeoxynucleotide probes, it is very unlikely that there is a common protein or mature mRNA from which $E_1\alpha$ and $E_1\beta$ are derived. The possibility that a large deletion could result in loss of genetic material from two proximal loci is not consistent with the observation that small amounts of $E_1\alpha$ and $E_1\beta$ were present, especially in cultured skin fibroblasts. There was no evidence for accumulation of a higher molecular weight precursor protein in the extracts of whole tissues.[2] Resolution of the mechanism of this combined defect awaits further application of techniques for examining the respective genes and their products.

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[2] *In vitro* translation experiments have indicated that both $E_1\alpha$ and $E_1\beta$ are initially synthesized as precursor peptides whose molecular weights are about 10% larger, as determined by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (Raefsky C, Patel M, unpublished data).