Pediatr. Res. 17: 514–517 (1983)

Biochemical Studies in the Liver and Muscle of Patients with Zellweger Syndrome

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Summary

Biochemical studies have been performed in muscle, liver, leukocytes, and fibroblasts from patients suffering from the Zellweger syndrome. Oxidation rates of [1-^{14}C]pyruvate, [U-^{14}C]malate, and [1-^{14}C]2-oxoglutarate were strongly reduced in skeletal muscle homogenate. Oxygen consumption in isolated skeletal muscle mitochondria could only be stimulated by ADP in the presence of ascorbate and N,N,N^1,N^1-tetramethyl-*p*-phenylenediamine. Cytochrome contents in heart muscle and liver mitochondria were normal. A very low activity of succinate-ubiquinone oxidoreductase was found in liver homogenate of two patients. From the effect of 2-thenoyltrifluoroacetone on the succinate-phenazine methosulphate oxidoreductase activity, a nearly competitive inhibition with respect to phenazine methosulphate was demonstrated in contrast with a non-competitive inhibition in controls. Normal oxidation rate of [1-^{14}C]pyruvate and [2-^{14}C]pyruvate was found in leucocytes and fibroblasts. Lactate and pyruvate levels were normal in serum and cerebrospinal fluid and β-hydroxybutyrate and acetoacetate levels were normal in blood. The ratios lactate/pyruvate and β-hydroxybutyrate/acetoacetate were normal as well. These findings point to a defect in the electron transport chain at the succinate-ubiquinone oxidoreductase level. This defect might be related to the absence of peroxisomes in the cells of Zellweger patients.

Abbreviations

DCIP, dichlorophenol indophenol
PMS, phenazine methosulphate
TMPD, N,N,N^1,N^1-tetramethyl-*p*-phenylenediamine
TTFA, 2-thenoyltrifluoroacetone

A disturbance in the metabolism of pipecolic acid has been reported (14) in three patients suffering from Zellweger Syndrome. Zellweger syndrome is also characterized by an increased urinary excretion of trihydroxycoprostanic acid and dihydroxycoprostanic acid (6, 9).

Mitochondrial defect in the brain, liver, and kidney of patients with this Syndrome was first established by Goldfischer *et al.* (4). Oxygen consumption of mitochondrial preparations from brain and liver with succinate and with substrates reducing nicotinamide adenine dinucleotide was markedly diminished but normal oxygen consumption was found with ascorbate and with TMPD. The authors concluded that there is a defect in the electron transport chain before the step involving cytochromes.

A metabolic disorder similar to the Zellweger syndrome was described by Versmold *et al.* (15). No pipecolic acid excretion, however, was found at 11 months of age. Mitochondrial studies revealed normal succinate and glutamate oxidation and normal coupling of respiration to phosphorylation. A functional abnormality of cytochrome b was established in patient's liver mitochondria. Hepatocytes were lacking peroxisomes.

A deficiency of peroxisomes in hepatocytes and in renal proximal tubulus was demonstrated by Goldfischer *et al.* (4) in two patients suffering from the Zellweger syndrome. Similar findings were also reported by Brun *et al.* (2) and by Pfeifer and Sandhage (11) in hepatocytes.

In the present paper, results are reported on the oxygen consumption in skeletal muscle mitochondria of a Zellweger patient. Oxidation rates of several substrates are measured in muscle homogenate of one patient as well as in leukocytes and fibroblasts from several patients. Experimental results in liver tissue show a defect in the electron transport chain of mitochondria, probably due to a deficiency of peroxisomes resulting in a damage of the interaction of succinate dehydrogenase with ubiquinone.

Patients

Patients 1–3 refer to patients described in a previous study (14). Patient 4 is a recently discovered patient who was studied at the age of 2½ years. This patient has been diagnosed as a Zellweger patient on the ground of the typical clinical features and biochemical abnormalities such as disturbed metabolism of pipecolic acid and of bile acids (tri- and dihydroxycoprostanic acids), which are specific for the Zellweger Syndrome. Patient 5 is a patient from the department of Pediatrics, University Hospital, Amsterdam. Diagnosis was based on the same criteria as mentioned for patient 4; moreover, deficiency of peroxisomes was demonstrated in liver tissue by electronmicroscopic examination (Dr. Dingemans, University Hospital, Amsterdam, personal communication). Patient 6 is a patient from the department of Pediatrics, University of Munich, W. Germany. Like in patient 5, no peroxisomes could be demonstrated in liver cells.

MATERIALS AND METHODS

Biochemical studies were performed with liver (patients 3, 5, and 6), skeletal muscle tissue (patients 1, 3, and 4), heart muscle (patient 3), fibroblasts (patients 1 and 4), and leukocytes (patients 2, 3, and 4).

Cytochrome content was measured in mitochondria isolated from heart muscle and liver, which were obtained immediately after death (patient 3).

Oxygen consumption was measured in a fresh muscle biopsy specimen from patient 1 and oxidation rates of several substrates were determined in a fresh muscle biopsy specimen from patient 4. Oxidation rate of succinate and glutamate and malate was measured in a liver homogenate of patient 5 (biopsy specimen) and patient 6 (autopsy specimen).

Succinate dehydrogenase ubiquinone interaction was studied in a liver homogenate from patients 5 and 6. A needle biopsy was performed in patient 5 and the material was immediately frozen at −70°C during 5 days preceding analysis. The control for patient 5 was a patient who died at the age of 4 months due to heart disease. Liver specimen of this control was obtained 1.5 h after

death and thereafter frozen at −70°C for 3 months until the assay was performed. In patient 6 liver autopsy was carried out immediately after death and the liver specimen was stored for 6 months at −70°C. Thereafter homogenization was performed and the obtained homogenate was stored for 6 months at −25°C. The control for patient 6 was also a patient with heart disease. The liver autopsy was performed within 2 h after death. The liver specimen was immediately frozen at −70°C and stored for 4 months before the assay was performed.

Muscle and liver tissue from control patients were obtained at operation for other purposes. Informed consent was obtained from parents of all patients tested as well as from controls. Heart muscle and liver mitochondria were isolated as described previously (1, 18).

Skeletal muscle homogenate was incubated in sealed glass vials at 37°C in a shaking water-bath. The basic incubation medium contained the following in a final volume of 500 μl: 30 mM potassium phosphate, 1.8 mM EDTA, 2 mM ADP, 0.9 mM Tris, 5 mM $MgCl_2$, 75 mM KCl, 25 mM sucrose, 20 mM glucose, 1 unit hexokinase, heparin (5 unit/ml incubation mixture). The final pH was 7.4. Final concentrations of substrates were: [1-^{14}C]pyruvate, 1.0 mM (0.25 μCi); malate, 1 mM (in combination with [1-^{14}C] pyruvate); malonate, 5 mM; DL-carnitine, 5 mM; [U-^{14}C]malate, 1 mM (0.25 μCi); pyruvate, 1 mM (in combination with [U-^{14}C] malate); acetylcarnitine, 2 mM; arsenite, 2 mM; and [1-^{14}C]2-oxoglutarate, 1 mM (0.25 μCi).

Incubations were started by addition of muscle homogenate (0.15–0.30 mg protein). Small plastic tubes containing 200 μl Hyamine hydroxide were inserted into the vials in order to trap [$^{14}CO_2$] produced (16). Incubations were terminated after 15 min at 37°C by addition of 200 μl 3 M $HClO_4$. The vials were left for an additional 30 min in the shaking water-bath to ensure complete trapping of the [$^{14}CO_2$].

Thereafter, the small plastic tubes with the Hyamine were transferred into 20 ml glass counting vials containing 10 ml toluene and 40 mg Omnifluor. Radioactivity was measured in a Nuclear Chicago Mark I liquid scintillation counter.

Isolation of leukocytes, cultivation of fibroblasts, and determination of pyruvate oxidation rate in these cells were performed as described previously (17).

Oxygen consumption in isolated muscle mitochondria was measured with an oxygen electrode in a final volume of 550 μl with pyruvate (2.5 μmoles) and malate (2.5 μmoles) or succinate (2.5 μmoles) and rotenone (200 ng), or ascorbate (2.0 μmoles) and TMPD (1.2 ng) as substrates at 37°C in the medium described by Max *et al.* (8). ADP was added to a final concentration of 0.9 mM.

Cytochrome c oxidase activity was measured according to van Buuren *et al.* (3) in muscle homogenate from patient 4 (a biopsy specimen). The content of cytochromes in mitochondria isolated from heart, muscle, and liver, which were stored at −70°C, was determined according to Bookelman *et al.* (1). Succinate-DCIP oxidoreductase activity and succinate-PMS oxidoreductase activity (coupled to DCIP reduction) were measured spectrophotometrically by following the absorbance change at 600 nm. For the calculations, a millimolar absorbance coefficient of 20 for the difference oxidized DCIP minus reduced DCIP was used. Protein concentration was determined according to Lowry *et al.* (7). Lactate, pyruvate, β-hydroxybutyrate, and acetoacetate levels were measured enzymatically (18).

Reagents were purchased as follows: rotenone from Boehringer and Söhne, Mannheim, W. Germany; Omnifluor from NEN Chemicals GmbH, Frankfurt, W. Germany; the hydroxide of Hyamine 10X from Packard, Instrument Co., Chicago, IL, U.S.A.; phenazine methosulphate from Sigma Chemical Company, Saint Louis, MO, U.S.A. and 2-thenoyltrifluoroacetone from BDH Chemicals Ltd, Poole, England. [^{14}C]-Labeled substrates were obtained from the Radiochemical Centre, Amersham, England. Hexokinase (freeze-dried, 40 units/mg) and DL-carnitine and acetylcarnitine were purchased from Koch Light, Colnbrook, England.

RESULTS

Biochemical studies in muscle, liver, leucocytes and fibroblasts. [$^{14}CO_2$]Production rate was studied in fresh skeletal muscle homogenate obtained by biopsy from patient 4 using [1-^{14}C]pyruvate, [U-^{14}C]malate, and [1-^{14}C]2-oxoglutarate as substrates. Oxidation of [1-^{14}C]pyruvate was measured in the presence of either malate or carnitine as acetyl-CoA acceptor. Oxidation of [U-^{14}C]malate was measured in the presence of either pyruvate or acetylcarnitine as acetyl-CoA donor; moreover, malonate was added in these experiments to prevent recycling of malate through the cycle. In one experiment oxidation of [U-^{14}C]malate was studied in the presence of acetylcarnitine and arsenite. In this case [$^{14}CO_2$] is liberated only at the isocitrate dehydrogenase level whereas in the absence of arsenite [$^{14}CO_2$] is liberated both at isocitrate dehydrogenase as well as at 2-oxoglutarate dehydrogenase. Reduced values were measured with patient's muscle homogenate for all substrates tested (Table 1).

In patients 5 and 6, oxidation rates were measured in liver homogenates with succinate or glutamate and malate as substrates. The measured values amounted to 10% of the deep frozen controls.

Oxygen consumption was measured with isolated skeletal muscle mitochondria from patient 1 with pyruvate and malate, succinate and rotenone, and ascorbate and TMPD as substrates. Addition of substrates did not result in an increase of oxygen consumption, which was measured with endogenous substrates only (transition from State 2 to State 3 condition) except for ascorbate and TMPD. Mitochondria isolated from muscle specimens of controls appeared to be normally functioning because respiratory control values of 4.6 ± 1.0 and P/O values (the ratio between the number of ATP molecules formed and the number of oxygen atoms consumed) of 2.7 ± 0.2 were found with 1 mM pyruvate and 1 mM malate as substrates.

Cytochromes were measured in mitochondria from a frozen-stored heart muscle and liver specimen of patient 3 (Table 2).

Normal values were obtained for cytochrome b and cytochrome $c + c_1$ and a rather high value for cytochrome aa_3.

The succinate-ubiquinone oxidoreductase activity of a liver homogenate from patients 5 and 6 was measured using DCIP as electron acceptor after the homogenate was freed from unbroken cells and nuclei by centrifugation for 5 min at 2000 × *g*. This activity was very low, in agreement with the low rate of oxidation of succinate or glutamate plus malate by oxygen. To obtain a more defined picture of the registered defect, the succinate-PMS oxidoreductase activity was measured and the effect of the inhibitor TTFA on this activity was determined. It can be seen from Figures 1 and 2 that the rate of reduction of PMS is not affected in the material from the patient, but the effect of TTFA is very close to the effect of a competitive inhibitor; however the inhibition in control homogenate has the features of a noncompetitive inhibition. Similar results as presented in Figures 1 and 2 were obtained with the liver biopsy specimen from patient 5.

Storage at −20°C for 6 months has no effect on the nature of the inhibition by TTFA, only the activities, both for the patient's homogenate and for the control homogenate, are lowered about 6-fold. Cytochrome c oxidase activity in a skeletal muscle homogenate (patient 4, biopsy specimen) was 11.6 nmole/mg protein · min (controls: 20.9–45.5; $n = 6$). Pyruvate oxidation rates in intact leukocytes from patients 2, 3, 4 and in intact fibroblasts from patients 1 and 4 measured with [1-^{14}C]pyruvate and [2-^{14}C]pyruvate as substrates revealed normal values (Table 3).

Measurement of lactate, pyruvate, β-hydroxybutyrate and acetoacetate. In view of the disturbance in oxidative metabolism established in mitochondria, serum and cerebrospinal fluid levels of lactate, pyruvate, β-hydroxybutyrate and acetoacetate were measured in three patients. Normal values were obtained for all metabolites both in serum and cerebrospinal fluid. From the results obtained, the ratios lactate/pyruvate and β-hydroxybutyrate/acetoacetate were calculated. These ratios reflect the extra- and intra-mitochondrial ratios of NADH/NAD^+ respectively.

The lactate/pyruvate ratios in blood varied between 13.3–19.0

Table 1. *[$^{14}CO_2$]Production rate from [1-^{14}C]-pyruvate, [U-^{14}C]-malate and [1-^{14}C]-2-oxoglutarate in a skeletal muscle homogenate from a Zellweger patient (see "Patients," patient 4) and from control subjects (expressed as nmole/min unit cytochrome oxidase)*

Substrate	Patient	Controls	
		Range	Mean ± S.D.
[1-^{14}C]-pyruvate + malate	20	72–197 (8)[1]	131 ± 52
[1-^{14}C]-pyruvate + D,L-carnitine	13	68–192 (8)	124 ± 47
U-^{14}C]-malate + pyruvate + malonate	34	92–219 (8)	148 ± 47
[U-^{14}C]-malate + acetylcarnitine + malonate	44	94–161 (7)	110 ± 31
[U-^{14}C]-malate + acetylcarnitine + arsenite	20	41– 84 (7)	64 ± 16
[1-^{14}C]-2-oxoglutarate	28	120–254 (8)	164 ± 51

[1] In parentheses: the number of control values.

Table 2. *Cytochrome content (pmole/mg protein) of heart muscle and liver mitochondria from patient 3*

	aa_3	b	$c + c_1$
Heart muscle	652 (440 ± 24)[1]	225 (249 ± 8)[1]	485 (473 ± 22)[1]
Liver	139 (85; 96)	80 (58; 68)	86 (58; 80)

[1] In parentheses: mean value controls ± S.D. (n = 10).

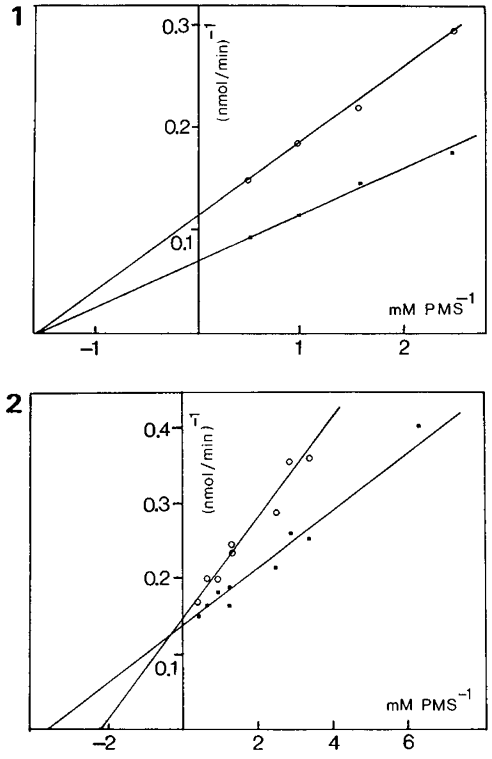

Fig. 1. (*upper panel*): control and Fig. 2 (*lower panel*): Zellweger patient. Succinate-phenazine methosulphate oxidoreductase activity of 100 μl liver homogenate (derived from 5 mg liver tissue) was measured by following the reduction of dichlorophenol indophenol by phenazine methosulphate (PMS) at 600 nm. The medium (2.5 ml) contained 0.25 M sucrose, 25 mM Tris-HCl buffer (pH 7.3), 50 μM DCIP, 20 mM succinate, 2 mM KCN and varying concentrations of phenazine methosulphate. For both experiments homogenate stored for 6 months at −20°C was used. (■—■), minus 2-thenoyltrifluoroacetone and (○—○) plus 250 μM 2-thenoyltrifluoroacetone.

(normal values 16 ± 3) and in cerebrospinal fluid from 10.8–15.8 (normal values 15 ± 3). The ratio of β-hydroxybutyrate/acetoacetate in blood varied between 0.7–2.2 (normal values <3).

DISCUSSION

Considering the results of our biochemical investigations concerning oxygen consumption measurement, substrate oxidation rates, and determination of cytochrome content we can conclude that there is a defect in the electron transport chain before the oxidative step involving cytochromes as was suggested previously (4). Unfortunately we did not have the opportunity to study all of these aspects in a single patient. Disturbances in the respiratory chain (patients 4, 5, and 6) and deficiency of peroxisomes (patients 5 and 6) were established. All six patients studied showed increased pipecolic acid excretion and disturbed bile acid metabolism. We were not able to measure the cytochrome content in skeletal muscle, in which most of the substrate oxidation experiments were performed, because, for unknown reasons, homogenization of the skeletal muscle of Zellweger patients was extremely difficult, resulting in a very low recovery of mitochondria. The findings of a normal cytochrome content in heart muscle mitochrondria as well as in liver mitochondria are in accordance with the suggestion that the defect is located before the oxidative step involving the cytochromes (4). Versmold *et al.* (15) found a decreased cytochrome content (aa_3, b, and c) in liver mitochondria from a Zellweger-like syndrome patient who also showed a deficiency of peroxisomes in hepatocytes. The authors concluded that the decreased cytochrome content may be a secondary phenomenon caused by any alteration of mitochondrial protein synthesis.

Table 3. *[$^{14}CO_2$]Production rate from [1-^{14}C]- and [2-^{14}C]-pyruvate in leucocytes and cultured fibroblasts from Zellweger patients and control subjects. Values for leucocytes were expressed as nmole/h 10^6 cells and for fibroblasts as nmole/h mg protein*

Substrate	Patients				Controls
	1	2	3	4	Mean ± S.D.
Leucocytes					
[1-^{14}C]-pyruvate		4.0	2.3	2.4	2.8 ± 0.9 (11)[1]
[2-^{14}C]-pyruvate		1.9	1.4	1.6	2.1 ± 0.8 (12)
Fibroblasts					
[1-^{14}C]-pyruvate	56.4			54.6	37 ± 14 (10)
[2-^{14}C]-pyruvate	37.5			25.5	18 ± 7 (5)

[1] In parentheses: the number of control values.

The decreased activity of cytochrome c oxidase measured in skeletal muscle homogenate might be due to the difficult homogenization procedure because activity is expressed on total protein present in homogenate. In this manner a low recovery of mitochondria in the homogenate results in a decreased specific activity of mitochondrial enzymes.

The nearly competitive inhibition of the succinate-PMS oxidoreductase activity by TTFA indicates that the interaction of succinate dehydrogenase with ubiquinone is largely destroyed. In intact systems, TTFA inhibits the V_{max}, but does not change the K_m of PMS, whereas in systems without ubiquinone or without interaction with ubiquinone the inhibition is competitive (13). Recently, it has been shown that in submitochondrial particles the interaction of the succinate dehydrogenase with ubiquinone is damaged by incubation with lipoxygenase, resulting in a change in the inhibition of the succinate-PMS oxidoreductase activity by TTFA from noncompetitive to competitive (12). Analogous to the effect of lipoxygenase, we then propose that the damage of the

interaction between succinate dehydrogenase and ubiquinone (probably a damage of the Q-binding protein in the succinate-Q oxidoreductase) is related to the absence of peroxisomes in the cells of Zellweger patients, resulting in an increased level of peroxides. Furthermore, we propose that the same damage has occurred at the interaction site of NADH dehydrogenase with ubiquinone. The abnormally high oxidation state of cytochrome b in the controlled state, as found by Versmold *et al.* (15), might be a secondary consequence of the proposed damage of succinate-Q-oxidoreductase.

The disturbance in the electron transport chain might also be explained in an alternative manner. Recently, Hajra *et al.* (5) demonstrated that the enzyme acyl-CoA:dihydroxyacetone-phosphate acyltransferase, which catalyses the biosynthesis of acyldihydroxyaceton phosphate, was primarily localized in rat liver peroxisomes. Because acyldihydroxyaceton phosphate is a precursor of glycerolipids it is involved in biosynthetic pathways of components of biologic membranes. Deficiency of peroxisomes can thus lead to a disturbance in the structure of the inner mitochondrial membrane, causing defects in the electron transport chain. Further investigation will be necessary to find support for this hypothesis.

Although several arguments have been provided in favour of the presence of a defect in the electron transport chain we found no change in the ratio of lactate/pyruvate as well as β-hydroxybutyrate/acetoacetate in blood. This finding could indicate that *in vivo* the proposed defect in the electron transport chain does not exist. The normal oxidation rates of [1-^{14}C]pyruvate and [2-^{14}C] pyruvate in intact cells, *e.g.*, in leukocytes and fibroblasts, demonstrate a normally functioning electron transport chain in these cells. On the other hand, a partly or even greatly damaged respiratory chain in mitochondria of liver and muscle is compatible with a normal lactate/pyruvate ratio. The lactate/pyruvate ratio is not a very sensitive parameter for the capacity of the mitochondria to oxidize NADH, because the *in vivo* respiratory rate is determined largely by the phosphate potential in the cell and is far below the maximal capacity. A drastic lowering of the oxidative capacity is needed to cause a change in the NADH/NAD^+ or lactate/pyruvate ratio under normal conditions. On the other hand, it might be possible that *in vivo* the level of peroxides is lower than *in vitro*, owing to perfusion of catalase-containing red cells to the tissues. In this manner, damage of the respiratory chain by hydrogenperoxide could be partly prevented by circulating red cells. A further explanation for the somewhat contrasting results between *in vivo* and *in vitro* observations could be based on the assumption that the damage of the electron transport chain, present *in vivo*, is increased *in vitro* during preparation of patient's material.

In fibroblasts the catalase activity is normally quite low. Effective protection against peroxides will be obtained by other mechanisms in these cells. The very low number of mitochondria in both fibroblasts and leukocytes will result in a low production of hydrogenperoxide (except for the condition of phagocytosis).

It seems reasonable to postulate that all biochemical abnormalities associated with the Zellweger syndrome, including disturbances in pipecolic acid metabolism, in bile acid metabolism and in the electron transport chain are related to the deficiency of peroxisomes (10). These authors showed that the conversion of trihydroxycoprostanic acid into cholic acid occurs in the peroxisomal fraction of rat liver.

REFERENCES AND NOTES

1. Bookelman, H., Trijbels, J. M. F., Sengers, R. C. A., and Janssen, A. J. M.: Measurement of cytochromes in human skeletal muscle mitochondria isolated from fresh and frozen stored muscle specimens. Biochem. Med., *19:* 366 (1978).
2. Brun, A., Gilboa, M., Meeuwisse, G. W., and Nordgren, H.: The Zellweger syndrome: subcellular pathology, neuropathology and the demonstration of pneumocystis carinii pneumonitis in two siblings. Eur. J. Pediatr., *127:* 229 (1978).
3. van Buuren, K. J. H., Zuurendonk, P. F., van Gelder, B. F., and Muysers, A. O.: Biochemical and biophysical studies on cytochrome aa_3: V. Binding of cyanide to cytochrome aa_3. Biochim. Biophys. Acta, *256:* 243 (1972).
4. Goldfischer, S., Moore, C. L., Johnson, A. B., Spiro, A. J., Valsamis, M. P., Wisniewski, H. K., Ritch, R. H., Norton, W. T., Rapin, I., and Gartner, L. M.: Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science, *182:* 62 (1973).
5. Hajra, A. K., Burke, C. L., and Jones, C. L.: Subcellular localization of acetyl coenzyme A: dihydroxyacetone phosphate acyltransferase in rat liver peroxisomes (microbodies). J. Biol. Chem., *254:* 10896 (1979).
6. Hanson, R. F., Szczepanik-van Leeuwen, P., Williams, G. C., Grabowski, G., and Sharp, H. L.: Defects of bile acid synthesis in Zellweger's Syndrome. Science *203:* 1107 (1979).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
8. Max, S. R., Garbus, J., and Wehmann, H. J.: Simple procedure for rapid isolation of functionally intact mitochondria from human and rat skeletal muscle. Anal. Biochem., *46:* 576 (1972).
9. Monnens, L., Bakkeren, J., Parmentier, G., Janssen, G., van Haelst, U., Trijbels, F., and Eyssen, H.: Disturbances in bile acid metabolism of infants with the Zellweger (cerebro-hepato-renal) syndrome. Eur. J. Pediatr., *133:* 31 (1980).
10. Pedersen, J. I. and Gustafsson, J.: Conversion of 3α, 7α, 12α-trihydroxy-5β-cholestanoic acid into cholic acid by rat liver peroxisomes. Febs Letters, *121:* 345 (1980).
11. Pfeifer, U. and Sandhage, K.: Licht und elektronenmikroskopische Leberbefunde beim Cerebro-Hepato-Renalen Syndrom nach Zellweger (Peroxisomen-Defizienz). Virchows Arch. A. Path. Anat. and Histol., *384:* 269 (1979).
12. Schewe, T., Albracht, S. P. J., and Ludwig, P.: On the side of action of the inhibition of the mitochondrial respiratory chain by lipoxygenase. Biochim. Biophys. Acta, *636:* 210 (1981).
13. Singer, T. P. and Edmondson, D. E.: In Methods in Enzymology, vol. 53 (S. Fleischer and L. Packer, Eds). p. 397. (Acad. Press, New York, 1978).
14. Trijbels, J. M. F., Monnens, L. A. H., Bakkeren, J. A. J. M., van Raay-Selten, A. H. J., and Corstiaensen, J. M. B.: Biochemical studies in the Cerebro-Hepato-Renal Syndrome of Zellweger. A disturbance in the metabolism of pipecolic acid. J. Inher. Metab. Dis., *2:* 39 (1979).
15. Versmold, H. T., Bremer, H. J., Herzog, V., Siegel, G., von Bassewitz, D. B., Irle, U., von Voss, H., Lombeck, I., and Brauser, B.: A metabolic disorder similar to Zellweger syndrome with hepatic acatalasia and absence of peroxisomes, altered content and redox state of cytochromes, and infantile cirrhosis with hemosiderosis. Eur. J. Pediatr., *124:* 261 (1977).
16. Willems, J. L., Monnens, L. A. H., Trijbels, J. M. F., Veerkamp, J. H., Meyer, A. E. F. H., van Dam, K., and van Haelst, U.: Leigh's encephalomyelopathy in a patient with cytochrome c oxidase deficiency in muscle tissue. Pediatrics, *60:* 850 (1977).
17. Willems, Hans, L., de Kort, Ted, F. M., Trijbels, Frans, J. M., Monnens, Leo, A. H., and Veerkamp, Jacques, H.: Determination of pyruvate oxidation rate and citric acid cycle activity in intact human leucocytes and fibroblasts. Clin. Chem., *24:* 200 (1978).
18. Willems, H.: Disturbances in pyruvate metabolism. A biochemical and clinical investigation. Thesis, University of Nijmegen, The Netherlands (1978).

19. The authors thank Prof. Dr. W. Tegelaers and Dr. W. Endres for providing liver specimens from Zellweger patients.

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21. Received for publication December 8, 1981.
22. Accepted for publication September 17, 1982.

0031-3998/83/17-0514$02.00/0

Printed in U.S.A.