Pediatr. Res. *16:* 861–868 (1982)

C_6–C_{10}-Dicarboxylic Aciduria: Investigations of a Patient with Riboflavin Responsive Multiple Acyl-CoA Dehydrogenation Defects

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Summary

The abnormal metabolites–adipic, suberic, and sebacic acids–were detected in large amounts in the urine of a boy during a Reye's syndrome-like crisis. Substantial amounts of 5-OH-caproic acid, caproylglycine, glutaric acid, and 3-OH-butyric acid and moderately elevated amounts of ethylmalonic acid, methylsuccinic acid, 3-OH-isovaleric acid, and isovalerylglycine were also found. These metabolites were consistently present in urine samples collected in the boy's habitual condition after the attack. 1-[^{14}C]-Palmitic acid was oxidized at a normal rate, whereas U-[^{14}C]-Palmitic acid was oxidized at a reduced rate in cultured skin fibroblasts from the patient, thus indicating a defect at the level of medium- and/or short-chain fatty acid oxidation. Riboflavin medication (100 mg three times a day) significantly reduced the excreted amounts of pathologic metabolites, suggesting a flavineadeninedinucleotide-related acyl-CoA dehydrogenation defect as the cause of the disease.

Carnitine in plasma was low in the patient (6 μmole/liter, controls 26–74 μmole/liter), suggesting carnitine deficiency as a secondary effect of the acyl-CoA dehydrogenation deficiency.

Speculation

The present patient, who presented with a Reye's syndrome-like attack, suffers from impaired dehydrogenation of acyl-CoA resulting in accumulation of acyl-CoA in the cells. Attacks with similar symptoms are seen in other acyl-CoA dehydrogenation deficiencies, such as glutaric aciduria types I and II, other types of C_6–C_{10}-dicarboxylic acidurias and isovaleric acidemia. Reduced flow through the acyl-CoA dehydrogenation steps may therefore be an ethiologic factor in Reye's syndrome. Several of the accumulated acyl-CoA's are toxic and may be responsible for some of the symptoms. The low carnitine level in plasma and the elevated esterified carnitine excretion in the present patient indicate that acyl-CoA accumulation may cause a functional carnitine deficiency by sequestration of carnitine as acyl-carnitines. As the inborn defect, systemic carnitine deficiency may exhibit symptoms like those of Reye's syndrome, it may be speculated whether functional carnitine deficiency in patients with accumulated acyl-CoA is another causal factor in the development of the symptoms during attacks.

Since the first report of patients with saturated C_6–C_{10}-dicarboxylic aciduria due to possible β-oxidation defect (13), several patients with similar clinical and biochemical characteristics have been described (15, 25, 27, 36). All the patients reported have had one or more acute attacks, most frequently characterized by a Reye-like syndrome (37) with vomiting and increasing lethargy that eventually leads to coma. Biochemical characteristics includes metabolic acidosis, hypoglycemia, fatty infiltration of the liver, and large amounts of the C_6–C_{10}-dicarboxylic acids, *i.e.*, adipic, suberic, and sebacic acids in the urine. In spite of the similarities between the clinical pictures during the acute attacks in these patients, the biochemical presentation exhibited a high degree of heterogeneity within the group. The precise location of the enzyme defect has not been found in any of the patients, but preliminary enzyme studies and the excretion pattern of organic acids suggest that the defect in the patients described by us (15), by Truscott and coworkers (36) and by Naylor and coworkers (27) is localized to the dehydrogenation of medium-chain fatty acids, whereas the defect in the patient reported by Mantagos *et al.* (25) is restricted predominantly to the dehydrogenation of the short-chain fatty acids. During the attack the case described here exhibited clinical symptoms very similar to the above-mentioned cases including large amounts of C_6–C_{10}-dicarboxylic acids in the urine. Unlike the other cases, this patient excreted substantial amounts of these metabolites together with 3-OH-butyric acid in the habitual condition. We are, therefore, possibly dealing with a new inborn error of the β-oxidation of fatty acids. The aim of the present report is to characterize the disease clinically and biochemically and to describe the first successful attempt to treat one of these diseases with vitamins. Both clinically and biochemically he responded positively to riboflavin treatment, and as a result the boy is now receiving permanent riboflavin medication.

MATERIALS AND METHODS

Family history. The patient (P.J.) is a male, now aged 5 years and 6 months. A brother, (J.J.), who is 10 years older had an attack of Reye's syndrome, verified by liver biopsy, when he was 2 years old. He suffered from a slight hypotonia during the following 2 years, but since then he has been clinically normal. In the routine screening of the family members, a slight organic aciduria was found in J.J. (Table 1) indicating a β-oxidation defect (see below).

The patient (P.J.) has another older healthy brother (B.J.) and a twin sister (S.J.) with prurigo besnier, who is otherwise clinically healthy. The mother suffer from rheumatoid arthritis. The father is healthy.

Case history. The patient (P.J.) was born 2 wk before term after an uncomplicated pregnancy. Birthweight was 2050 g. In the neonatal period, he was once admitted to a hospital with pneumonia and slight icterus. During early childhood, he suffered from

Table 1. The urinary excretion of organic acids from the patient (P.J.) during the attack and in the habitual condition, and from his family (μmole/mmole creatinine)

	Patient (P.J.)			Brother J.J.	Brother B.J.	Sister S.J.	Mother I.J.	Father H.C.J.	
	Attack	Habitual							
	Aug. 24, 1979	Feb. 28, 1980	May 13, 1980	May 13, 1980	Sept. 16, 1979	Sept. 16, 1979	Sept. 16, 1979	Sept. 16, 1979	Control[f]
Adipic acid[c]	3970	520	420	51	2	10	5	2	2–12
Suberic acid[c]	440	89	96	21	1	4	2	1	1–6
Sebacic acid[c]	530	23	57	11	nd[1]	2	1	nd	1–4
Unsaturated Suberic acid[a]	510	65	66	21	ND[2]	ND	ND	ND	ND
Butyrylglycine[b]	nd	nd	2	nd	nd	nd	nd	nd	nd
Caproylglycine[b]	70	110	140	20	nd	6	nd	nd	nd
Caprylylglycine[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caprylglycine[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-OH-caproic acid[d]	390	215	260	6	5	4	7	6	5–8
Ethylmalonic acid[d]	9	45	93	139	1	7	6	2	1–6
Methylsuccinic acid[d]	8	32	45	30	2	5	3	1	2–3
Suberylglycine[e]		ND	ND						ND
Isovalerylglycine[b]	5	21	17	3	nd	1	1	nd	nd
3-OH-isovaleric acid[a]	38	76	85	24	ND	12	ND	ND	10–13
Isobutyrylglycine[b]		34	26	9	nd	nd	nd	nd	nd–2
2-Me-butyrylglycine[b]		1	3	nd	nd	nd	nd	nd	nd
Glutaric acid[a]	360	28	81	24	ND	ND	ND	ND	ND
Lactic acid[a]	85	24	256	ND	18	18	15	15	20–60
3-OH-butyric acid[a]	475	173	690	65	13	23	8	25	70–120

[1] nd, not detectable, limit of detection 1 μmole/mmole creatinine.
[2] ND, not detectable, limit of detection 10 μmole/mmole creatinine.
[a] Quantitated by GC with 2-OH-caproic acid as the internal standard.
[b] Quantitated by SIM (CI) with heptanoylglycine as the internal standard.
[c] Quantitated by SIM (EI) with diethylglutaric acid as the internal standard.
[d] Quantitated by SIM (EI) with 2-OH-caproic acid as the internal standard.
[e] Judged from metylated metabolic profile.
[f] Four children 4–9 years.

frequent upper respiratory tract infections. One year before the present admission he had an episode of vomiting, lethargy, and fever. He recovered without treatment. Physically he was less developed than his twin sister. He suffered from a slight reduction in muscle strength and muscle tonus and he was 16 months old when he first walked alone. At the age of 2 years and 8 months he was admitted to the hospital after 2 wk of weariness, loss of appetite, and occassional vomiting. The last 3 days before admission his clinical condition deteriorated with frequent attacks of vomiting. His temperature was 38°C. At admission (August 22, 1979), he was drowsy, with normal respiration and lightly infected in the respiratory tract. Hepatomegaly was not present. Plasma glucose and pH were normal (Table 2). He was treated with i.v. glucose and electrolytes. The following day his condition deteriorated. He became more drowsy and developed Cheyne-Stokes respiration. His temperature was normal. Blood glucose was decreasing and a metabolic acidosis developed. Spinal glucose was low. The liver was not enlarged but the activity of liver enzymes in serum was very high (Table 2). Two days after admission (August 24, 1979) he was very lethargic and difficult to contact. Hepatomegaly was now pronounced with 100-fold increased liver enzymes. Consent for liver biopsy was not given by the parents. He suffered now from hypoglycemia and the metabolic acidosis was not fully compensated. Plasma ammonia concentration was just below the upper control limit (Table 2), and he was still undergoing treatment with glucose and electrolytes. The following day (August 25, 1979), his condition improved (Table 2). The hepatomegaly gradually decreased, but 4 wk later when he was discharged, the liver could still be palpated 7 cm below the right curvature. As before admission he suffered from a slight to moderate muscle hypotonia, but creatine kinase was within control limits, as it was during the crisis.

Liver enzymes were normalized. One wk after the crisis the ammonia level was 37 μmole/liter. One month after discharge, the clinical condition was satisfactory, although he had reduced muscle strength, was slightly hypotonic and had slight hepatomegaly.

Before the trials with riboflavin (100 mg 3 times a day) thorough clinical investigations secured the fact that the patient was in his habitual condition. At the time of the first trial the habitual condition was characterized by a slight hypotonia, but no hepatomegaly. The level of aminotransferases were slightly above controls and a slight metabolic acidosis existed (February 28, 1980 in Table 2). After both trials, the riboflavin treatment was terminated.

The patient had a second attack of drowsiness and vomiting (July, 28, 1980). On admission, he was not hypoglycemic and metabolic acidosis was not present, but the liver was enlarged (4 cm below the curvature) and liver enzymes concentration were increased (Table 2). Riboflavin treatment was instituted at once. Within 1 wk, the patient was in a satisfactory condition again (Table 2), without having gone through lethargic and hypoglycemic stages. One and one-half months later *i.e.*, after 1½ month of riboflavin treatment, he was in a very good condition (Table 2). Since this last attack, the patient has been treated with 100 mg riboflavin 3 times a day, except for a short period in January 1981. After 1 wk without riboflavin the patient started vomiting, whereafter riboflavin treatment was instituted again. A few days later he was free of symptoms.

During the riboflavin treatment the patient has been in a satisfactory clinical condition and is now developing normally; however, occassionally he has been fatigued and has lost his appetite. The hypotonia has decreased and the hepatomegaly has not been present since the second attack.

Biochemical methods. Blood glucose, standard bicarbonate, pH, base-excess, serum aspartate aminotransferase (ASAT) and serum alanine aminotransferase (ALAT) were analysed by means of standard methods. Amino acids in serum and urine were measured with an automatic amino acid analyzer (6). Free and esterified carnitines were determined by the carnitine acetyltransferase method described by McGarry and Foster (10). Organic acids, as trimethylsilylderivatives, were determined by gas chromatography

Table 2. *Laboratory values for P.J. during the attack, in the habitual condition and in a mild relapse period*

	Attack				Habitual	Relapse	Habitual	95% reference intervals
	Aug. 22, 1979	Aug. 23, 1979	Aug. 24, 1979	Aug. 25, 1979	Feb. 28, 1980	July 28, 1980	Aug. 4, 1980	Sept. 15, 1980	
Plasma glucose	4.4	3.3	1.7	4.4		3.3	5.0	4.1	3.3–5.6 mmole/liter
Spinal glucose		1.4							2.8–4.4 mmole/liter
Serum aspartate aminotransferase		4560	4680		57	960	125	48	10–40 U/liter
Serum alanine aminotransferase			3060		42				10–40 U/liter
Blood pH	7.35	7.37	7.32	7.35	7.31	7.36	7.37	7.39	7.36–7.42
Base excess	−4.2	−6.6	−8.3	−6.8	−8.6	−2.8	−1.8	−0.4	−3 to +3
Serum bicarbonate		18.9	17.6	18.8	17.4	22.0	22.9	23.9	21.3–25.8 mmole/liter
Plasma ammonia			75						12–76

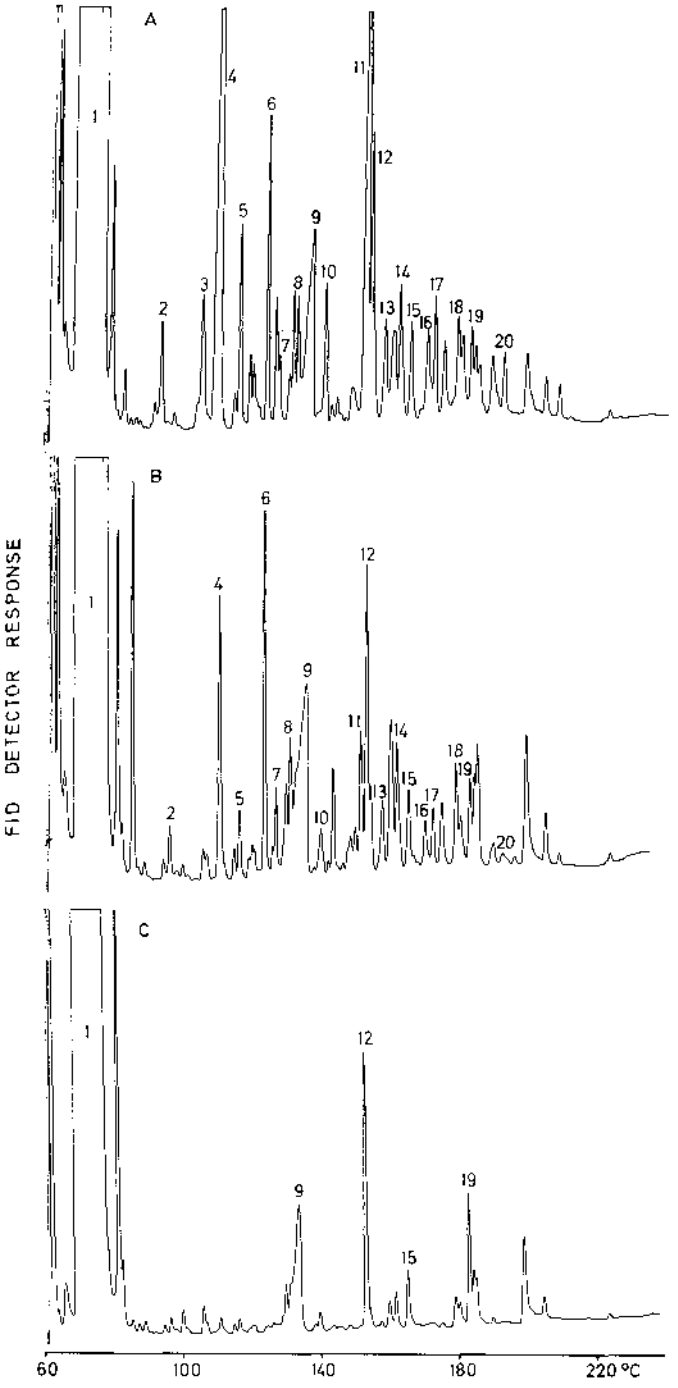

Fig. 1. Gaschromatograms (Flame ionisation detector response) of hydroxylamine-treated and silylated extracts from the urine of: (*A*) the patient in the habitual condition before riboflavin treatment (130580); (*B*) the patient during the second riboflavin trial (290580); and (*C*) a child without metabolic disorders. The column was a 180 cm × 3 mm (i.d.) glass coil packed with 3% Dexsil 300 on Chromosorb W-HP, programmed from 60°C at 4°/min. Helium carrier flow-rate was 20 ml/min. The peaks are: (1) solvent; (2) lactic acid; (3) 2-OH-butyric acid; (4) 3-OH-butyric acid; (5) 3-OH-isovaleric acid; (6) 2-OH-caproic acid (internal standard); (7) ethylmalonic acid; (8) 2-oxo-caproic acid (internal standard); (9) urea + methylsuccinic acid + 5-OH-caproic acid; (10) glutaric acid; (11) adipic acid; (12) phenylbutyric acid (internal standard); (13) 2-OH-glutaric acid; (14) 2-oxo-glutaric acid; (15) 4-OH-phenylacetic acid; (16) unsaturated suberic acid; (17) suberic acid; (18) aconitic acid; (19) citric acid; and (20) sebacic acid.

and gas chromatography/mass spectrometry (Selected Ion Monitoring) by means of methods described previously (15), except for the acylglycines, which were analysed as the methylesters by chemical ionisation selected ion monitoring. The monitored ions were for all the compounds, including the internal standard (heptanoylglycine), M + 1. The calibration curves were linear from 0–150 μmole/mmole creatinine, with a coefficient of variation of 10–15%.

In vitro studies on the β-oxidation of fatty acids were performed on cultured fibroblasts from the patient (P.J.), his brother (J.J.) and from six control children (aged 1–9 years). The fibroblasts were grown in Eagels medium containing 10% human serum. Oxidation of 1-[^{14}C]-palmitic acid and U-[^{14}C]-palmitic acid was measured by incubating the PBS (Phosphate Buffer Saline, Gibco) buffer-washed cells in monolayers (200–400 μg protein) in 1.5 ml PBS containing 40 μM 1-[^{14}C]-palmitic acid (specific activity 10 μCi/mmole) or U-[^{14}C]-palmitic acid (specific activity 10 μCi/mmole). After 45 min at 37°C, the reaction was terminated by addition of 200 μl citrate buffer (0.08 M citrate, 0.05 M phosphate, pH 2). The [$^{14}CO_2$] evolved was trapped on Protosol-coated filter paper using the method described by Gliemann (12) and the [^{14}C]-labeled perchloric acid soluble reaction products (SCFA) were isolated according to the technique of van Hinsbergh and coworkers (21). The [^{14}C]-activity was measured in a liquid scintillation counter.

RESULTS

Amino acids. During the first and most serious acute attack, metabolic profiles of amino acids in serum and urine and organic acids in urine were investigated. Amino acids in serum were within control limits except for phenylalanine, which was slightly above upper control range. Taurine and ethanolamine were excreted in urine in elevated amounts, whereas all other amino acids were within control range.

Organic acids. The urinary metabolic profile of organic acids was very disturbed during the attack (Table 1). Identities of the various compounds were established by means of gas-chromatography and mass spectrometry. Compounds related to the β-oxidation of fatty acids were especially elevated. These were adipic acid, suberic acid, unsaturated suberic acid, sebacic acid, 5-OH-caproic acid, and caproylglycine. Ethylmalonic acid and methylsuccinic acid were also excreted in enhanced amounts. The branched-chain amino acid-derived metabolites, isovalerylglycine and 3-OH-isovaleric acid, were excreted in slightly elevated quantities. Glutaric acid, a metabolite of the lysine catabolism, was also excreted in excessive amounts. From the elevated excretion of 3-OH-butyric acid, it appears that the slight metabolic acidosis (Table 2) is accompanied by ketosis. It should be mentioned that lactic aciduria was not pronounced.

During the months that followed the acute attack, several urine samples were collected for organic acid profile analyses. Two such organic acid profiles from 24-h urine samples, which also served as pretreatment profiles in the riboflavin medication trials (see below), are shown in Table 1 (Feb. 28, 1980 and May 13, 1980). The gas chromatographic representation of one of these is depicted in Fig. 1A. The excretion pattern of organic acids during these clinically quiet periods (the habitual condition) is qualitatively the same as the one during the acute attack. In these urine samples, isobutyrylglycine and 2-Me-butyrylglycine were also measured and found to be significantly elevated (Table 1). The excreted amounts of the three dicarboxylic acids-adipic, suberic, and sebacic acids-were 5–10 times higher during the attack than in the habitual condition; caproylglycine and 5-OH-caproic acid were only a little higher; and ethylmalonic acid and methylsuccinic acid were present in even smaller amounts during the attack than during the habitual condition. It is noteworthy that the C_8–C_{10}-acylglycines, caprylylglycine and caprylglycine, were not excreted in detectable amounts either during the attack nor in the habitual condition. It is further noteworthy that the habitual condition at Feb. 28, 1980 was characterized by a slight metabolic acidosis with ketosis and slight elevations in serum concentration of liver enzymes.

Riboflavin treatment. Because of the possibility of an acyl-CoA dehydrogenation defect as the underlying metabolic error in the patient, riboflavin, the precursor of the coenzyme flavineadeni-

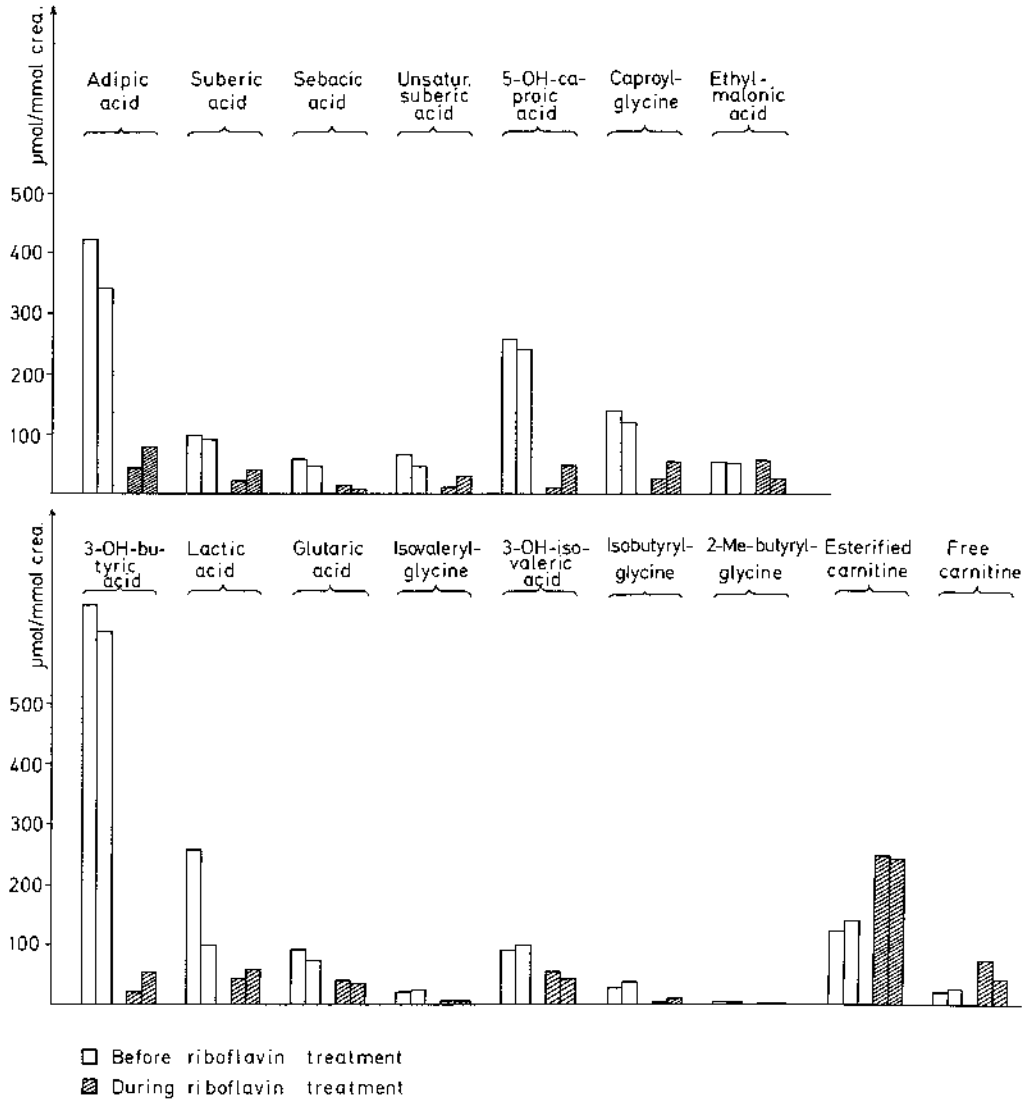

Fig. 2. Urinary excretions of organic acids together with free and esterified carnitine in 24-h urines before (130580 and 140580, *white bars*) and during riboflavin treatment (290580 and 300580, *hatched bars*).

nedinucleotide (FAD) for the acyl-CoA dehydrogenation enzymes (see below), was given during two periods separated by 2 months. At both occasions the amounts of the various metabolites in two 24-h urine samples collected during the wk before the riboflavin medication was defined as the habitual condition [Fig. 1A and Fig. 2 (open bars)]. The urine samples analyzed during the trials represented two 24-h urines collected 1 wk after the medication was started (Fig. 1B and Fig. 2 (hatched bars)). During both periods, the improvement of the metabolic profiles was significant compared to those in the habitual condition. The improvement can be substantiated as follows: (1) judged from the excretion of 3-OH-butyric acid, ketosis disappeared; (2) caproylglycine, apparently an indicator of fatty acid β-oxidation blockage (see discussion), was excreted in far lower amounts during the treatment than before; (3) excretion of the dicarboxylic acid, adipic acid and the ω-1-OH-acid, 5-OH-caproic acid was far lower during the treatment; (4) the urinary excretion of the other dicarboxylic acids, suberic, sebacic, and unsaturated suberic acids, also diminished during treatment; (5) the representatives for the branched-chain amino acid metabolism, isovalerylglycine, 3-OH-isovaleric acid, isobutyrylglycine, and 2-Me-butyrylglycine also diminished; (6) the same is true for the lysine metabolite, glutaric acid; (7) from these measurements of ethylmalonic acid, it is not possible to decide whether the excretion of this compound diminished during treatment; and (8) judging from lactic acid excretion, the lactic acidosis, which was not very pronounced in the habitual condition, also seems to have diminished during treatment.

Family studies of organic acid profiles. The metabolic profiles of organic acids from two brothers (J.J. and B.J.), one twin sister (S.J.) and from the parents (H.C.J. and I.J.) were investigated. The profile from one of the brothers (J.J.) was slightly abnormal, characterized by the same metabolites as those found in the patient (Table 1). Except for ethylmalonic acid, the excreted amounts of the unusual metabolites were small, but significantly elevated. The other family members exibited normal profiles of organic acids (Table 1).

Carnitine measurements. Very late during the present study, we were able to determine carnitine in plasma and urine. At that time only one plasma sample from the period before the permanent riboflavin medication was available. This was from the second attack just before the institution of the permanent treatment (July 28, 1980). The sample is therefore presumably representative of carnitine concentrations during the initial phase of an acute attack. The results are shown in Table 3. Free carnitine in the plasma was very low, whereas the total carnitine (free and esterified) was within the control range.

Urinary esterified carnitine was elevated both in a urine sample from September 13, 1979 and from the habitual condition previous to the last trial with riboflavin (Table 3 and Fig. 2). From the present results, it is not possible to decide whether free carnitine in the urine was significantly decreased. Riboflavin treatment had the effect of further elevating the excretion of esterified carnitine and significantly enhancing free carnitine excretion (Fig. 2).

Fibroblast enzyme studies. Table 4 shows the results from the

Table 3. *Serum and urine concentration of free and esterified carnitine in the patient*

	P-carnitine (μmole/liter)		U-carnitine (μmole/mole creatinine)			
	July 28, 1980		Sept. 13, 1979		May 13, 1980	
	Esterified	Free	Esterified	Free	Esterified	Free
P.J.	32	6	59	14	122	21
Control (five children)	17–33	26–74	23–30	24–42		

Table 4. *Production of CO_2 and short-chain fatty acids (SCFA, percloric acid soluble materials) in monolayers of cultured fibroblasts from 1-[^{14}C]-palmitic acid and U-[^{14}C]-palmitic acid (nmole/mg protein/45 min)*

	1-[^{14}C]-palmitic acid			U-[^{14}C]-palmitic acid		
	CO_2	SCFA[1]	Total	CO_2	SCFA[1]	Total
Patient P.J.	0.301	1.880	2.181	0.06	0.53	0.58
Brother J.J.	0.356	1.594	1.950	0.14	0.99	1.13
Controls (6 children 1–9 years) range	0.126–0.297	1.208–1.937	1.334–2.164	0.10–0.14	0.84–1.26	0.93–1.52

[1] SCFA, perchloric acid soluble reaction products.

investigation of β-oxidation of palmitic acid in cultured fibroblasts. The study of the oxidation of U-[^{14}C]-palmitic acid shows that the production rate of CO_2 and short-chain fatty acids (perchloric acid soluble acids) in the patient's (P.J.) fibroblasts was approximately 50% of that of the rate found in six control cultures. Thus, the β-oxidation pathway is impaired in the patient. The normal productions of CO_2 and short-chain fatty acids from 1-[^{14}C]-palmitic acid shows that the impairment of the β-oxidation is not caused by a defect in the transport of fatty acids through the mitochondrial membrane and/or probably not by a serious defect in the dehydrogenation of long-chain fatty acids (see "Discussion").

DISCUSSION

Reye's syndrome is a well-defined clinico-pathologic disease entity (29, 35, 37); however, considerable variation exists in the presentation and severity of the disease. It is generally accepted that the syndrome may not represent the manifestation of a single ethiologic factor (29, 35).

The Reye's syndrome-like presentation of the present patient, in whom we have documented a β-oxidation defect, suggests an impairement of the β-oxidation as a possible ethiologic factor in Reye's syndrome. The same causal relationship is indicated in Jamaican Vomiting Sickness (33), which shows symptoms similar to Reye's Syndrome and is caused by the inhibition of the fatty acid oxidation by a metabolite of Hypoglycin-A, and in two recently published cases of Reye's syndrome with C_6–C_{10}-dicarboxylic aciduria due to a possible inhibition of the β-oxidation by a toxin similar to Hypoglycin-A (4).

Another ethilogic factor in Reye's syndrome seems to be carnitine deficiency. Some recent cases of the syndrome have been diagnosed as systemic carnitine deficiencies (5, 11), a condition that causes impaired oxidation of long-chain fatty acids at the level of carnitine-dependant transport through the mitochondrial membrane (9). Both β-oxidation impairment and carnitine deficiency, (judged from the low carnitine level in plasma) were found in the present patient. The studies of fibroblast enzymes indicate very strongly that the patient suffer from a β-oxidation defect. The indicated carnitine deficiency is therefore most probably secondary. The finding of caproylglycine and ethylmalonic acid in the urine from the patient further indicates that the defect is localized to the dehydrogenation of the medium- and short-chain acyl-CoA, because these two compounds can be derived from accumulated caproyl-CoA and butyryl-CoA, respectively (20, 24) (Fig. 3). This is supported by the finding of the same two compounds in urine from patients with Jamaican Vomiting Sickness (33), in whom it is known that the acyl-CoA dehydrogenases are inhibited by methylenecyclopropylacetyl-CoA, a metabolite of Hypoglycin-A (28). The excretion of isovalerylglycine, 3-OH-isovaleric acid, isobutyrylglycine, 2-Me-butyrylglycine and glutaric acid shows that the acyl-CoA dehydrogenation defect in the patient is not isolated to the dehydrogenation of fatty acids (Fig. 3). On the contrary all the known acyl-CoA dehydrogenation processes are affected, except for the long-chain acyl-CoA dehydrogenation, judged from the normal production of [$^{14}CO_2$] and perchloric acid soluble compounds in fibroblasts incubated with 1-[^{14}C]-palmitic acid. This result must be evaluated with caution, because the further oxidation of 1-[^{14}C]-palmitic acid after the liberation of the first radioactive acetyl-CoA–in contrast to that in the controls–is impaired by the medium- and short-chain acyl-CoA dehydrogenation defect. A defect in the dehydrogenation of long-chain acyl-CoA cannot at present be excluded; however, a serious reduction of long-chain acyl-CoA dehydrogenation would show up in this assay. Fibroblasts from a patient with glutaric aciduria type II (16) showed only 20% of control ability to produce [$^{14}CO_2$] and radioactive perchloric acid soluble compounds from 1-[^{14}C]-palmitic acid (7). This patient suffers from a defect in all the acyl-CoA dehydrogenation processes, localized to the common electron-transport system from acyl-CoA dehydrogenases to the electron transport chain (7). This means that a possible defect of the long-chain acyl-CoA dehydrogenation in the present patient cannot be serious and that the common factor indicated to be defective, must effect the various acyl-CoA dehydrogenation system in a different way.

The acyl-CoA dehydrogenation systems is comprised of acyl-CoA dehydrogenases, of which there exist several specific enzymes (1, 19), electron-transfer flavoprotein (EFT) (18) and electron-transfer flavoprotein dehydrogenase (ETF DH) (31), both of which are common electron transporters for all the acyl-CoA dehydrogenation systems. A defect in either ETF or ETF DH cannot be excluded at present, but the low oxidation rate of 1-[^{14}C]-palmitic acid in the fibroblasts from the above mentioned patient with a defect in ETF or ETF DH, contraindicates these possibilities. The significant biochemical and clinical improvements during the riboflavin medication periods point, however, to a defect that can be partly repaired by increasing the intracellular concentration of FAD. Riboflavin is a precursor for FAD, which is a coenzyme for acyl-CoA dehydrogenase, ETF and ETF DH, and therefore also a common factor in the acyl-CoA dehydrogen-

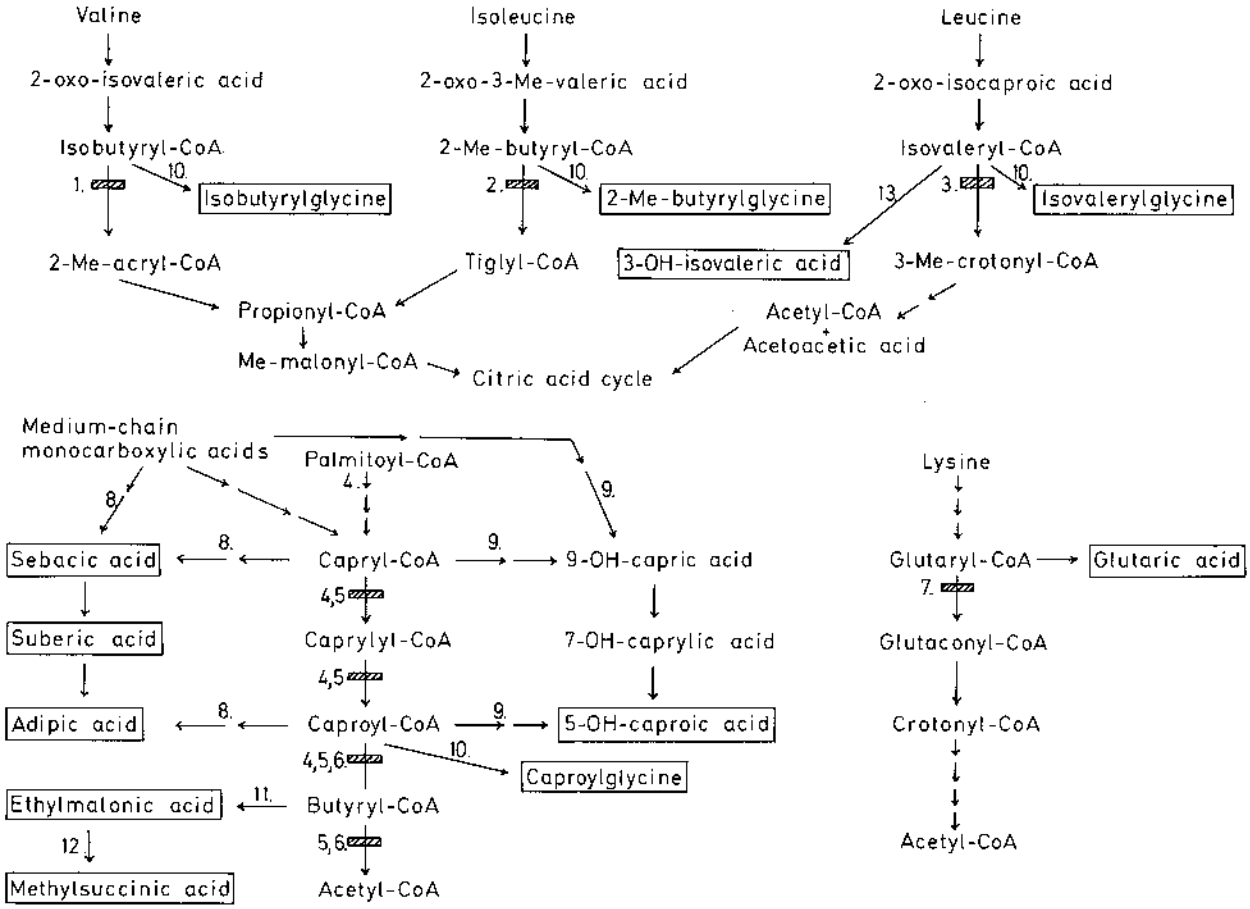

Fig. 3. The proposed defective intermediate metabolism in the patient. The defective steps are marked with hatched squares: (1, 2, 6) short-chain acyl-CoA dehydrogenation. At present it is not known if the dehydrogenases involved in these steps are different enzymes, (3) isovaleryl-CoA dehydrogenation, (4) long-chain acyl-CoA dehydrogenation, (5) general acyl-CoA dehydrogenation, and (7) glutaryl-CoA dehydrogenation. The compounds found elevated in the urine from the patient are marked with squares. The enzymes indicated to be involved in the production of these compounds: (8), ω-oxidation enzymes, (9) ω-1-oxidation enzymes, (10) glycine-N-acylase, (11) propionyl-CoA carboxylase, (12) methylmalonic acid mutase, and (13) not known at present.

ation processes. From measurements in liver mitochondria from riboflavin deficient rats, which have been found to exhibit an organic aciduria very similar to the one presented here (17), it is known that buturyl-CoA dehydrogenase is affected more than palmitoyl-CoA dehydrogenase by the resulting FAD deficiency (17, 23). A defect related to the synthesis and transport of FAD or a binding defect of FAD to one or more of the enzymes of the acyl-CoA dehydrogenation systems in the patient may therefore be compatible with the fibroblasts studies and with the excretion pattern of organic acids in the urine. The case described by Mantagos and coworkers (25) exhibited biochemical characteristics similar to those in the case presented here. In fibroblasts from Mantagos' patient, butyric acid oxidation was decreased (25), eventually caused by decreased activity of butyryl-CoA dehydrogenase, which in an assay without added FAD was low (50% of controls) (30). This might be compatible with an FAD related defect of acyl-CoA dehydrogenases, a situation we have proposed as one of the possibilities in the present patient. Before more studies are performed at the enzymatic level in both patients it is not possible to decide whether we are dealing with two disease entities or variants of the same molecular disease.

The brother, (J.J.), to the present patient most probably suffers from the same defect of the acyl-CoA dehydrogenation. This is indicated by urinary excretion of the unusual metabolites, and by the diminuation of the amounts of unusual metabolites after a riboflavin medication trial identical to those discribed for P.J. (results not shown). It was, however, not possible to detect the enzymatic defect in the enzyme assay and the boy has been asymptomatic for 10 years after his first and only attack and is still in good clinical condition. Thus the consequenses of the defect are not as serious as those in his brother. At present nothing can be said about the reason for this.

The low level of carnitine in plasma from P.J. and the elevated urinary excretion of esterified carnitine may be explained as follows. The equilibrium constant between short-chain acyl-CoA and acyl-carnitines have been measured *in vitro* to be in the order of 1 (3). The accumulation of acyl-CoA in the cells of the patient may therefore result in accumulation of acyl-carnitines, which are excreted in the urine and thereby cause functional carnitine deficiency. The further enhancement of the urinary excretion of both esterified carnitines and of free carnitine during the riboflavin treatment is at present unexplained; however, the derangement of the carnitine and acyl-carnitine metabolism in this patient is presently under investigation.

The biosynthetic pathway for the production of adipic, suberic, and sebacic acids in patients with C_6–C_{10}-dicarboxylic aciduria has not yet been fully elucidated. Results obtained recently from rats showed that the precursors of dicarboxylic acids are most probably medium-chain monocarboxylic acids (26). If these results are relevant to humans, this means that the pathway for production of sebacic acid in the patient is most probably ω-oxidation of medium-chain monocarboxylic acids, *e.g.*, capric acid (Fig. 3), accumulated because of the enzyme deficiency at medium-chain level. The urinary excretion of caproylglycine indicate that caproyl-CoA is accumulated in substantial amounts, and direct ω-oxidation of caproic acid to adipic acid may therefore take place. The main production pathway for adipic acid and also for suberic acid is probably β-oxidation of sebacic acid (Fig. 3) because the

affinity of the ω-oxidation system towards capric and caprylic acid is low (26). The mechanism for the synthesis of 5-OH-caproic acid is possibly the same as that for adipic acid, because the ω-1-oxidation of monocarboxylic acids most probably parallels that of the ω-oxidation qualitatively (2, 8) (Fig. 3). The elevated excretion of 3-OH-butyric acid, indicating ketosis, was unexpected in this patients with defective β-oxidation. One possible explanation, however, might be that the accumulated isovaleryl-CoA, indicated by the excretion of isovalerylglycine and 3-OH-isovaleric acid, inhibits the citric acid cycle by inhibiting 2-keto-glutarate oxidation (14), thus channeling acetyl-CoA preferentially towards ketone body production.

The enzymatic investigations and the urinary profile data suggest–in addition to isovaleryl-CoA–accumulation of isobutyryl-CoA, 2-Me-butyryl-CoA, and possibly of higher straight-chained acyl-CoA intracellularly. Some of these acyl-CoA thioesters have been shown to be toxic both *in vivo* (32, 34) and *in vitro* (22). It is therefore highly probable that the mechanism underlying the Reye-like pathophysiologic manifestation in the patient directly or indirectly are connected to the accumulation of these acids or their coenzyme-A derivatives.

REFERENCES AND NOTES

1. Besrat, A., Polan, C. E., and Henderson, L. M.: Mammalian metabolism of glutaric acid. J. Biol. Chem., *244:* 1461 (1969).
2. Björkhem, I. and Danielsson, H.: ω- and ω-1-oxidation of fatty acids by rat liver microsomes. Eur. J. Biochem., *17:* 450 (1970).
3. Bremer, J.: Pyruvate dehydrogenase, substrate specificity and product inhibition. Eur. J. Biochem., *8:* 535 (1969).
4. Chalmers, R. A., Lawson, A. M., Whitelow, A., and Purkiss, P.: Twin siblings with a Reye's-like Syndrome associated with an abnormal organic aciduria, hypoglycemia, diarrhea and vomiting with close similarities to Jamaican Vomiting Sickness. Pediatr. Res., *14:* 1097 (1980).
5. Chapoy, P. R., Angelini, C., Brown, W. J., Stiff, J. E., Shug, A. L., and Cederbaum, S. D.: Systemic carnitine deficiency–A treatable inherited lipid-storage disease presenting as Reye's syndrome. N. Engl. J. Med., *303:* 1389 (1980).
6. Christensen, E. and Hertel, J.: Amino acid analysis with fluorimetric detection in the investigation of inborn errors of metabolism. In: J. M. Rattenburny, Ed.: Amino acid analysis. (Ellis Horwood Ltd., Chichester, England, 1981).
7. Christensen, E., Kølvraa, S., and Gregersen, N. Unpublished results.
8. Ellin, Å., Orrenius, S., Pilotti, Å., and Swahn, C. G.: Cytochrome P-450 of rat kidney cortex microsomes: Further studies on its interaction with fatty acids. Arch. Biochem. Biophys., *158:* 597 (1973).
9. Fritz, I. B. and Yue, K. T. N.: Long chain carnitine acyl transferase and the role of acylcarnitine derivatives in the catalytic increase of long chain fatty acid oxidation. J. Lipid Res., *4:* 279 (1963).
10. McGarry, J. D. and Foster, D. W.: An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J. Lipid Res., *17:* 277 (1976).
11. Glasgow, A. M., Eng, G., and Engel, A. G.: Systemic carnitine deficiency simulating recurrent Reye syndrome. J. Pediatr., *96:* 889 (1980).
12. Gliemann, J.: Insulin-like activity of diluted human serum assayed by an isolated adipose cell method. Diabetes, *14:* 643 (1965).
13. Gregersen, N., Lauritzen, R., and Rasmussen, K.: Suberylglycine excretion in the urine from a patient with dicarboxylic aciduria. Clin. Chim. Acta, *70:* 417 (1976).
14. Gregersen, N.: Studies on the effect of saturated and unsaturated short-chain monocarboxylic acids on the energy metabolism of rat liver mitochondria. Pediatr. Res., *13:* 1227 (1979).
15. Gregersen, N., Rosleff, F., Kølvraa, S., Hobolth, N., Rasmussen, K. and Lauritzen, R.: Non-ketotic C_6-C_{10}-dicarboxylic aciduria: Biochemical investigation of two cases. Clin. Chim. Acta, *102:* 179 (1980).
16. Gregersen, N., Kølvraa, S., Rasmussen, K., Christensen, E., Brandt, N. J., Ebbesen, F., and Hansen, F. H.: Biochemical studies in a patient with defects in the metabolism of acyl-CoA and sarcosine: Another possible case of glutaric aciduria type II. J. Inher. Metab. Dis., *3:* 67 (1980).
17. Gregersen, N. and Kølvraa, S.: The occurrence of C_6–C_{10}-dicarboxylic acids, ethylmalonic acid, 5-OH-caproic acid, butyrylglycine, caproylglycine, isovalerylglycine, isobutyrylglycine, 2-Me-butyrylglycine and glutaric acid in the urine of riboflavin deficient rats. J. Inher. Metab. Dis., Vol. 5 (1982).
18. Hall, C. L. and Kamin, H.: The purification and some properties of electron transfer flavoprotein and general fatty acyl-CoA dehydrogenase from pig liver mitochondria. J. Biol. Chem., *250:* 3476 (1975).
19. Hall, C. L.: Acyl-CoA dehydrogenases from pig liver mitochondria. In: J. M. Lowenstein, Ed.: Methods in enzymology, Vol. 71, p. 375 (Academic Press, New York, 1981).
20. Hegre, C. S., Halenz, D. R., and Lane, M. D.: The enzymatic carboxylation of butyryl coenzyme A. J. Am. Chem. Soc., *81:* 6526 (1959).
21. van Hinsbergh, V. W. M., Veerkamp, J. H., and van Moerkerk, H. T. B.: An accurate and sensitive assay of long-chain fatty acid oxidation in human skeletal muscle. Biochem. Med., *20:* 256 (1978).
22. Hird, F. J. R. and Weidemann, M. J.: Oxidative phosphorylation accompanying oxidation of fatty acids by rat liver mitochondria. Biochem. J., *98:* 378 (1966).
23. Hoppel, C., DiMarco, J. P., and Tandler, B.: Riboflavin and Rat hepatic cell structure and function. J. Biol. Chem., *254:* 4164 (1979).
24. Kølvraa, S. and Gregersen, N.: Unpublished results.
25. Mantagos, S., Genel, M., and Tanaka, K.: Ethylmalonic-adipic aciduria: In vivo and in vitro studies indicating deficiency of activities of multiple acyl-CoA dehydrogenases. J. Clin. Invest., *64:* 1580 (1979).
26. Mortensen, P. B. and Gregersen, N.: The biological origin of ketotic dicarboxylic aciduria: In vivo and in vitro investigations of the ω-oxidation of C_6–C_{16}-monocarbocylic acids in unstarved, starved and diabetic rats. Biochem. Biophys. Acta, *666:* 394 (1981).
27. Naylor, E. W., Mosovich, L. L., Guthrie, R., Evans, J. E., and Tieckelmann, H.: Intermittent non-ketotic dicarboxylic aciduria in two siblings with hypoglycaemia: An apparent defect in β-oxidation of fatty acids. J. Inher. Metab. Dis., *3:* 19 (1980).
28. Osmundsen, H. and Sherratt, H. S. A.: The effect of pent-4-enoate and methylenecyclopropylacetate on some enzymes of β-oxidation in extracts of liver mitochondria. Biochem. Soc. Trans., *3:* 330 (1975).
29. Reye, R. D. K., Morgan, G., and Baral, J.: Encephalopathy and fatty degeneration of the viscera: a disease entity in childhood. Lancet *2:* 749 (1963).
30. Rhead, W., Mantagos, S., and Tanaka, K.: Glutaric aciduria type II: In vitro studies on substrate oxidation, acyl-CoA dehydrogenases, and electron transferring flavoprotein in cultured skin fibroblasts. Pediatr. Res., *14:* 1339 (1980).
31. Ruzicka, F. and Beinart, H.: A new ironsulphur flavoprotein of the respiratory chain. J. Biol. Chem., *252:* 8440 (1977).
32. Samson, F. E., Dahl, N., and Dahl, D. R.: A study on the narcotic action of the short chain fatty acids. J. Clin. Invest., *35:* 1291 (1956).
33. Tanaka, K., Kean, E. A., and Johnson, B.: Jamaican vomiting Sickness: Biochemical investigation of two cases. N. Engl. J. Med., *295:* 461 (1976).
34. Teychenne, P. F., Walters, I., Claveria, L. E., Calne, D. B., Price, J., MacGilivary, B. B., and Gompertz, D.: The encephalopathic action of five-carbon-atom fatty acids in the rabbit. Clin. Sci. Mol. Med., *50:* 463 (1976).
35. Thaler, M. M.: Clinical and enzymatic indices of hepatic dysfunction in Reye's syndrome. In: J. F. S. Crocker, Ed.: Reye's Syndrome II, p. 115 (Grune and Stratton, Inc., New York, 1979).
36. Truscott, R. J. W., Hick, L., Pullin, C., Halpern, B., Wilcken, B., Griffiths, H., Silink, M., Kilham, H., and Grunseit, F.: Dicarboxylic aciduria: Responce to fasting. Clin. Chim. Acta, *94:* 31 (1979).
37. DeVivo, D. C. and Keating, J. P.: Reye's syndrome. Adv. Pediatr., *22:* 175 (1976).

38. The authors thank Mrs. Vibeke Winter, Anne Marie Holm and Inga Knudsen for skilled technical assistance.
39. The work was supported by grants from The Danish Medical Research Council.

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41. Received for publication February 9, 1982.
42. Accepted for publication April 21, 1982.

0031-3998/82/1610-0861$02.00/0