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Research Article

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Methods were developed to study his metabolic abnormality in dilute suspensions of white blood cells and cultured skin fibroblasts, as well as in cell-free sonicates of fibroblasts. Oxidation of pyruvic acid-1-^{14}C and pyruvic acid-2-^{14}C by his cells and pyruvate decarboxylase activity in sonicates of his cells were less than 20% of those in cells from control subjects. Oxidation of glutamic acid-U-^{14}C, acetate-1-^{14}C, and palmitate-1-^{14}C was normal, as was incorporation of alanine-U-^{14}C into protein.

The rate of oxidation of pyruvic acid by the father's cells and the activity of pyruvate decarboxylase in the father's sonicated fibroblasts were intermediate between those of the patient and those of controls. Values for the mother were at or just below the lower limits of the ranges in controls. Kinetic data suggested the posibility of several forms of pyruvate decarboxylase in this family.

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A Defect in Pyruvate Decarboxylase in a Child with an Intermittent Movement Disorder

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ABSTRACT A patient with an intermittent movement disorder has been found to have an inherited defect in pyruvate decarboxylase ((2-oxo-acid carboxy-lyase, E.C. 4.1.1.1.). The patient is a 9 yr old boy who since infancy has had repeated episodes of a combined cerebellar and choreoathetoid movement disorder. He has an elevated level of pyruvic acid in his blood, an elevated urinary alanine content, and less marked elevations in blood alanine and lactate.

Methods were developed to study his metabolic abnormality in dilute suspensions of white blood cells and cultured skin fibroblasts, as well as in cell-free sonicates of fibroblasts. Oxidation of pyruvic acid-1-^{14}C and pyruvic acid-2-^{14}C by his cells and pyruvate decarboxylase activity in sonicates of his cells were less than 20% of those in cells from control subjects. Oxidation of glutamic acid-U-^{14}C, acetate-1-^{14}C, and palmitate-1-^{14}C was normal, as was incorporation of alanine-U-^{14}C into protein.

The rate of oxidation of pyruvic acid by the father's cells and the activity of pyruvate decarboxylase in the father's sonicated fibroblasts were intermediate between those of the patient and those of controls. Values for the mother were at or just below the lower limits of the ranges in controls. Kinetic data suggested the possibility of several forms of pyruvate decarboxylase in this family.

Possible mechanisms relating the chemical abnormality and the clinical symptoms in this patient are discussed.

INTRODUCTION

A variety of inherited ataxias are known, and in several of these specific biochemical abnormalities have been found (1–6). Recently, we have demonstrated an inherited defect in the oxidative decarboxylation of pyruvic acid in a boy with an intermittent ataxic syndrome (7, 8). The patient appears to resemble a child studied by Lonsdale and associates (9–11). Details of the studies in our patient which revealed a hereditary defect in pyruvate decarboxylase (2-oxo-acid carboxy-lyase, E.C. 4.1.1.1.) are presented below.

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METHODS

Clinical studies

The patient, an 8 yr old boy, has had documented episodes of ataxia two to six times a year since he was 16 months old. Most attacks have followed nonspecific febrile illnesses or other stresses. Symptoms have lasted a few hours to over a week, and in severe attacks he has been bedridden. Extensive evaluations at several medical centers have revealed a cerebellar and choreoathetoid movement disorder during attacks but only generalized "clumsiness" at other times.

There was no family history of ataxia, no evidence of consanguinity, and no history of anoxia or significant head injury. Pregnancy and delivery were unremarkable.

On admission, the patient had definite cerebellar ataxia, mild but definite choreoathetosis, irregular "wandering" eye movements without true nystagmus,[1] and minimal dystonic posturing. His symptoms cleared, and after 4 days only mild cerebellar signs remained.

The concentration of pyruvic acid in the patient's blood was about three times the normal level, whereas the concentration of alanine was about twice normal, and that of lactic acid slightly increased (Table I). The urinary alanine content was elevated; analyses by paper chromatography have shown a similar elevation.[2] No other abnormality in plasma or urinary amino acids was noted. The blood pyruvic acid levels in the father were usually within the normal range although occasional values were elevated. The single slightly elevated value for the mother was from blood obtained in clinic and may not represent a truly basal level. The pyruvic acid concentration in the brother's blood, and the alanine levels in the urine from the brother and both parents, were normal.

[1] Podos, S. M. 1969. Neuro-ophthalmologic abnormalities in hyperpyruvicemia with hyper-α-alanemia. In press.

[2] Menkes, J. H. Personal communication.

TABLE I

Levels of Pyruvic and Lactic Acids in Blood, and Alanine in Blood and Urine from the B. R. Family

Subject	Pyruvate	Lactate	Alanine	
			Blood	Urine
	mg/100 ml	*mg/100 ml*	*μmoles/liter*	*μmoles/day*
Patient	2.74, 2.04, 1.68	28, 19, 18	938, 910, 462	1496, 1568
Father	1.00, 0.86, 0.60	11, 11, 5	426	303
Mother	0.94	4	336	397
Brother	0.78	7	280	136
Normal range	0.39–0.86	9–16	100–472	12–500

Pyruvic (12) and lactic (13) acids were measured in the NIH clinical laboratories, on fasting blood samples deproteinized with cold trichloracetic acid, by enzymatic methods in routine use. Samples from the patient and his father were obtained under basal conditions on several different days, and from the mother and brother during a single clinic visit. Alanine was measured with an automatic amino acid analyzer as described previously (14). Results with normal subjects agreed with those of previous workers, and values for the normal rangse are derived from those quoted by several authors (15–17).

Further details of the clinical history and examinations and of other laboratory studies, including muscle biopsy, will be reported elsewhere.[8]

Metabolic studies

Methods and materials. L-Glutamic acid-U-^{14}C (218 mCi/mmole) and L-alanine-U-^{14}C (125 mCi/mmole) from New England Nuclear Corporation were repurified by paper chromatography. Sodium pyruvate-1-^{14}C from New England Nuclear (2.89 and 3.27 mCi/mmole) and from Amersham-Searle (23.8 mCi/mmole) and sodium pyruvate-2-^{14}C from Tracerlab (3.16 mCi/mmole) were stored at −20°C in a vacuum dessicator and used without further purification. Sodium acetate-1-^{14}C from New England Nuclear (31.0 mCi/mmole) and sodium bicarbonate-^{14}C from Amersham-Searle (44.4 mCi/mmole) were also used without further purification. The palmitic acid-1-^{14}C (55.2 mCi/mmole) from Amersham-Searle was at least 98% radiopure by gas-liquid chromatography.

Thiamine hydrochloride was obtained from Nutritional Biochemicals Corp., DL-carnitine (A-grade) from Calbiochem, crystallized human serum albumin from Pentex Corp., bovine serum albumin (fraction V) from Armour Pharmaceutical Co., methylcellulose (15 centipoise) from Fisher Scientific Company, and dextran T-500 from Pharmacia Fine Chemicals, Inc. Hyamine hydroxide, PPO (2,5-diphenyloxazole), and POPOP (1,4-bis[2-(phenyloxazolyl)]benzene) were from the Packard Instrument Corp., and NAD (grade III yeast), adenosine triphosphate (ATP) (A grade), and dithiooctanoic acid (oxidized and reduced forms) from Sigma Chemical Co. Other reagents were analytical grade. In experiments with cell suspensions, plastic or siliconized glassware was used unless otherwise noted.

White blood cell incubations. Venous blood (10–30 ml) was mixed with 1/5 volume of a solution of 5% dextran, 1.5% ethylenediaminetetraacetate (EDTA) (pH 7.4), 0.7% NaCl (18) and left in an ice bath for 45 min. The supernatant fluid was removed with a glass pipette, centrifuged (600 *g* for 10 min at 4°C), and the cell pellet suspended in 1 ml of 0.11 M NaCl, 0.04 M phosphate buffer (pH 7.4). Water (3 ml) was added to lyse contaminating red cells; exactly 90 sec later 1 ml of 3.6% NaCl was rapidly pipetted into the solution (18). The cells were washed three times more by centrifugation and resuspension in about 30 ml of buffered saline each time. The final pink pellet was suspended in buffered saline, and the cells in a sample counted in a Coulter Counter.

Cells were incubated at 37°C in 20-ml polyethylene vials, usually for 1 hr. Each vial contained 1.5–5.0 × 10^6 cells in 3 ml of buffered saline, and either radioactive pyruvic acid (0.16 μmoles), glutamic acid (0.0029 μmoles), sodium acetate (0.016 μmoles), or alanine (0.0015 μmoles). Vials were closed with serum caps from which were suspended disposable plastic center wells (Kontes Glass Corp.) containing filter paper wicks. After incubations with pyruvate and glutamate, 0.2 ml of 1 M hyamine hydroxide in methanol was added to each well and 0.4 ml of 50% H_2SO_4 to each vial; vials were shaken for 1 hr more, the wells transferred to counting vials containing 15 ml of 0.6% PPO in toluene, and radioactivity measured in a Packard model PR-1 scintillation counter. With acetate as substrate, acidification was avoided to prevent collection of volatile substrate with the $^{14}CO_2$; each plastic well then contained 0.18 ml of 8% NaOH throughout the incubation, and samples were counted in 0.4% PPO, 0.05% POPOP in toluene-methanol 2:1. To measure incorporation of radioactive amino acids into protein, the incubated cell suspension was added to an equal volume of 10% trichloroacetic acid; the vial was rinsed with 0.5 ml of 1% bovine serum albumin solution and then with a few drops of water, and the washings were added to the trichloroacetic acid. Proteins were prepared by the method of Siekevitz (19), heated in counting vials for 20 min at 60°–70°C with 1 ml of 1 M hyamine hydroxide in methanol, and counted after cooling and addition of 15 ml of 0.6% PPO in toluene. Duplicate

[8] Blass, J. P., R. P. Kark, and W. K. Engel. 1969. In preparation.

control vials containing boiled cells or no cells were incubated simultaneously with active preparations and results corrected accordingly.

Fibroblast incubations. Fibroblasts derived from skin punch biopsies were cultured by previously described techniques (20) in Eagle's minimal essential medium, modified to contain 0.16% $NaHCO_3$, nonessential amino acids, neomycin (50 μg/ml), and 0.01% Phenol Red (21, 22). Cells were harvested by treatment for 10–15 min with 1–3 ml of a solution of 0.05% trypsin, 0.2% methylcellulose, 0.01% EDTA in buffered saline (0.11 M NaCl, 0.04 M phosphate buffer, pH 7.4). After addition of 20–30 ml of cold buffered saline, cells were collected by centrifugation (1000 *g* for 10 min at 4°C) and washed twice more by resuspension and recentrifugation. The cells in a sample of the final washed cell suspension were counted in a Coulter Counter and protein assayed by the modified biuret technique (23).

Plastic vials containing 1.3–1.4 ml of buffered saline, 150,000–700,000 fibroblasts, and 0.1 μc of the appropriate substrate were usually incubated for 90 min. The palmitic acid-1-^{14}C was used complexed to crystalline human serum albumin in a molar ratio of 4:1. The $^{14}CO_2$ was usually collected in hyamine after acidification and counted as in the white cell studies. In later experiments, $^{14}CO_2$ was collected without acidification; this method gave complete collection of $^{14}CO_2$ with lower "blank" values for oxidation of pyruvic acid in vials containing boiled or no cells.

Cell-free fibroblast preparations. Washed cell pellets were suspended in 1.0 ml of distilled water in plastic test tubes in an ice–salt water bath, and disrupted by sonication using the microtip of the Branson Sonifier (Branson Instruments Co., Stamford, Conn.). Sonication at 2 A current was in six bursts of 5 sec each separated by 10- to 15-sec cooling periods. No cells were seen in these preparations under phase microscopy, and activity was similar whether preparations were used as such or only after a centrifugation which would have removed unruptured cells.

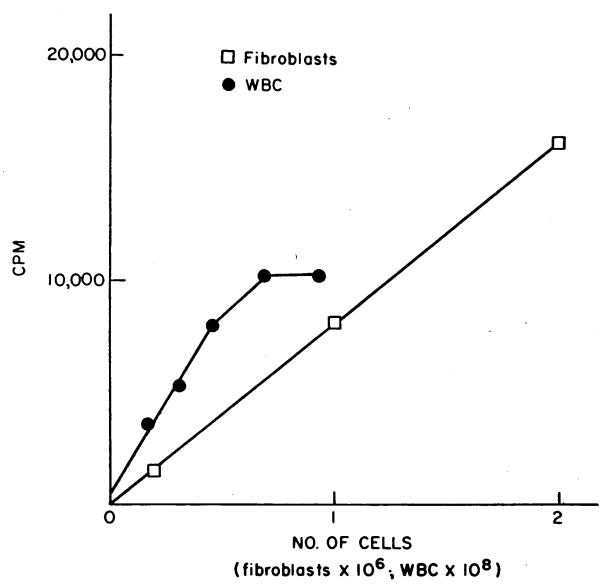

FIGURE 1 Conversion of pyruvic acid-1-^{14}C to $^{14}CO_2$ by white blood cells and fibroblasts; cpm vs. number of cells. Varying numbers of fibroblasts from a single normal culture were incubated with 10^5 cpm of pyruvic acid-1-^{14}C for 90 min; varying numbers of white blood cells from a single normal preparation were incubated with 5×10^5 cpm of pyruvic acid for 1 hr. Each point represents the mean of duplicate determinations less the value for nonenzymic oxidation, measured in simultaneous incubations with boiled cells and with no added cells. See text for details.

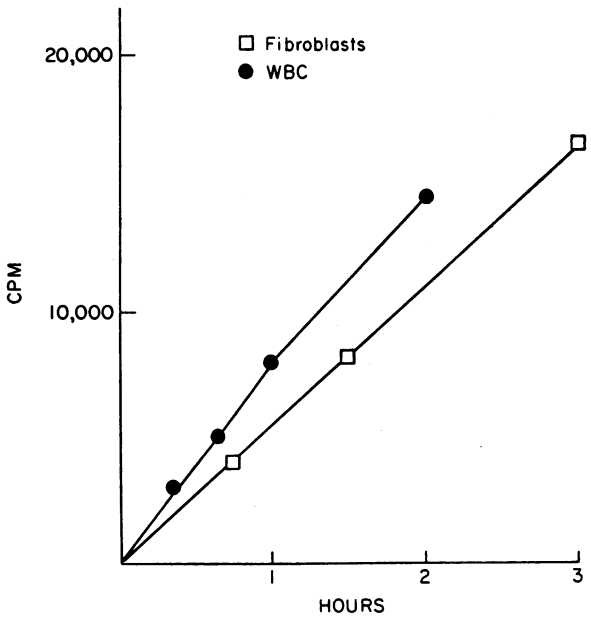

FIGURE 2 Conversion of pyruvic acid-1-^{14}C to $^{14}CO_2$ by white blood cells and fibroblasts; cpm vs. time. Vials containing either 10^5 fibroblasts from a single normal culture and 10^5 cpm of pyruvic acid-1-^{14}C or 2.3×10^6 white blood cells from a single normal preparation and 5×10^5 cpm of pyruvic acid were incubated for varying lengths of time. Each point represents the mean of duplicate determinations less the value for nonenzymic oxidation, which was measured simultaneously and was similar with boiled cells and with no added cells. See text for details.

Pyruvate decarboxylase activity was measured by a modification of the method of Reed and Willms (24). Incubations were in duplicate for 30 min at 30°C. Each test tube contained, in a volume of 0.14 ml, 25–200 μg of cell protein as measured by the method of Lowry (25); 15 μmoles of potassium phosphate buffer, pH 6.0: 0.03 μmoles of $MgCl_2$; 2.5 μmoles of potassium ferricyanide; thiamine pyrophosphate, usually 0.02 μmoles; and an appropriate amount of pyruvic acid-1-^{14}C (0.0007–0.084 μmoles). The $^{14}CO_2$ was collected in NaOH without acidification. From 0.5 to 1.0% of the added substrate was converted to $^{14}CO_2$, 3–12 times as much as in controls containing no tissue or tissue killed with ethanol.

RESULTS

White cell studies. The oxidation of pyruvic acid-1-^{14}C to $^{14}CO_2$ proceeded linearly with time (Fig. 1) and in proportion to the number of white cells added (Fig. 2) provided less than 2 of% of the added substrate was oxidized. Results were similar for oxidation of other substrates and for incorporation of alanine into

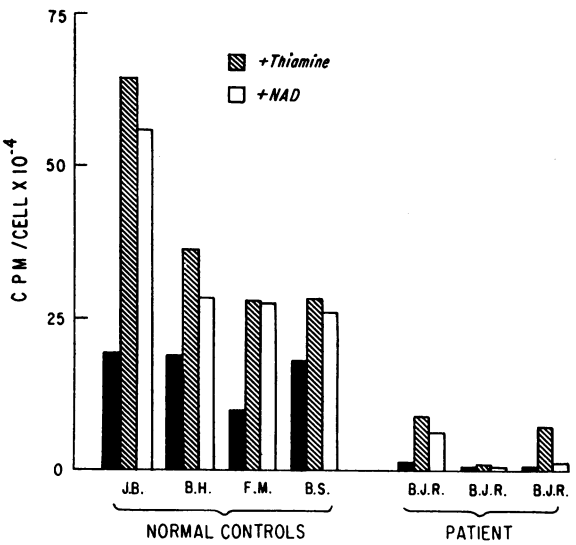

FIGURE 3 Effect of added thiamine and NAD on conversion of pyruvic acid-1-^{14}C to $^{14}CO_2$ by white blood cells from the patient and from normal controls. Each group of bars represents results with cells from a single culture flask; final concentrations of thiamine and NAD (when added) were 5.7 mmoles/liter. See text for details.

protein. Experimental conditions were chosen accordingly, to allow measurement of metabolic rates.

Oxidation of pyruvic acid by the patient's white cells was less than 20% of that in the lowest control (Table II); oxidation of glutamic acid and sodium acetate and incorporation of alanine into protein were comparable to those of normal cells. Oxidation of pyruvic acid by white blood cells from the parents was within the lower limits of the normal range.

With addition of thiamine (Fig. 3), the maximum rate in the patient's cells approached the lower limit of the range for unfortified normal cells, but the defect persisted relative to thiamine-enriched cells from normal individuals.

Fibroblast studies. As with white blood cells, metabolism by fibroblasts was proportional to the number of cells added and linear with time under the experimental conditions chosen; Figs. 1 and 2 show the results with pyruvic acid-1-^{14}C. Radioactivity measured in $^{14}CO_2$ or in protein was at least twice the level in boiled cell controls with pyruvate as substrate and at least five times control levels with other substrates.

Oxidation of pyruvic acid-1-^{14}C by the patient's fibroblasts was less than 10% of that by control cells (Table III); oxidation of glutamic acid and palmitic acids and incorporation of alanine into protein appeared normal. Addition of more cells allowed demonstration of the low rate of pyruvate oxidation by the patient's fibroblasts, but normal cells then metabolized 30% of the added substrate, and metabolism was no longer proportional to cell number or to time. Average values for oxidation of pyruvic acid by fibroblasts from the parents were

TABLE II

White Blood Cell Metabolism in the B. R. Family and in Control Subjects

Subject	Substrate			
	$^{14}CO_2$ produced from:			Protein-^{14}C from Alanine-U-^{14}C
	Pyruvate-1-^{14}C	Glutamate-U-^{14}C	Acetate-1-^{14}C	
	cpm/10⁴ cells			*cp/m10⁴ cells*
Controls Ranges	24.9 ±3.0 (16) [10.0–46.6]	15.5 ±3.7 (12) [5.0–50.0]	15.2 ±2.5 (11) [5.5–24.0]	2.9 ±0.5 (12) [0.7–4.0]
Patient B. J. R. Ranges	0.9 ±0.6 (5) [0–2.0]	28.9 ±15.2 (4) [8.8–55.5]	15.0 ±3.3 (3) [10.0–21.4]	1.1; 4.9*
Father (B. R.) Range	15.9	30.6	10.0 ±1.5 (3) [7.6–12.6]	3.6
Mother (L. R.)	12.0	43.5	No determination	2.9

White blood cells were prepared from the patient, his family, normal volunteers, and adults and children with neurologic diseases (Dawson's encephalitis, ataxia telangectasia, and inherited neuromuscular diseases). After incubation with appropriate substrate, radioactivity was counted either in $^{14}CO_2$ or in proteins. See text for details. Values represent cpm/10,000 cells ±SE, and numbers in brackets represent the ranges. The number of control subjects studied or the number of preparations from a member of the B. R. family are shown in parentheses. Values can be converted to μmoles by multiplying by 2.14×10^{-7} μmoles/cpm for pyruvate, by 2.78×10^{-9} for glutamate, by 2.13×10^{-8} for acetate, and by 4.85×10^{-9} for alanine.

* Two preparations examined.

TABLE III

Metabolism in Fibroblasts from the B. R. Family and from Control Subjects

Subject	Substrates			
	$^{14}CO_2$ from:			Protein-^{14}C from Alanine-U-^{14}C
	Pyruvic acid-1-^{14}C	Glutamic acid-U-^{14}C	Palmitic acid-1-^{14}C	
	cpm/10^3 cells			*cpm/10^3 cells*
Controls (all)	8.6 ±1.5	5.4 ±1.3	0.74 ±0.13	1.00 ±0.24
J. S.	10.7 ±4.2 (4)	8.3 ±2.7 (4)	0.93 ±0.24 (3)	1.49 ±0.41 (3)
E. R.	8.7 ±3.5 (3)	3.2, 10.0 (2)	0.50 ±0.19 (3)	0.07 ±0.24 (3)
L. D. R.	10.6 ±3.6 (5)	1.9, 6.2 (2)	0.96, 1.01 (2)	0.73 ±0.47 (3)
E. B.	4.2 ±0.8 (4)	3.3, 1.3 (2)	0.53 ±0.14 (3)	0.74, 2.81 (2)
Patient (B. J. R.)	<0.4 (5)	2.4, 6.0 (2)	0.54 ±0.32 (3)	0.88 ±0.50 (3)
Father (B. R.)	2.0 ±0.8 (5)	2.8 (1)	0.58 ±0.14 (3)	0.79, 4.42 (2)
Mother (L. R.)	3.6 ±1.2 (5)	4.2 (1)	0.88 ±0.28 (3)	0.81, 2.17 (2)
Brother (D. R)	8.4 ±2.4 (5)	3.0 (1)	Not determined	1.26, 2.60 (2)

Fibroblasts were prepared and incubated with various substrates and the radioactivity in $^{14}CO_2$ or in protein counted, as described in detail in the text. Values represent cpm/10^3 cells, followed by SE (when three or more cultures were studied). Results for the total control group were calculated from the means for each individual. Values in parentheses represent the number of cultures studied from each cell line. Control cultures were from clinically normal individuals. Values can be converted to μmoles using the factors in Table II.

intermediate between values for the patient and those for control subjects, although some individual values for the parents fell within the lower limit of the normal range. Metabolism by the brother's cells appeared normal. Addition of thiamine, NAD, and lipoic acid did not significantly increase pyruvate oxidation by the patient's or by normal fibroblasts.

TABLE IV

Oxidation of Pyruvic Acid-1-^{14}C to $^{14}CO_2$ by Fibroblasts from the B. R. Family and Controls in Media Containing Carnitine

Subject	$^{14}CO_2$
	cpm/μg cell protein
Controls (7 subjects)	25.2 ±2.4 (10.5–44.5)
E. B.	23.8 ±10.4 (11.8–44.5)
J. P.	27.7 ±1.3 (25.1–29.6)
L. R.	19.0 ±6.3 (10.5–31.3)
E. R.	18.8 ±3.9 (11.4–24.4)
J. S.	26.9 ±2.5 (22.6–31.4)
M. M.	17.5 ±3.3 (14.1–24.0)
K. M.	43.3 ±1.7 (39.2–44.4)
Patient (B. J. R.)	2.0 ±0.8 (0.6– 3.6)
Father (B. R.)	6.8 ±0.7 (5.8– 8.0)
Mother (L. R.)	12.5 ±2.4 (8.2–16.7)

Fibroblasts were incubated as described in detail in the text, in media containing 0.87 mM DL-carnitine. $^{14}CO_2$ was collected without acidification. K. M. and M. M. had a myopathy; other controls were clinically normal. Values represent means ±SE (and range) for three cultures from each individual and for all controls treated as one population.

The low rate of oxidation of pyruvic acid-1-^{14}C by the patient's fibroblasts was clearly demonstrated when carnitine was added to prevent accumulation of acetyl coenzyme A (26) and $^{14}CO_2$ was collected without acidification (Table IV). Oxidation by the father's

TABLE V

Pyruvate Decarboxylase Activity in Sonicated Fibroblasts from the B. R. Family and Controls

Subject	Pyruvate decarboxylase activity
	μmoles/g protein per 30 min
Controls	2.37 ±0.27 (5)
E. W.	1.50 ±0.25 (4)
K. S.	2.28 ±0.47 (6)
M. M.	2.11 ±0.54 (4)
K. M.	2.34 ±0.58 (5)
J. S.	3.16 ±0.80 (6)
Patient (B. J. R.)	0.41 ±0.03 (8)
Father (B. R. Sr.)	1.00 ±0.24 (4)
Mother (L. R.)	1.50 ±0.26 (6)
Brother (D. R.)	2.24 ±0.65 (5)

The activity of pyruvate decarboxylase was assayed by a modification of the method of Reed and Willms (24) in sonicated fibroblasts. Activities shown are with 0.2 mM pyruvate as substrate. See text for details. K. M. and M. M. had a myopathy, and K. S. and J. S. Refsum's disease (3); E. W. was clinically normal. Values represent mean ±SE (followed by the number of cultures studied for each individual or the number of control subjects studied).

fibroblasts was intermediate between values for the patient and those for control subjects. The mean value for the mother's fibroblasts was slightly below the control range, although some values with her cells fell within the range of individual values from controls. The defect in the patient's cells was evident whether activities were calculated per unit cell protein, as in Table V, or per cell, as shown in Tables II–IV. There were approximately 200 cells/μg protein.

With pyruvic acid-2-^{14}C as substrate (Fig. 4), oxidation by the patient's cells was again low, and by his parent's cells was just below the normal range.

Cell-free preparations. Pyruvate decarboxylase activity was proportional to the amount of fibroblast protein added, under the experimental conditions used (Fig. 5). Ferricyanide was used as an electron acceptor to avoid measurement of the later oxidative steps in the pyruvate dehydrogenase complex (24). The concentration of pyruvic acid, 0.2 mmoles/liter, was about twice that in normal blood under basal conditions (12).

Pyruvate decarboxlase activity in sonicated fibroblasts from the patient was less than 20% of that in preparations from controls (Table V). The value for the father was again intermediate between that of the patient and those of controls, and the value for the mother coincided with the lowest control value. The activity of this enzyme was also low in homogenized muscle from the patient (20 nmoles/g protein per 30 min), compared to muscles from five controls (167 ±40 nmoles/g protein per 30 min, mean ±SE).

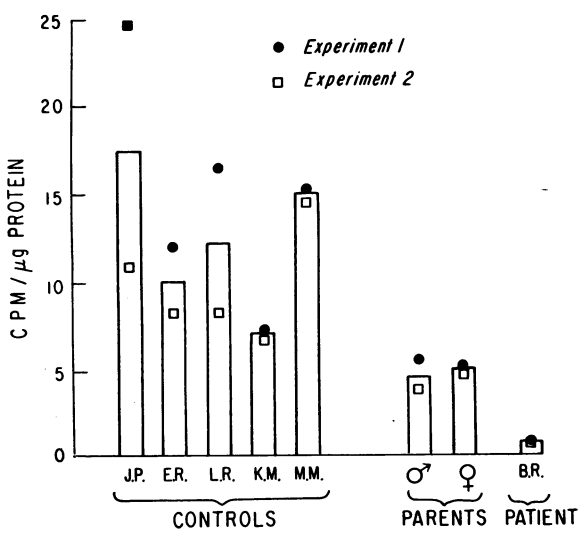

FIGURE 4 Conversion of pyruvic acid-2-^{14}C to $^{14}CO_2$ by fibroblasts from the B. R. family and controls. Points represent values for single cultures and bars mean values for single individuals. J. P., E. R., and L. R. were clinically normal; K. M. and M. M. had a myopathy. $^{14}CO_2$ was collected in NaOH without acidification. See text for details.

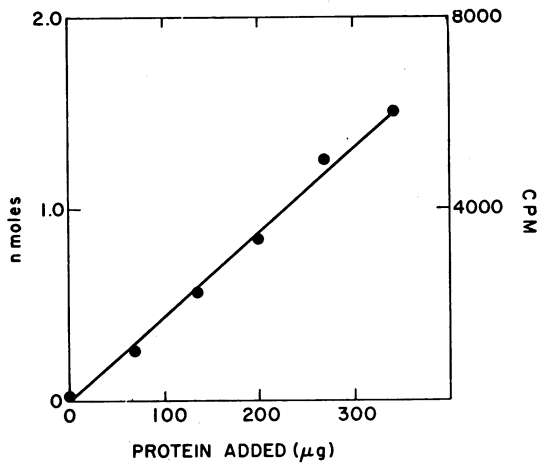

FIGURE 5 Pyruvate decarboxylase activity in sonicated fibroblasts; activity vs. amount of sonicate. Fibroblasts from four control subjects were sonicated together, and the pyruvate decarboxylase activity in varying amounts of the sonicate was measured with ferricyanide as electron acceptor. Each point represents the mean of duplicate determinations. See text for details.

Excess thiamine pyrophosphate was routinely added to the cell-free preparations in which the defect was demonstrated. In preparations from both the patient and controls, the absence of added coenzyme was associated with 20–30% lower activity, whereas the addition of twice the usual amount of coenzyme caused no significant change (Table VI). Complete dissocation of pyruvate decarboxylase apoenzyme from the coenzyme has been shown usually to require alkaline conditions (*27*, *28*).

TABLE VI

Effect of Added Thiamine Pyrophosphate on Pyruvate Decarboxylase Activity in Sonicated Fibroblasts from B. R. Family and Controls

Subject	Pyruvate decarboxylase activity		
	No added TPP	0.143 mM TPP	0.286 mM TPP
		%	
Controls	79 ±4	(100)	100 ±3
E. W.	64 ±8	(100)	101 ±12
K. S.	90 ±9	(100)	109 ±4
M. M.	85 ±4	(100)	93 ±2
K. M.	78 ±11	(100)	96 ±9
J. S.	81 ±6	(100)	102 ±10
Patient (B. J. R.)	72 ±13	(100)	103 ±11
Father (B. R. Sr.)	73 ±15	(100)	119 ±9
Mother (L. R.)	69 ±5	(100)	95 ±3
Brother (D. R.)	72 ±4	(100)	91 ±13

Pyruvate decarboxylase activity was compared in individual fibroblast sonicates incubated with 0.286, 0.143, and no added thiamine pyrophosphate (TPP), and the percentage of the activity with 0.143 mM coenzyme calculated. See text for details. Values represent the mean ±SE for three cultures from each individual and for the group of controls.

TABLE VII

Pyruvate Decarboxylase Activity in Mixtures of Fibroblast Sonicates from Patient B. R. and a Control Subject

Protein added		Pyruvate decarboxylase activity		
B. R.	Control	Experimental	Calculated	Ratio, Calc:Exp
μg		*μmoles/g protein per 30 min*		%
140	0	0.41	—	—
105	25	1.13	1.14	101
70	75	1.86	1.86	100
35	100	2.59	2.59	100
0	150	—	3.32	—

Pyruvate decarboxylase activity was measured, as described in detail in the text, in fibroblast sonicates from the patient B. R., from control K. M., and in mixtures of the two. Results of mixing sonicated cells from the patient and from two other controls were similar.

Mixing experiments (Table VII) provided no evidence for an inhibitor of pyruvate decarboxylase in the patient's cells. The deficit in pyruvate decarboxylase activity in the patient's sonicated fibroblasts persisted over a range of substrate concentrations (Fig. 6). Again, values for the father were usually below, for the brother above, and for the mother approximately at the lower limit of the range in controls. Inflections in the substrate-activity curves for members of the affected family and one of the controls suggested that fibroblasts from each of these individuals might contain more than one pyruvate decarboxlase enzyme. The Michaelis-Menten constants calculated from these curves could be arranged into several groups on the basis of small differences and might be consistent with the possible existence of different forms of the enzyme (Table VIII).

TABLE VIII

Michaelis-Menton Constants for Pyruvate Decarboxylase in Sonicated Fibroblasts from the B. R. Family and Controls

Subject	Michaelis-Menton constants				
	K_{m1}	K_{m2}	K_{m3}	K_{m4}	K_{m5}
	moles/liter $\times 10^{-4}$				
Controls					
E. W.			1.6		
K. S.					5.1
M. M.		0.6			
K. M.		0.7			
J. S.		1.1		2.3	
Patient (B. J. R.)			1.7	2.4	
Father (B. R. Sr.)		1.1	1.7		
Mother (L. R.)	0.2			2.2	
Brother (D. R.)	0.3	0.8			

Michaelis-Menton constants (K_m) were derived from the curves shown in Fig. 6 by the method of double reciprocals (29).

DISCUSSION

The experimental studies described above demonstrate a defect in pyruvate decarboxylase in the patient B. R.

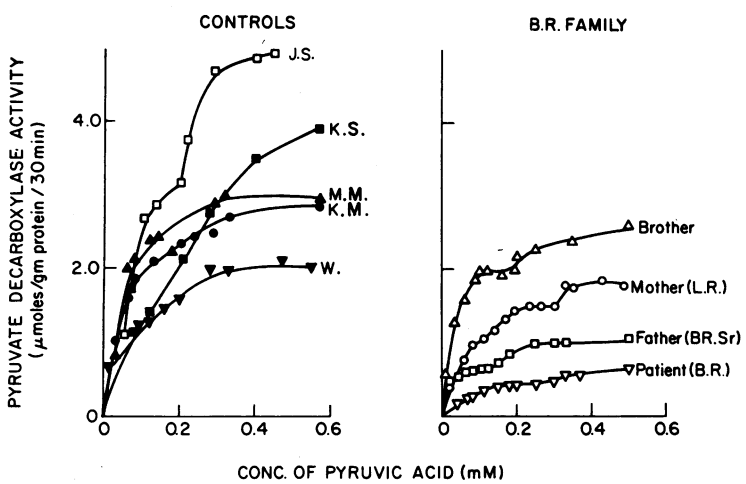

FIGURE 6 Pyruvate decarboxylase activity in sonicated fibroblasts from the B. R. family and controls at different concentrations of pyruvic acid substrate. Pyruvate decarboxylase activity was measured, as described in the text, with different concentrations of pyruvic acid substrate. The curves represent the average of results with at least three different preparations for each individual; standard errors were about 25%, similar to those shown in Table V. The inflections in the curves for the affected family and for control J. S. were repeatedly demonstrated in single preparations of sonicated cells from these individuals.

The activity of this enzyme in cell-free sonicates of the patient's fibroblasts, measured by a specific assay, was 10–20% of that in control preparations, and activity also appeared low in a single sample of his muscle. A quantitatively similar defect in the oxidation of pyruvic acid was found in suspensions of his white blood cells and cultured fibroblasts. Oxidation of palmitate and acetate, which like pyruvate enter the tricarboxylic acid cycle after conversion to acetyl coenzyme A, and of glutamate were normal. Under the experimental conditions used, fixation of CO_2 to form dicarboxylic acid was found to be less than 0.1% of the rate of pyruvate oxidation (30), and a block in this pathway did not seem to account for the defect observed in the patient's cells. Alanine, the transamination product of pyruvic acid, was incorporated into protein at a normal rate. Pyruvic-glutamic transaminase activity in his serum (16 U) was within the normal range (5–35 U) (31).

The abnormalities in this patient did not appear to result from defective thiamine metabolism. Pyruvate decarboxylase activity was low in his tissues despite addition of excess thiamine pyrophosphate. Varying the amount of coenzyme added caused proportionally similar effects in preparations from the patient and controls, suggesting similar binding of the coenzyme in the patient's and control tissues. No evidence for an inhibitor of thiamine pyrophosphate action was found. In the patient's cells, oxidation of glutamic acid, which involves a thiamine pyrophosphate–dependent enzyme, appeared normal. Despite the addition of excess thiamine to suspensions of the patient's cells, the defect persisted in comparison to thiamine-enriched control cells. The addition of thiamine did increase the rate of oxidation of pyruvic acid by white cells from both the patient and control. Several investigators (32–34) have demonstrated incomplete saturation of apoenzymes with coenzymes in clinically normal individuals, and added thiamine might increase pyruvate oxidation without a necessary defect in thiamine metabolism.

The pattern of inheritance of pyruvate decarboxylase in the affected family remains difficult to evaluate. Although the results of kinetic studies in crude preparations are compatible with the existence of several, perhaps allelic forms of the enzyme, structurally different types have not been demonstrated. The existence of several forms of the enzyme could complicate interpretation of the genetic mechanisms in the affected family in terms of classic Mendelian concepts (35), but would perhaps not be surprising in view of the apparent widespread ccurrence of genetic polymorphism (36–39). Further studies of pyruvate decarboxylases in human and other tissues may clarify some of these problems.

The clinical manifestations in the patient may be related to the defect in pyruvate decarboxylase. Elevation of pyruvic acid concentration was the most marked abnormality in the patient's blood. The accumulation of alanine and lactic acid could be secondary to hyperpyruvicemia. The genetic defect in the patient may involve brain as well as fibroblasts, white cells, and muscles. In rats, where cerebral tissues were available for study, the K_m values for pyruvate decarboxylase were similar for brain, $2.1 \pm 0.2 \times 10^{-5}$ mole/liter, and for muscle, $4.5 \pm 2.5 \times 10^{-5}$ mole/liter (mean ±SE, four determinations in each tissue). Reduction in cerebral pyruvate decarboxylase is associated with a movement disorder in thiamine-deficient rats (40), and in patients pyruvic acid levels frequently rise during fever, perhaps because of relative thiamine deficiency (41–43). Fever might be expected to precipitate a movement disorder in a patient whose capacity to decarboxylate pyruvic acid was already low.

The combination of clinical and biochemical findings in the patient described above distinguish him from other patients with chronic hyperpyruvicemia or intermittent ataxia. The combination of characteristics is distinct from those described in lactic acidosis (44–50), thiamine deficiency (51–55), pyruvate carboxylase deficiency (5, 56), the subacute sclerosing leukoencephalopathy of Leigh (5, 57–61), or in the Friedreich-like syndrome described by Dunn, Perry, and Dolman (10, 62). Lonsdale and associates (9–11) have recently studied a mentally slow child with hyperpyruvicemia and persistent hyperalanuria and hyperpyruvicemia; the enzymatic defect in this patient has not been described. Intermittent ataxia has been reported in both sporadic (63) and familial (64, 65) distributions, and has been associated with inherited abnormalities in amino acid metabolism in Hartnup disease (2) and in late onset branched-chain aciduria (6, 66). Intermittent ataxia with hyperpyruvicemia, with hereditary pyruvate decarboxylase deficiency, may constitute still another biochemically defined type of ataxia.

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