Clinical and Biochemical Variation and Family Studies in the Multiple Acyl-CoA Dehydrogenation Disorders

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ABSTRACT. We report clinical and biochemical studies in patients with multiple acyl-CoA dehydrogenation disorders (MAD) and their parents. A severely affected (MAD:S) patient presented with neonatal acidosis leading to death and excreted a wide range of straight- and branched-chain acyl CoA derivatives. Two patients with mild variants of the same disorder (MAD:M) presented with neurologic abnormalities, acidotic coma, and/or poor growth; they primarily excreted ethylmalonate and variable amounts of adipate. Fibroblasts from the MAD:S patient demonstrated severely defective radiolabeled substrate oxidation, while the MAD:M cells had milder oxidative defects. Fibroblasts from two other MAD:M and six other MAD:S patients demonstrated analogous defects in substrate oxidation. As a group, MAD:S cells deficient in electron transfer flavoprotein:ubiquinone oxidoreductase had significantly lower residual oxidative activities than did MAD:S cells deficient in electron transfer flavoprotein. Fibroblasts from the parents of four MAD:S patients oxidized radiolabeled substrates significantly less effectively than did normal infant controls but were indistinguishable from normal adult cells. We found relatively higher residual oxidative activities in maternal than in paternal cells. Amniocytes from a fetus at risk for MAD:S catabolized labeled substrates normally; the infant has been clinically and biochemically normal up to 30 months of age. (*Pediatr Res* 21: 371–376, 1987)

Abbreviations

ETF, electron transfer flavoprotein
ETF:QO, ETF:coenzyme Q oxidoreductase
MAD, multiple acyl-CoA dehydrogenation disorder
MAD:M, MAD, mild variant
MAD:S, MAD, severe variant
MCT, medium-chain triglycerides
FBS, fetal bovine serum

MAD include glutaric aciduria type II (1–9), ethylmalonic-adipic aciduria (10), and C_6–C_{10} dicarboxylic aciduria responsive to riboflavin (11). Most MAD:S patients present shortly after birth with severe hypoglycemia and profound metabolic acidosis but without ketosis (1–8, 12). Hypotonia and seizures are common symptoms (1–7, 12). Some patients have "sweaty-foot" or acrid odor (1–3, 5, 7); most do not survive the neonatal period (1, 3–5, 7) although some have lived for months (6, 12). MAD:M patients have presented at 7 wk to 19 yr of age with less severe and more variable clinical symptoms, including intermittent hypoketotic hypoglycemia, acidosis, and coma (9–11). The characteristic metabolites elevated in most MAD patients include glutaric acid, ethylmalonic acid, and adipic acid in widely varying proportions (1–12). The volatile short-chain fatty acids, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, and hexanoic acid (1, 3–5, 7, 10, 12) and their glycine conjugates (2, 4, 6, 10–12) are elevated in many cases, as is the amino acid sarcosine (2–4, 12). The clinical and biochemical heterogeneity of these disorders reflect varying deficiencies of either ETF or its dehydrogenase (ETF:QO), thought to be the primary biochemical defects (13). Herein we describe the clinical features and metabolite elevations of one new MAD:S and two new MAD:M patients with widely differing clinical presentations. We also report oxidation studies using fibroblasts from these three patients, from eight previously identified MAD patients and from eight parents of four of the MAD:S patients.

CASE REPORTS

Patient 1401 (C.W) was a 3600 g term infant born to a gravida 4 para 4 mother after an uneventful pregnancy, labor, and delivery. At 24 h of age, he developed postprandial vomiting and hypoglycemia. Shortly thereafter he developed increasing metabolic acidosis, respiratory distress, and suffered a cardiopulmonary arrest. He was resuscitated, ventilated, and transferred to the University of Kentucky Medical Center. At admission, he was noted to have a dark brown urine with a distinct musty odor. A work-up for inborn errors of metabolism was begun. An initial physical examination was remarkable only for unresponsiveness. Arterial pHs were 7.29 or below. Peripheral blood glucose was 58 mg/100 ml with a cerebrospinal fluid glucose of 14 mg/100 ml. Blood ammonia was 210 μM. During the 2nd hospital day, he developed irregular ventricular rhythms and episodic hypotension. He was transiently stabilized with cardioversion and lidocaine, but developed clonic seizures. A second episode of ventricular tachycardia was successfully treated but irreversible hypotension developed and he died at 3 days of age

after he developed a idioventricular rhythm. Postmortem biochemical studies of urine demonstrated the organic aciduria of MAD:S (see "Results"). An autopsy revealed diffuse fatty changes affecting virtually all hepatocytes and focal pulmonary hemorrhage. Facial features and microscopic examination of the kidneys were normal. This child has three siblings in good health; the parents are of Northern European origin and are nonconsanguineous.

Patient 840 (M.L.) is a 6-yr-old boy born by an uncomplicated cesarean section for breech presentation. At birth, he weighed 9 lb 2 oz and Apgars were 9 and 9 at 1 and 5 min. His milestones were slow and he walked at 18 months. At 34 months his speech was limited to single words. At age 22 months, he had a febrile illness followed by gait difficulty and irritability for 1 wk. Over the next 7 months he had two episodes of febrile illnesses followed by 12-h periods of shaking and fine tremors. At age 34 months he was admitted to the hospital when he developed seizures and coma after a febrile illness. He was mildly acidotic with a serum bicarbonate level of 16 mEq/liter (normal 24–28). His urine ketones were 1+. His blood ammonia and glucose were normal. Serum and urine amino acid screens were normal. Urinary organic acids were abnormal (see "Results"). When the diagnosis of MAD:M was suspected, he was started on a low fat diet consisting of Vivonex HN, supplemented initially with safflower oil but currently with fruits, cereal, and yogurt. A trial with oral riboflavin supplements was performed (see "Results"). While he has had slow improvement in his neurological status, 16 months after admission he continues to have quadriparesis and ankle clonus. He has no recognizable speech, although he appears to hear. He is able to swallow from a cup, but drools a great deal. He takes daily phenobarbital. He has been admitted on two subsequent occasions for pneumonia but had no signs of metabolic decompensation. Total plasma carnitine was 34 μM (normal 50 ± 10 μM), with free and esterified fractions of 25 μM (normal 45 ± 10 μM) and 9 μM (normal 5 ± 5 μM), respectively. The mother is of Irish, Scotch, German, and Norwegian ancestry, and the father is of Yugoslavian ancestry; they are nonconsanguineous. This child has two older brothers who have been evaluated for hyperkinesis, poor coordination, and academic difficulties. They and their parents excrete no abnormal organic acids (data not shown).

Patient 930 (P.K.) is a 3½-yr-old American boy of Greek ancestry whose major problems have been slow physical and mental development. He was a 36-wk, 2268-g baby born to a 42-yr-old mother following a pregnancy complicated by placenta previa. The mother was hospitalized 3 months prior to delivery for impending premature delivery. Delivery was by cesarean section with no perinatal distress. In the newborn period, he fed poorly and failed to breast-feed successfully.

Following birth, he grew poorly; at 1 yr of age, his weight age was 3 months and his height age was 6 months. Since 1 yr of age he has remained far below the fifth percentile in both weight and height. At 3 yr of age, his weight age is 10 months and his length age is 18 months. His head circumference has increased along the 25th percentile since birth.

He has had delayed psychomotor development, especially noticeable since 6 months of age, and has lost some previously acquired abilities. He smiled at 2 months of age, rolled over at 4 months, and sat at 8 months. At 2 yr of age he had a 25-word vocabulary, mostly understandable to family only, but by 2½ yr of age he was no longer speaking. At 3½ yr of age he is beginning to speak again (five words only). At 13 months of age, he became mobile by scooting in a sitting position on his buttocks and moving forward on his hands. At 15 months of age he began pulling himself to standing position but stopped standing at 2 yr of age. At 3 yr of age he began standing and walking and now at 3½ yr of age he can run.

He has had no episodes of vomiting, lethargy, acidosis, ketosis, or hypoglycemia. Physical examination has been remarkable only for mild hyperreflexia in the lower extremities. Routine laboratory investigations have all been within normal limits. CT scans of the head at 1½ and 3 yr of age have been normal. An EEG at 3 yr of age was normal. Ethylmalonic and adipic acids were first found in the urine at 1½ yr of age; at that age he tolerated challenges of 450 mg of MCT oil/kg/day (divided three times a day) and 180 mg of lysine/kg/day without any vomiting, lethargy, or ketoacidosis. His mental and physical development was not improved by an 8-month trial of 250 mg of glycine/kg/day supplementation or by a low fat diet (with poor compliance). Total plasma carnitine was 84 μM, with free and esterified fractions of 62 and 22 μM, respectively.

METHODS

Metabolite analyses. Nonvolatile urinary organic acids were analyzed by sequential liquid partition chromatography, gas chromatography, and gas chromatography-mass spectrometry as previously described (14). Volatile short-chain fatty acids were quantified by direct injections of acidified urine and plasma and gas chromatography as previously described (14).

Materials. All chemicals used were reagent grade (Sigma Chemical Company, St. Louis, MO). All tissue culture reagents and media were from Kansas City Biologicals, Kansas City, MO. [1-^{14}C]butyrate, [1-^{14}C]octanoate, and [1,4-^{14}C]succinate were obtained from New England Nuclear Corporation, Boston, MA and [9,10(n)-^{3}H]palmitate and L-[2-^{14}C]leucine from Research Products International, Arlington Heights, IL.

Cell culture. Untransformed cultures of normal diploid skin fibroblasts were established by accepted techniques immediately after biopsy. Skin biopsies were obtained at necropsy 6 to 18 h after death from six adults without clinical evidence of inborn errors of metabolism, a 29-yr-old man (line S-1), a 54-yr-old man (S-3), and 57-, 74-, 71-, and 64-yr-old women (S-5, -6, -7, -8); cultures S-2 and -4 were established from skin biopsies of a healthy 35-yr-old man and 24-yr-old woman, respectively. Control cell cultures were derived from the foreskins of 10 randomly selected, healthy full-term male infants. Skin fibroblasts were obtained from seven MAD:S patients who presented in the neonatal period, three female (1540, Ref. 4; 1441, Ref. 6; 1520, Ref. 15) and four males (1400, Ref. 1; 1430, Ref. 12; 1515, Ref. 5; 1401). The MAD:M patients included one female (493, Ref. 10) and three males (741, patient B.K. in Ref. 16; 840 and 930), who all presented in infancy. Cells were subcultured serially (1:4) at 7- to 9-day intervals in Eagle's minimal essential medium containing 10% FBS, 2 mM glutamine, 140 μM penicillin, 86 μM streptomycin, and 162 μM neomycin. Cells in passages 6 to 20 were used in all experiments. Amniocyte culture was performed using the methods described for fibroblasts.

^{14}C-substrate oxidation to ^{14}CO$_2$ by intact fibroblasts in suspension was performed precisely as described earlier, using ^{14}C-substrates at previously reported specific activities and concentrations (17, 18). Oxidation studies were done 7 to 9 days after subculture (1:4) when the monolayers were confluent. Less than 5% of the label from any ^{14}C-substrate was converted to ^{14}CO$_2$ in these experiments (17). [9,10(n)-^{3}H]palmitate oxidation was quantitated by measuring ^{3}H released into water by cell monolayers in 2 cm^2 microtiter wells. The specific activity, concentration and incubation time were 380 mCi/mmol, 22 μM and 2 h, respectively. The ^{3}H$_2$O produced was separated from unreacted substrate by anion exchange chromatography on AG-1 (Biorad) as described earlier (19). Less than 2% of the ^{3}H on the substrate was converted to ^{3}H$_2$O (Moon A, Rhead W, unpublished data).

^{14}C incorporation from labeled substrates into cellular protein was determined by the method described previously (17); the culture medium used was Leibowitz L-15 containing 10% FBS and 20 mM HEPES, pH 7.2; the specific activities and concentrations of both [1-^{14}C]butyrate and -octanoate were 10 mCi/mmol and 0.1 mM, respectively. The specific activity and concentration of [1,4-^{14}C]succinate were 1.0 mCi/mmol and 1.0 mM, respectively. Less than 7% of the label from any ^{14}C-

substrate was incorporated into cellular protein in these experiments (17). Incubation time was 22 h; protein was determined by a fluorometric method (20). All p values cited are two-tailed and calculated by Student's t test, and compare infant's or parent's values to control values, unless stated otherwise.

RESULTS

ETF-deficient patient 1401 had the large elevations of short-chain fatty acids in serum and urine characteristic of MAD:S (Tables 1 and 2). Glycine conjugates of these acids were not detected. The most highly elevated acid in urine obtained postmortem was glutaric acid; ethylmalonic and adipic acids were also elevated (Table 2). The levels of other elevated acids in μmol/mg creatinine were: 1.29 methylsuccinic acid, 0.45 suberic

acid, 0.10 sebacic acid, 4.33 2-hydroxyglutaric acid, 80.18 hippuric acid, 37.13 4-hydroxyphenyllactic acid, and 21.49 lactic acid. Sarcosine was not detected.

The initial urine specimen of ETF-deficient patient 840, obtained when he was comatose, contained large amounts of 4-hydroxyhippuric acid (> 15 μmol/mg creatinine) and a small elevation of ethylmalonic acid without an elevation of glutaric or adipic acids (Table 2). Treatment with riboflavin (100–500 mg/day) appeared to decrease initially the excretion of ethylmalonic acid with a later return to pretreatment levels.

ETF-deficient patient 930 initially had very small elevations of ethylmalonic and adipic acids (Table 2). Administration of 3.3 g MCT in three divided doses caused a moderate increase in the excretion of ethylmalonic, adipic, and glutaric acids. Simultaneous administration of glycine abolished the increase seen with MCT alone, although hexanoylglycine was not detected. Administration of an oral load of lysine did not cause increased excretion of glutaric acid. A fat-free diet decreased the excretions of the acids to below the limit of detection.

Cells from these and other MAD patients oxidized radiolabeled substrates less effectively than did normal controls (Table 3). MAD:M cells oxidized [1-^{14}C]butyrate at 28% (493) to 57% (930) of control levels and averaged 46% of control levels ($p < 0.05$). With [9,10(n)-^{3}H]palmitate as substrate, activities ranged from 31% (493) to 63% (741) of control. As a group, the MAD:M cells oxidized ^{3}H-palmitate at 48% of control levels ($p = 0.01$). Analogously, ETF-deficient cells 840 and 930 oxidized [1-^{14}C] octanoate at 47 and 55% of control levels ($p < 0.05$). Higher levels of residual activity were seen in the L-[2-^{14}C]leucine oxidations. MAD:M cells oxidized this substrate at 27% (493) to

Table 1. *Short-chain fatty acid levels in patient 1401**

	Isobutyric	Butyric	2-Methylbutyric	Isovaleric
	Serum (μmol/liter)			
Patient 1401	4740	1091	921	564
Normals	<1.8	<2.5	<0.3	<0.4
	Urine (μmol/mg creatinine)			
Patient 1401	2.72	5.08	0.31	0.80
Normals	<0.08	<0.08	<0.08	<0.08

* Normal values from Sweetman *et al.* (3).

Table 2. *Urinary levels of organic acids*

Patient log no.	Collection date	Treatment	Glutaric	Ethylmalonic	Adipic
			μmol/mg creatinine		
ETF-deficient patient 1401	DOB 11/11/79				
7547	11/14/84	Postmortem	83.55	3.50	5.32
ETF-deficient patient 840	DOB 5/4/79				
A617	Recd 3/31/82		*	0.773	
A662	4/5/82		*	1.132	0.092
A789	4/27/82		*	0.583	0.069
A885	5/21/82		*	0.313	0.074
A1539	10/19/84	Baseline	<0.010	0.057	<0.010
A1697	11/29/82 (24-h urine)	Riboflavin: 50 mg bid 3 wk	<0.010	0.048	
A1698	11/29/82 (24-h urine)	50 mg bid 3 wk	<0.010	0.090	0.040
A1970	1/20/83	500 mg qD	<0.010	0.197	0.023
A1971	1/20/83	500 mg qD	*	0.324	0.024
ETF-deficient patient 930	DOB 3/28/81				
A1342	8/25/82		<0.010	0.151	0.245
A1362	9/2/82		<0.010	0.105	0.020
A1507	10/11-12/82	Baseline: Isomil	<0.10	0.105	<0.010
A1508	10/12-13/82	MCT: 1.1 g tid	*	0.390	0.361
A1526	10/15-16/82	MCT: 1.1 g tid	*	0.160	0.055
		Glycine: 250 mg/kg/day			
A1527	10/16-17/82	Lysine: 60 mg/kg/day	<0.010	0.180	0.156
A1528	10/17-18/82	Lysine: 600 mg/kg/day	<0.010	0.038	0.014
A1769	12/13-14/82	Low fat; glycine 250 mg/kg/day	*	0.031	<0.010
A1873	1/7/83	No fat	*	<0.010	<0.010
A2157	3/4/83		<0.010	0.056	<0.010
A2469	4/30/83		*	0.346	0.112
A2828	7/19/86	18% fat	*	0.209	0.092
A3174	10/3/83		*	0.117	0.019
A4078	2/5/84	Well	*	0.129	0.039
A4079	3/10/84	Sick	<0.010	0.153	0.058
Normal ($n = 10$)			<0.10	<0.010	<0.100

* No glutarate was detected in these urines, although its elution near the internal standard prevented its quantitation.

Table 3. *Oxidation of labeled substrates by intact patient fibroblasts**

Cell cultures	[1-^{14}C]butyrate	[9,10(n)-^{3}H]palmitate	L-[2-^{14}C]leucine	[1-^{14}C]octanoate	[1,4-^{14}C]succinate
	nmol $^{14}CO_2$ or ^3H_2O produced/mg protein/h ± SEM				
Normal infant controls	5.54 ± 0.48	0.40 ± 0.036	1.87 ± 0.14	2.77 ± 0.26	1.82 ± 0.21
MAD:M					
ETF:QO deficient					
493	1.55 ± 0.33	0.12 ± 0.022	0.50 ± 0.20	ND	1.35 ± 0.21
741	3.10 ± 0.35	0.25 ± 0.021	1.19 ± 0.32	ND	1.64 ± 0.27
ETF deficient					
840	2.33 ± 0.29	0.22 ± 0.009	0.88 ± 0.17	1.30 ± 0.22	1.78 ± 0.22
930	3.16 ± 0.48	0.18 ± 0.032	1.40 ± 0.15	1.52 ± 0.25	1.91 ± 0.23
MAD:S					
ETF deficient					
1400	ND	0.07 ± 0.007	ND	ND	ND
1401	0.50 ± 0.09	0.02 ± 0.004	0.30 ± 0.08	ND	2.00 ± 0.49
1430	0.55 ± 0.16	0.03 ± 0.017	0.32 ± 0.10	ND	2.26 ± 0.45
1441	0.50 ± 0.11	0.04 ± 0.002	0.22 ± 0.13	ND	1.71 ± 0.73
ETF:QO deficient					
1515	0.22 ± 0.10	0.01 ± 0.002	0.15 ± 0.08	ND	2.07 ± 0.51
1520	0.17 ± 0.05	0.01 ± 0.003	0.14 ± 0.05	ND	1.27 ± 0.26
1540	0.33 ± 0.14	0.01 ± 0.001	0.22 ± 0.05	ND	2.31 ± 0.79

* The number of determinations ranged from 14 to 43 for control cultures and two to 15 for individual MAD cultures; ND, not determined.

Table 4. *^{14}C incorporation from labeled substrates into cellular protein of intact patient fibroblasts*

Cell cultures	[1-^{14}C]butyrate	[1-^{14}C]octanoate	[1,4-^{14}C]succinate
	nmol ^{14}C incorporated/mg protein/h ± SEM		
Normal infant controls	0.68 ± 0.06	0.23 ± 0.02	0.23 ± 0.03
MAD:M			
ETF:QO deficient			
493	0.28 ± 0.09	0.12 ± 0.03	0.17 ± 0.08
632	0.39 ± 0.10	0.18 ± 0.02	0.20 ± 0.06
741	0.42 ± 0.08	0.11 ± 0.03	0.20 ± 0.03
MAD:S			
ETF deficient			
1400	0.07 ± 0.01	0.02 ± 0.01	0.22 ± 0.08
1401	0.13 ± 0.02	0.05 ± 0.01	0.25 ± 0.09
1430	0.06 ± 0.03	0.04 ± 0.02	0.21 ± 0.07
1441	0.15 ± 0.02	0.04 ± 0.01	0.17 ± 0.06
ETF:QO deficient			
1515	0.07 ± 0.02	0.03 ± 0.02	0.18 ± 0.10
1520	0.15 ± 0.01	0.03 ± 0.02	0.18 ± 0.08
1540	0.08 ± 0.02	0.04 ± 0.01	0.26 ± 0.05

* The number of determinations ranged from 25 to 44 for controls and two to 10 for individual MAD cultures.

75% (930) of control levels and averaged 54% of control ($p = 0.03$).

MAD:S cells demonstrated more severe defects in substrate oxidation, oxidizing radiolabeled butyrate, palmitate, and leucine at 3–10, 3–16, and 7–17% of control levels, respectively ($p = 0.001$). With both labeled butyrate and palmitate, ETF-deficient MAD:S cells 1400, 1401, 1430, and 1441 demonstrated less severe oxidative defects (9–10% of control) than did ETF:QO deficient cells 1515, 1520, 1540 (3–4% of control; $p = 0.02$). With L-[2-^{14}C]leucine as substrate, oxidation in the former cells was 15% of control, in the latter 9% of control, and did not differ significantly between the two groups ($p = 0.16$).

We obtained similar results upon measuring the incorporation of ^{14}C from radiolabeled fatty acids into the cellular protein of intact fibroblasts (Table 4). In the ETF:QO-deficient MAD:M patients 493, 632, and 741, ^{14}C incorporation from ^{14}C-butyrate and -octanoate averaged 55 and 57% of control, respectively ($p = 0.05$). In MAD:S patients, residual activities were somewhat

higher than those in the $^{14}CO_2$ production assay, ranging from 9 to 22% of control with both substrates ($p < 0.01$). This less sensitive assay system did not clearly distinguish the ETF-deficient from the ETF:QO-deficient MAD:S patients.

We also performed fibroblast oxidation studies with the parents of both ETF- and ETF:QO-deficient MAD:S patients, comparing these parental cells to both normal infants and normal adults (Table 5). [1-^{14}C]butyrate oxidation in all parental cells was indistinguishable from that in adult controls, but averaged 51% of the infant controls ($p < 0.05$). Similarly, parental cultures catabolized L-[2-^{14}C]leucine at 60% of infant control levels ($p < 0.05$), but were indistinguishable from the normal adults. We found somewhat higher residual oxidative activities for both substrates in maternal cultures from three of the four families. This difference is most marked in the mothers of ETF:QO-deficient patients 1515 and 1520, whose activities were significantly higher than in the corresponding paternal cells ($p < 0.05$). ^{14}C-succinate oxidation was identical in all cells. In parental cells,

Table 5. $^{14}CO_2$ production from labeled substrates by intact parental fibroblasts*

Cell cultures	[1-^{14}C]butyrate	L-[2-^{14}C]leucine	[1,4-^{14}C]succinate
	nmol $^{14}CO_2$ produced/mg protein/h ± SEM		
Normal infant controls	6.59 ± 0.45	1.93 ± 0.17	1.79 ± 0.23
Normal adult controls	3.61 ± 0.56†·‡	0.85 ± 0.09‡·§	1.82 ± 0.16§
ETF-deficient MAD:S			
Proband:1401			
Mother	3.69 ± 0.40	1.10 ± 0.12	2.27 ± 0.34
Father	3.10 ± 0.46	1.25 ± 0.16	1.27 ± 0.30
Proband:1441			
Mother	2.04 ± 0.46	1.14 ± 0.21	1.58 ± 0.13
Father	2.50 ± 0.39	0.98 ± 0.19	1.95 ± 0.07
ETF:QO-deficient MAD:S			
Proband:1515			
Mother	7.45 ± 1.73	1.58 ± 0.16	2.68
Father	2.83 ± 0.50	0.81 ± 0.09	2.60
Proband:1520			
Mother	2.97 ± 0.37	1.60 ± 0.24	1.55 ± 0.31
Father	2.10 ± 0.26	0.87 ± 0.13	1.32 ± 0.26

* The number of determinations ranged from 15 to 29 for pooled control cells and from one to 11 for individual parental cultures.
† Cultures S-1,-2,-4.
‡ Difference from infant controls significant at $p < 0.05$.
§ All S cells.

^{14}C incorporation into cell protein from ^{14}C-butyrate and -octanoate averaged 60 and 45% of infant controls ($p < 0.05$), but was indistinguishable from the normal adults (data available on request). As in the $^{14}CO_2$ production studies, higher residual activities were found in maternal than in paternal cells in more than three-quarters of the studies. This distinction again appeared more marked in the parents of ETF:QO-deficient patients 1515 and 1520.

We also performed ^{14}C-incorporation studies in amniocytes from a subsequent pregnancy of the mother of ETF-deficient MAD:S patient 1401; activity was greater than 106% of five amniocyte controls with ^{14}C-butyrate, -octanoate and -succinate as substrates (data not shown). Organic acid analyses of the amniotic fluid and mother's urine revealed no abnormal metabolites (courtesy of Kay Tanaka, M.D.). The pregnancy was carried to term and the male infant has no clinical or biochemical abnormalities at age 30 months. Fibroblast cultures were not established on this child.

DISCUSSION

The findings in the ETF-deficient MAD patients 1401, 840, and 930 clearly demonstrate the wide range of clinical and biochemical heterogeneity in patients with this enzymatic defect. Patient 1401 presented in the neonatal period with acidosis and early death. His clinical course, pathological findings, and urinary metabolites are virtually identical to those of other MAD:S patients and clearly assign him this diagnosis. Patient 840 is neurologically devastated following an episode of acidotic coma and seizures. This patient consistently excreted ethylmalonic acid. His total plasma carnitine levels were in the low normal range and the ratio of esterified to free carnitine was moderately elevated. No precursor loads were performed and riboflavin administration did not produce a lasting decrease in ethylmalonate excretion. Patient 930's findings are more unusual. He excreted only ethylmalonate; adipate excretion was above normal in several samples and increased on MCT loading. Since he had no documented episodes of unexplained coma, lethargy, hypoglycemia, ketosis, or acidosis, his developmental delay may result from some unrecognized prenatal or perinatal insult. While his limited responses to precursor loads seem unexpected in disorders with generalized blocks in acyl-CoA dehydrogenation,

ETF:QO deficient MAD:M patient 741 did not excrete glutarate or medium chain dicarboxylic acids after lysine and MCT challenges (16). The MAD patient described by Verjee and Sherwood (8), who presented at age 6 months, also had a persistent ethylmalonic aciduria, while excretion of adipate and glutarate were intermittent. In summary, the findings in these patients underscore the variability of the MAD:M.

The three patients reported herein (1401, 840, 930) have not been described previously. In studying their fibroblasts, we thought it informative to investigate MAD cells not yet studied with these techniques (741, 1441, 1520, and 1540) and compare them to MAD cells 493, 1400, 1430, and 1515, studied by others previously (1, 10, 12, 21).

In the MAD:M cells, we find moderate and highly variable defects in oxidation of multiple radiolabeled substrates catabolized via acyl-CoA intermediates. Frerman (personal communication) found intermediate ETF:QO deficiencies in patients 493 and 741 (13 and 51% of control, respectively). We recently demonstrated ETF deficiency in cells 840 and 930 (29 and 38% of control, respectively; $p < 0.05$; Ref. 22). It is not known whether ETF or ETF:QO is rate limiting for electron flux from the acyl-CoA dehydrogenases to coenzyme Q; their measured specific activities cannot be compared directly, due to profound differences in the fibroblast subfractions and assays employed (13, 22). However, the activity of each is clearly lower than the summed activities of the six acyl-CoA dehydrogenases from which they accept electrons. Thus, moderate reductions in the activity of either could lead to significant acyl-CoA accumulation, producing clinical symptoms and a characteristic MAD organic aciduria.

The MAD:S cells demonstrated severe and more consistent defects in substrate oxidation, catabolizing labeled butyrate, palmitate, and leucine at less than 17% of control levels. These results are similar to those of other investigators, where residual oxidation of these and related substrates ranged from 3 to 22% of control (1, 10, 12, 21). Interestingly, the oxidative defect in the ETF:QO-deficient cells (1515, 1520, 1540; Ref. 13) is 2- to 3-fold more severe than in the ETF-deficient cells (1400, 1401, 1430, 1441; Ref. 22). Lehnert et al. (7) also found similar differences in residual oxidative activities between MAD:S patients KII (ETF:QO deficiency) and 1400 (ETF deficiency). While the etiology of the morphologic abnormalities in the

ETF:QO-deficient MAD:S patients is not understood, it could be related to the severity of the metabolic block, rather than to its site. Although all known ETF-deficient MAD:S patients have specific abnormalities of the ETF α-subunit (23), the clinical significance and/or inheritance of lesions involving the ETF β-subunit cannot be predicted.

Our studies with parental fibroblasts from four MAD:S families demonstrate that oxidative activities in parents are generally lower than those in normal infants but indistinguishable from normal adults. We find the relatively higher maternal residual oxidative activities perplexing, although no ready explanation is apparent in the literature. These observations also underscore the necessity of using age-matched controls in these and related studies. Frerman and Goodman (13) have demonstrated intermediate levels of ETF:QO activity in fibroblasts derived from parents of ETF:QO-deficient MAD:S patients, consistent with autosomal recessive inheritance in these families. There have been several females affected with ETF deficiency, including patient 1441, a female sibling of patient 1430 (12) (Steinmann B, personal communication), and one other MAD:S patient (B.C.; Dr. Y. Hsia, HI), to whom we have tentatively assigned the diagnosis of ETF deficiency using the ^{3}H-palmitate assay (0.03 ± 0.01 nmol/mg protein/h; 8% of control). Thus, both ETF:QO- and ETF-deficient MAD:S are most likely autosomal recessive disorders. To date, direct assay of ETF and ETF:QO has been necessary to confirm the diagnosis in suspected MAD patients. We have recently coupled the ^{3}H-palmitate oxidation assay to cell fusion and complementation analysis, providing an efficient means of assessing the degree of metabolic block and identifying the biochemical lesion without direct assay. We describe this assay and complementation system more fully elsewhere (24).

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