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3-Oxothiolase Activities and [^{14}C]-2-Methylbutanoic Acid Incorporation in Cultured Fibroblasts from 13 Cases of Suspected 3-Oxothiolase Deficiency

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ABSTRACT. Cultured fibroblasts from 13 patients with organic aciduria suggesting 3-oxothiolase deficiency were studied by measuring first the capacity of the isoleucine degradative pathways in whole cells, as the incorporation of 1-[^{14}C]-2-methylbutanoic acid into macromolecules, and, second, the activity of 3-oxothiolase in cell homogenates using specific 3-oxoacyl-CoA substrates to identify the different enzymes. Nine patients showed low incorporation by the macromolecular labeling assay, as well as deficiency of 2-methylacetoacetyl-CoA thiolase. In this group of patients, low activity by the macromolecular labeling assay was associated with clinically severe symptoms, and vice versa. Two patients showed reduced macromolecular labeling, but apparently normal 3-oxothiolase. Finally, two patients showed normal activities by either test, the reason for their particular organic aciduria being unknown. In conclusion, occurrence of urinary 2-methyl-3-hydroxybutyric acid and/or tiglylglycine is not an unequivocal indicator of the absence of the thiolase that metabolizes 2-methylacetoacetyl-CoA. Measurement of 1-[^{14}C]-2-methylbutanoic acid incorporation in cultured fibroblasts adds important information in studying possible defects of the isoleucine catabolic pathway. (*Pediatr Res* 28: 518–522, 1990)

Abbreviations

MMLA, macromolecular labeling assay
GCMS, gas chromatography mass spectrometry
MEM, minimal essential medium
NCS, newborn calf serum

An inborn error of isoleucine catabolism (Fig. 1) characterized by urinary excretion of 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid, tiglylglycine, and butanone was first described by Daum et al. (1). Since then, a number of cases have been reported, with a somewhat varying pattern of organic aciduria, but generally excretion of 2-methyl-3-hydroxybutyric acid and tiglylglycine (2–15). In some of these cases, a defect in the mitochondrial short chain-length-specific thiolase (EC 2.3.1.9) (McKusick 20375) has been observed (7, 9–12). Clinically, the patients show a highly variable symptomatology, some cases presenting in the 1st year of life with severe ketoacidosis and life-threatening disease, other cases presenting with mild attacks with lethargy and vomiting later in childhood. We are thus faced with a syndrome of highly variable clinical severity, as well as biochemical heterogeneity with a varying degree of organic aciduria. To clarify this situation, we developed an MMLA that measures the overall capacity of isoleucine degradation distal to 2-methylbutyryl-CoA, using [1-^{14}C]-2-methylbutanoic acid incorporation in cultured fibroblasts from skin biopsies. In parallel, we measured the fibroblast activities of 3-oxothiolase using 2-methylacetoacetyl-CoA as well as acetoacetyl-CoA and 3-oxohexanoyl-CoA to give positive identification of any decreased activities of the different cellular thiolases present. Some of the results have been briefly reported elsewhere (16, 17).

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MATERIALS AND METHODS

Dulbecco's MEM, nonessential amino acids, L-glutamine, penicillin, streptomycin, trypsin, and NCS were obtained from Flow Laboratories (Herts, England).

Sec-butylmagnesium chloride (catalog 22, 442-1, 100 mL), flex needle (catalog Z10, 091-9), round bottom storage flask with Teflon stopcock (500 mL, catalog Z10, 223-7, standard taper joint 24/40), septa (catalog Z12, 440-0), and nonradiolabeled 2-methylbutanoic acid (D,L) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Carbon dioxide (^{14}C, 1 mCi, sp act 55.9 mCi/mmol) in a breakseal tube was obtained from ICN Radiochemicals (Irvine, CA). Silicic acid was obtained from Sigma Chemical Co. (St. Louis, MO). 2-Methylbutan-2-ol and chloroform were obtained from American Burdick Jackson (Muskegon, MI). The omni glass column used for liquid partition analyses had the dimensions of 6.6 mm inner diameter × 250 mm (Rainin Instruments, Emeryville, CA). L-[4,5-^{3}H]lysine (sp act 98 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, UK).

Synthesis of [1-^{14}C]-2-methylbutanoic acid. [1-^{14}C]-2-methylbutanoic acid was synthesized via the Grignard reagent and [^{14}C]CO_2 (18). The 500-mL round-bottom storage flask was dried overnight in an oven at 140°C after removal of the Teflon

stopcock. An 18 (length) × 0.5 (width) cm glass rod was similarly dried. After cooling, the rod was inserted through a small needle hole in the center of a 24-mm septum. A magnetic stirbar and the ampoule of [^{14}C]CO_2 (with breakseal closure facing upward) were placed in the round-bottom flask and the septum with rod inserted was wired in place on the round-bottom flask with two turns of copper wire. The rod was then positioned 1–2 inches above the breakseal closure. The Teflon stopcock was opened and the entire system flushed with dry nitrogen gas. Nitrogen flow was halted and the stopcock closed. Approximately 2 mL (4 mmol) of *sec*-butylmagnesium chloride in ether were introduced through the septum by the double-tipped needle transfer procedure as previously described (19). Magnetic stirring was initiated and the breakseal closure shattered by plunging the rod downward. Reaction was carried out at room temperature for 24 h. After this period, 0.5 mL of aqueous 1 N NaOH was injected through the septum and stirred for 1 min, and the reaction vessel opened to the atmosphere to destroy excess Grignard. The reaction vessel was rinsed with three 10-mL aliquots of water, the pH of the rinses adjusted to 7 with 1 N NaOH, and the rinses lyophilized to dryness. The lyophilized fractions were taken to a pH below 1 with 5 N H_2SO_4 and a small piece of dry ice added to each fraction to remove excess radiolabeled CO_2 gas. The fractions were then adsorbed onto silicic acid that had been previously rinsed for 2 h with 9% 2-methylbutan-2-ol in chloroform (20). The column was packed with the silicic acid and the [1-^{14}C]-2-methylbutanoic acid eluted in 75 mL chloroform. The sodium salt of the [1-^{14}C]-2-methylbutanoic acid was prepared by addition of 100 mmol NaOH in methanol to the chloroform solution. The chloroform fraction was reduced to near dryness under a gentle stream of dry nitrogen. The concentrated [1-^{14}C]-2-methylbutanoic acid was taken up in 65% absolute methanol/35% water to a concentration of approximately 0.1 μCi/μL. Insoluble material was removed by centrifugation and the pH of the solution was approximately 6–7.

Determination of radiochemical purity and sp act of [1-^{14}C]-2-methylbutanoic acid. Identification of [1-^{14}C]-2-methylbutanoic acid and quantification of radiochemical purity was achieved by reversed-phase HPLC (21) and combined GCMS (22). The C18 HPLC column was previously standardized by analysis of 50 μmol of standard 2-methylbutanoic acid in absolute ethanol and monitoring of 1-min collected fractions at 212 nm in a Gilford recording spectrophotometer. The column flow rate was 1.45 mL/min. The column was equilibrated and eluted for 30 min with 0.05 M potassium phosphate buffer, pH 2.1 (buffer A), followed by elution with 85% A/15% methanol for the next 20 min, after which the column was returned to the starting conditions by elution with A for 30 min. Using this system, standard 2-methylbutanoic acid eluted at 42 min. For analysis of radiochemical purity, 1 μCi of [1-^{14}C]-2-methylbutanoic acid was applied to the column. Greater than 97% of the dpm eluted at the expected retention time for 2-methylbutanoic acid. For the determination of sp act, measurements were carried out on a DB-Wax polyethylene glycol gas chromatograph capillary column (0.53 mm × 30 m, J & W Scientific, Rancho Cordova, CA) with direct electron impact mass spectrometry. The GCMS was a Finnegan (San Jose, CA) Quadropole model 4021 with INCOS data system. The nonradiolabeled standard curve of 2-methylbutanoic acid ranged from 0 to 2 nmol. The 2-methylbutanoic acid was diluted 1:1 in 0.3 N formic acid in methanol, and the GCMS conditions were: injector temperature, 200°C; separator temperature, 300°C; ionizer temperature, 250°C; He, 2 psi, EI-Direct 1800 V, gas chromatography column programmed at 4 C/min with a starting temperature of 100°C. The gas chromatography column was connected directly into the source, with flow diverted for 50 s to avoid methanol/formic acid in the source. The 2-methylbutanoic acid eluted at 193 s. The major molecular ion for 2-methylbutanoic acid was m/z 74, with other significant ions being 57 and 87. For the synthesized [1-^{14}C]-2-methylbutanoic acid, major ions were m/z 76 and 89, corresponding to mass effects from C14 *versus* C12. For the determination of sp act, the ratios of C14/C12 at m/z 74, 76, 87, and 89 were determined from the reconstructed ion current for three separate injections of the synthesized [1-^{14}C]-2-methylbutanoic acid. With this procedure, we found an average of 82.4% of the theoretical sp act of our [1-^{14}C]-2-methylbutanoic acid, yielding a final sp act of 46.1 mCi/mmol. This value probably reflects a small amount of C12 in the nitrogen used in the Grignard reaction.

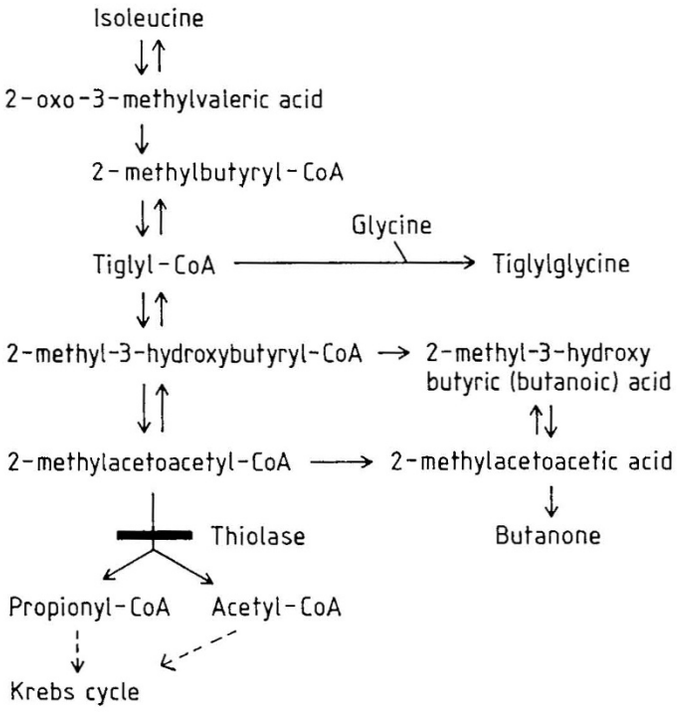

Fig. 1. Degradative pathway of isoleucine.

Patients and cells. Patients studied were those who had one or more abnormal elevations of 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid, or tiglylglycine in urine, either in random specimens or after a challenge with isoleucine. This pattern of urinary organic acids is generally considered to be indicative of a deficiency of 3-oxothiolase, provided that other metabolites characteristic of propionic or methylmalonic acidemia are not elevated (23). Fibroblasts derived from 13 patients were provided by a large group of investigators (see Acknowledgments). A brief summary of the major clinical characteristics of the patients is presented in Table 1 with literature references to the reported cases. The following brief case reports describe those not previously reported.

Case M.T. This patient is a sister of C.T. (7). The parents were nonconsanguineous and of French-Canadian heritage. She presented at 9 mo of age with an infection-provoked episode of severe ketoacidosis. Urine GCMS revealed 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetate, and tiglylglycine. The initial crisis episode was treated with high-dose i.v. glucose. She has required several hospitalizations for treatment with i.v. glucose, but has developed normally.

Case S.S. This female patient (U.S., white) presented with vomiting, poor feeding, ketoacidosis, and lactic acidosis at 3 mo of age. During the first 8 h after an isoleucine load, urinary 2-methyl-3-hydroxybutyrate was 23 mmol/mol creatinine and tiglylglycine 8 mmol/mol creatinine, suggesting the possibility of 3-oxothiolase deficiency (11). Subsequently, she had an extremely complicated history with epileptic seizures, mild optic nerve degeneration, insulin-dependent diabetes, and hypertrophic cardiomyopathy with supraventricular tachycardia. She died at the age of 2 y. Autopsy suggested subacute necrotizing encephalomyelopathy.

Table 1. *Clinical information of patients studied**

Identification	Sex	Ethnic background	Age at onset	Organic aciduria	Main clinical features	Reference
LV	M	Laos	10 mo	MHB, TG (Ile load)	Attacks of ketoacidosis	11
MM	F	Spain	16 mo	MHB, TG	Retarded development	11
ZM	M	US, white	4 mo	MHB, TG	Ketoacidosis, vomiting	24, case reports
SS	F	US, white	3 mo	MHB, TG (Ile load)	Poor feeding, lactic acidosis	Case reports
BM	M	US, white	3 mo	MHB, TG (Ile load)	Hypoglycemia, seizure	24, case reports
BB†	M	The Netherlands	11 mo	MHB, TG	Severe ketoacidosis	13
CB†	M	The Netherlands	11 mo	MHB, TG	Severe ketoacidosis	13
MAIP‡	F	Spain	5 y	MHB, MAA,TG	No symptoms	14
AIP‡	M	Spain	5 mo	MHB, MAA, TG	Metabolic acidosis, hepatomegaly	14
CT§	F	French-Canadian	8 mo	MHB, MAA, TG	Severe ketoacidosis	7
MT§	F	French-Canadian	9 mo	MHB, MAA, TG	Severe ketoacidosis	Case reports
WG 88	M	Dutch-Canadian	22 mo	MHB, MAA, TG (variable)	Metabolic acidosis	1
WG 138	F	Chile	12 mo	MHB, MAA, TG	Acetonuria, vomiting	3

* Abbreviations: MHB, 2-methyl-3-hydroxybutyrate; MAA, 2-methylacetoacetate; TG, tiglylglycine.
† Twins.
‡ Siblings.
§ Siblings.

Case B.M. This case is described in detail elsewhere (24). An isoleucine challenge was given because the patient's twin brother had slightly elevated 2-methyl-3-hydroxybutyrate. In the first 8-h urine after isoleucine challenge, B.M. had 2-methyl-3-hydroxybutyrate of 89 mmol/mol creatinine and tiglylglycine of 10 mmol/mol creatinine, suggesting 3-oxothiolase deficiency (11).

Case Z.E. This case is described in detail elsewhere (24). The patient was found by organic acid analysis to excrete 2-methyl-3-hydroxybutyrate at 8 mmol/mol creatinine and tiglylglycine at 68 mmol/mol creatinine, without an isoleucine challenge, suggesting the possibility of a deficiency of 3-oxothiolase (11).

MMLA. Fibroblasts from healthy individuals were used as controls. The cells were grown in Dulbecco's MEM supplemented with 10% NCS, penicillin, and streptomycin. Harvesting was done using 0.25% trypsin-EDTA. The labeling assay was derived from the method described by Willard *et al.* (25), in which abnormalities of propionate metabolism were studied by incubation with [1-^{14}C]propionic acid and by determining the incorporation of radioactivity into acid-precipitable proteins. Similar methods with ^{14}C-isovaleric acid have been reported (26–28). Fibroblasts were grown to approximately 90% confluency in MEM-NCS. The medium was removed and the cells were washed in Puck's saline F and incubated at 37°C for 20 h in Puck's saline F with the following final concentrations: 15% NCS, 5 mmol/L glucose, and 3.9 μmol/L [1-^{14}C]-2-methylbutanoic acid (180 μCi/L). After incubation, the medium was removed and the cells rinsed and harvested with trypsin.

After stopping the reaction of trypsin by the addition of MEM-NCS, the cell suspensions were transferred to 15-mL tubes and centrifuged for 5 min (1500 rpm) at 20°C. The cell pellet was extracted with cold 5% trichloroacetic acid, left for 10 min at 4°C, and centrifuged for 5 min at 4000 rpm. The precipitate was dissolved in 1 N NaOH. Aliquots were taken for liquid scintillation counting and determination of protein (29).

Enzyme analysis. Fibroblast extracts containing 10–50 μg protein were assayed in duplicate for protein, citrate synthase, and thiolase activity as described previously (10). Thiolase activity was determined with three substrates: acetoacetyl-CoA (C_4), 2-methylacetoacetyl-CoA (C_5), and 3-oxohexanoyl-CoA (C_6). The latter substrate (C_6) measures the wide specificity thiolase (EC 2.3.1.16) of mitochondria and peroxisomes. C_5 substrate measures the mitochondrial thiolase (EC 2.3.1.9), the absence of which causes 2-methyl-3-hydroxybutyric aciduria (McKusick 20375). The C_4 substrate is the common substrate for all the thiolases (cytoplasmic and mitochondrial EC 2.3.1.9, and mitochondrial EC 2.3.1.16) and is therefore not discriminatory. However, experience has shown that the activity ratio C_5/C_4 is the most reliable detector of heterozygotes for this disorder (30). The detection limit of the thiolase assay is approximately 0.1 nmol. When very low or zero activities were encountered, the assay was repeated *1*) with four times the original amount of protein to detect any significant activity and *2*) with four times the standard assay concentration of 2-methylacetoacetyl-CoA or CoASH to ensure that a Km defect was not responsible.

RESULTS

Macromolecular labeling with [1-^{14}C]-2-methylbutanoic acid. The incorporation of ^{14}C from 2-methylbutanoic acid into macromolecules is presented in Table 2. Fibroblasts from healthy subjects ($n = 6$) showed a mean incorporation of 2.64 pmol·h^{-1}·mg^{-1} of protein, the range being 1.83–3.65. This incorporation is about 10 times less than the incorporation from ^{14}C-isovaleric acid into macromolecules through the leucine pathway, but the relative SD are similar, being 24% for the former and 28% for the latter (28). When studying the incorporation in fibroblasts from 13 patients with 2-methyl-3-hydroxybutyric aciduria, two patients showed incorporation within the normal range (1.99–2.97 pmol·h^{-1}·mg^{-1}), whereas in 11 cases, the incorporation was clearly reduced and ranged from 0.05 to 1.06 pmol·h^{-1}·mg^{-1}, corresponding to 2–41% of the mean normal value.

The possibility was considered that a varying rate of protein synthesis by the mutant cells might influence the degree of macromolecular labeling by [1-^{14}C]-2-methylbutanoic acid. Therefore, in separate experiments, we monitored the rate of protein synthesis by the incorporation of ^{3}H-lysine. In six mutant cell lines with macromolecular labeling ranging from 2–32% of normal, the labeling of protein by ^{3}H-lysine showed a relative SD of 26.5%, and there was no correlation between the labeling of protein by ^{3}H-lysine and [1-^{14}C]-2-methylbutanoic acid.

3-Oxothiolase activities in broken cells. When thiolase activity was measured with three different substrates (C_4, C_5, and C_6, see Materials and Methods), the activity ratio C_5/C_4 has been shown to be the most reliable parameter in distinguishing patients, heterozygotes, and normals for McKusick 20375 (30). The C_5/C_4 ratio in fibroblasts from 15 controls was 1.18 ± 0.18 (mean ± SD). When measuring the enzyme in fibroblasts from 13

Table 2. *3-oxothiolase activities and [1-^{14}C]-2-methylbutanoic acid macromolecular labeling of cultured fibroblasts*

Subjects	3-oxothiolase activity*			[1-^{14}C]-2-methylbutanoic acid incorporation†	
	Substrate C_5	Ratio C_5/C_4	C_5/C_4 (% of normal)	pmol/h/mg	% of normal
Normals					
Number	15	15	15	6	6
Mean ± SD	36.3 ± 9.0	1.18 ± 0.18	100 ± 15	2.60 ± 0.63	100 ± 24
Range	19.7–53.3	0.9–1.67	76–142	1.83–3.65	70–140
Heterozygotes					
Number	10	10	10		
Mean ± SD	13.2 ± 4.1	0.51	43		
Range	2.9–30.6	0.29–0.64	25–54		
Patients: deficient thiolase activity and macromolecular labeling					
LV‡	6.6; 1.0	0.33; 0.13	19 (11–28)	0.83 ± 0.30 (8)	32
MM	ND§	ND		0.38 ± 0.13 (11)	15
BB	ND	ND		0.11 ± 0.08 (5)	4
CB	ND	ND		0.44 (0.20–0.63)	18
MAIP	ND	ND		0.81 (0.74–0.90)	31
AIP	ND	ND		0.33 ± 0.10 (4)	13
CT	ND	ND		0.05 (0.04–0.06)	2
MT	ND	ND		0.10 ± 0.02 (4)	4
WG 138	ND	ND		0.24 (0.19–0.29)	9
Patients: Normal thiolase activity, deficient macromolecular labeling					
ZM	30.9	0.96	81	0.53 ± 0.21 (5)	20
WG 88	33.5	1.06	90	0.85 (0.76–0.98)	32
Patients: Normal thiolase activity and macromolecular labeling					
SS	28.8	0.98	83	2.14 (2.05–2.22)	82
BM	47.5	1.30	110	1.99 ± 0.96 (5)	75

* 3-oxothiolase activities in fibroblast lysates determined with specific C_5 (2-methylacetoacetyl-CoA) and nonspecific C_4 (acetoacetyl-CoA) substrates. Units: nmol substrate removed/min/mg protein (mean of duplicates).

† Incorporation of [1-^{14}C]-2-methylbutanoic acid into macromolecules in intact fibroblasts. Units: pmol incorporated/h/mg protein. Numbers given as mean ± SD with number of determinations in parentheses. Mean and range given where only three separate determinations.

‡ Thiolase activities in lysates of LV fibroblasts were determined from cells grown on two separate occasions. Both times the activity was very low but detectable.

§ ND denotes no activity detectable even at four times protein and substrate concentration.

patients with suspected 3-oxothiolase deficiency, activity with C_5 substrate (and the C_5/C_4 ratio) was undetectable in eight patients. In patient L.V., the C_5/C_4 ratio was very low and variable, ranging from 0.13 to 0.33. The remaining four patients showed normal ratios ranging from 0.96 to 1.30. The mean C_5/C_4 ratio in fibroblasts from 10 heterozygotes was 0.51. In no case was there evidence of abnormally high Km for C_5 substrate. Also, no fibroblast extract from patients showed significant abnormality in thiolase activity with C_6 substrate, and all gave activities of citrate synthase within the normal range (data not shown).

Macromolecular labeling versus enzyme activities. When combining MMLA and enzyme activities from the different patients, a certain pattern emerged (Table 2). Nine patients showed deficient 3-oxothiolase as well as reduced activity by MMLA. Two patients exhibited clearly reduced activity by MMLA, but normal or near normal activities of 3-oxothiolase. Finally, two patients showed normal activities by either test.

DISCUSSION

The patients with suspected 3-oxothiolase deficiency that we studied clearly fall into three categories: *1*) patients with low incorporation by the MMLA and also low or undetectable 3-oxothiolase activity toward 2-methylacetoacetyl-CoA; *2*) patients with low activity by MMLA, but normal enzyme activities; and *3*) patients with normal MMLA as well as normal enzyme activities.

The patients in the first category (n = 9) clearly suffer from mitochondrial 3-oxothiolase deficiency that is manifest by enzyme measurements in broken cells, as well as in whole cells *in vitro*, as demonstrated by MMLA. However, although this group of patients with one exception showed no detectable enzyme activity, they presented a striking clinical heterogeneity. To some extent, the degree of clinical severity was reflected by MMLA. Thus, severe clinical symptoms were generally encountered in patients with less than 20% of normal activity by MMLA. Patient M.A.I.P., on the other hand, being a symptom-free sibling of the affected patient A.I.P., showed 31% of normal MMLA activity. Furthermore, patient L.V., with 32% of normal MMLA activity, remained well between episodes of ketoacidosis and showed normal growth and development. In this case, an isoleucine load was initially necessary to clarify the picture. Work is in progress to study the possibility of genetic heterogeneity within this group of patients.

The second group of patients (n = 2) raises extremely interesting questions. These patients had deficient isoleucine catabolism as measured by MMLA and abnormal profiles of organic aciduria, but enzyme measurement apparently excluded 3-oxothiolase deficiency. In particular, the normal thiolase activity in W.G. 88 cells was quite unexpected. These cells were from the original proband J.B., who led Daum *et al.* (1, 3) to suggest that the absence of thiolase activity was the cause of the symptoms. These authors showed that W.G. 88 cells metabolized isoleucine at only 44–46% of normal rate. In the early studies, J.B. did not have elevated tiglylglycine, but, interestingly, re-examination of this patient at age 28 revealed large amounts of tiglylglycine (Scriver CS, personal communication). W.G.138 cells, also originally studied by Daum *et al.* (3), clearly have abnormal thiolase activity. The mutant allele may therefore be different in the cell lines W.G. 88 and W.G. 138, as originally suggested (3).

In Z.M., the other patient in our category 2, there was intermittent excretion of tiglylglycine. We attempted to determine

whether the 2-methyl-3-hydroxybutyryl CoA dehydrogenase was deficient. Cell lysates were incubated with tiglyl-CoA, tritiated water, NAD, and avidin (5). The incorporation of label into propionate was normal, suggesting normal dehydrogenase. Patient Z.M. therefore seems to have a previously unexplained inhibition of the isoleucine pathway in intact cells. A possible explanation might lie in a functional defect at the thiolase step caused by the accumulation of an inhibitory metabolite due to a primary lesion somewhere else in the pathway of isoleucine catabolism. In any case, the occurrence of urinary 2-methyl-3-hydroxybutyric acid and/or tiglylglycine is not an unequivocal indicator of the absence of the thiolase that metabolizes 2-methylacetoacetyl-CoA.

The patients in the 3rd category ($n = 2$) apparently have no disorder of isoleucine catabolism distal to 2-methylbutyryl-CoA, and the reason for their particular pattern of organic aciduria is unknown.

Note added in proof: Since the submission of this paper we have had the opportunity to examine another fibroblast explant (W.G. 26) from patient J.B. (Ref. 1). In contrast to the cell line W.G. 88 previously examined, W.G. 26 shows no detectable enzyme activity with 2-methylacetoacetyl-CoA as substrate. Patient J.B. therefore most likely suffers from mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency, in accordance with the original suggestion (Ref. 1). However, the finding of normal thiolase activity in W.G. 88 cells was confirmed by repeated measurements made on another batch of these cells. The reason for the discrepancy between W.G. 88 and W.G. 26 is not understood at present, but will be further investigated.

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