J. Inher. Metab. Dis. 18 Suppl. 1 (1995) 90–100

Measurement of dihydroxyacetone-phosphate acyltransferase (DHAPAT) in chorionic villous samples, blood cells and cultured cells

R. J. A. WANDERS[1]*, R. OFMAN[1], G. J. ROMEIJN[1], R. B. H. SCHUTGENS[1], P. A. W. MOOIJER[1], C. DEKKER[1] and H. VAN DEN BOSCH[2]

[1]*University Hospital Amsterdam, Academic Medical Centre, Departments of Pediatrics and Clinical Chemistry, Laboratory of Pediatric Clinical Chemistry, Section for Clinical Enzymology;* [2]*Centre for Biomembranes and Lipid Enzymology, University of Utrecht, Utrecht, The Netherlands*

**Correspondence: University Hospital Amsterdam, AMC, Meibergdreef 9, Room F0-224, 1105 AZ Amsterdam, The Netherlands*

Summary: Dihydroxyacetone-phosphate acyltransferase (DHAPAT) is a peroxisomal enzyme catalysing the first step in ether-phospholipid biosynthesis. DHAPAT is deficient in cells from patients suffering from a variety of peroxisomal disorders. Accurate measurement of the activity of this enzyme is of great importance, especially since it is a central parameter in the prenatal diagnosis of the disorders of peroxisome biogenesis, rhizomelic chondrodysplasia punctata and DHAPAT-deficiency. We describe a straightforward and accurate assay allowing the activity of DHAPAT to be measured reliably in chorionic villus samples, blood cells, cultured skin fibroblasts, cultured chorionic villus fibroblasts and cultured amniocytes.

Ether-phospholipids are phospholipids which differ from the generally known diacylglycerophospholipids in one major aspect, which is the occurrence of an ether bond rather than an ester bond at the *sn*-1 position of the glycerol backbone. In mammals the main end products of ether-phospholipid biosynthesis are the plasmalogens (1-0-alk-1[1]-enyl-2-acylphosphoglycerides) with a double bond between the two carbon atoms adjacent to the ether bond. Plasmalogens have long been known to be widely distributed in mammalian membranes, making up 5–20% of total phospholipids. They are particularly abundant in electrically active tissues such as brain. The physiological function of plasmalogens and of ether-phospholipids in general has remained an enigma except for that of platelet activating factor (PAF), which has been implicated in a range of (patho-) physiological processes (Chung 1992).

As first discovered by Hajra and co-workers (see Hajra and Bishop (1982) for review) peroxisomes play an essential role in ether-phospholipid biosynthesis. Indeed, the enzymes responsible for the introduction of the typical ether bond in ether-phospholipids, i.e. dihydroxyacetone-phosphate acyltransferase (DHAPAT) (EC 2.3.1.42) and alkyldihydroxyacetone-phosphate synthase (alkyl-DHAP synthase) (EC 2.5.1.26) are localized

predominantly in peroxisomes (see Hajra and Bishop (1982) for review). DHAPAT has been found to be a membrane-bound protein with its active site facing the interior of the peroxisome (Jones and Hajra 1980; De Clerq et al 1984; Hardeman and Van den Bosch 1988). Purification of the enzyme protein has recently been achieved from guinea-pig liver (Webber and Hajra 1993) and human placenta (Ofman and Wanders 1994).

Measurement of DHAPAT activity is important for the correct identification of patients suspected to suffer from a peroxisomal disorder (Wanders et al 1988, 1993; Schutgens et al 1989). DHAPAT is deficient in patients suffering from disorders of peroxisome biogenesis that include Zellweger syndrome (ZS, McKusick 214100), neonatal adrenoleukodystrophy (NALD, McKusick 202370) and infantile Refsum disease (IRD, McKusick 266510). DHAPAT is also deficient in patients with the rhizomelic form of chondrodysplasia punctata (RCDP) (McKusick 215100), although residual activity is much higher in RCDP fibroblasts than in Zellweger fibroblasts (see Table 1). It should be noted that there are also other biochemical abnormalities in RCDP patients (Hoefler et al 1988; Van den Bosch et al 1992; Wanders et al 1993). Finally, DHAPAT is deficient in patients with a variant form of rhizomelic chondrodysplasia punctata characterized by an isolated deficiency of DHAPAT (Wanders et al 1992a; Barr et al 1993; Clayton et al 1994). Importantly, DHAPAT is also expressed in chorionic villus cells, either cultured or not (see Table 1), and in cultured amniocytes and is often used for prenatal diagnosis (Schutgens et al 1989; see also Table 1).

PRINCIPLE OF THE ASSAY METHOD USED TO MEASURE DHAPAT ACTIVITY

Acyl-CoA: dihydroxyacetone-phosphate acyltransferase (DHAPAT, EC 2.3.1.42) catalyses the reaction

$$\text{dihydroxyacetone-phosphate} + \text{acyl-CoA} \rightarrow \text{acyldihydroxyacetone-phosphate} + \text{CoA-SH}$$

Activity is relatively low in tissues and fibroblasts and hence the use of a radioactively labelled substrate is mandatory. Since radiolabelled dihydroxyacetone-phosphate (DHAP) is not available commercially, a method was developed for preparation of [^{14}C]DHAP from commercially available [^{14}C]glycerol-3-phosphate (Davis and Hajra 1979; Schutgens et al 1984, 1986; Wanders et al 1985; Ofman and Wanders 1994). This is done as shown schematically in Figure 1.

Owing to the unfavourable equilibrium constant of the *sn*-glycerol-3-phosphate dehydrogenase reaction (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8), pyruvate and lactate dehydrogenase (LDH, L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) have to be added to obtain full conversion of glycerol-3-phosphate to dihydroxyacetone-phosphate.

The [^{14}C]DHAP synthesized in a preincubation is then used for DHAPAT activity measurements, which are done at slightly acidic pH with palmitoyl-CoA as second substrate. Finally, the product acyl-[^{14}C]dihydroxyacetone-phosphate is separated from the substrate [^{14}C]DHAP and quantified.

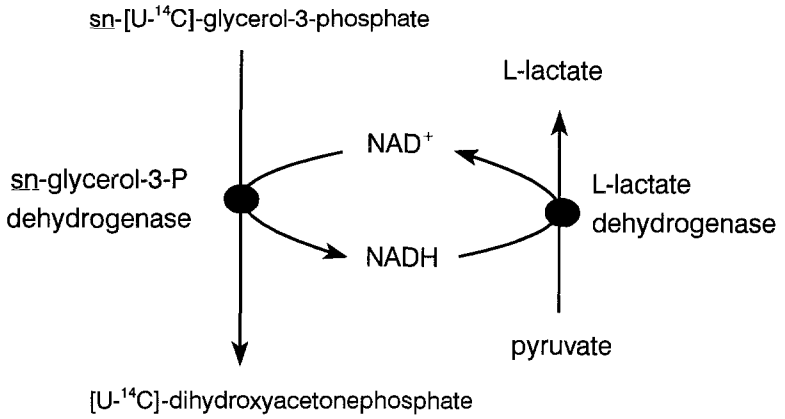

Figure 1 Schematic representation of the method used to prepare radiolabelled dihydroxyacetonephosphate (DHAP) from glycerol-3-phosphate (G3P)

EQUIPMENT, CHEMICALS AND SOLUTIONS

1. Equipment

Liquid scintillation counter; vortex mixer; low-speed centrifuge; thermostated water bath; glass test tubes (11/12×65 mm approx.) with caps; scintillation vials; adjustable automatic pipettes and tips; a pipette or syringe with a Teflon plunger for 100 and 600 μl; gloves; Pasteur pipettes and bulbs; timer; container with ice; rocking table; needles (1.1×40 mm); cellulose filter papers (2.1 cm diameter); sonicator with a small tip (tip diameter of 0.25 cm).

2. Chemicals

- Lactate dehydrogenase (LDH, L-lactate:NAD oxidoreductase, EC 1.1.1.27, from hog muscle, specific activity ~550 U/mg (25°C); Boehringer Mannheim, cat. no. 127221)
- Glycerol-3-phosphate dehydrogenase (G3PDH, *sn*-glycerol-3-phosphate:NAD^+ 2-oxidoreductase, EC 1.1.1.8, from rabbit muscle, specific activity ~170 U/mg (25°C); Boehringer Mannheim, cat. no. 127124)
- Triethanolamine hydrochloride (2,2′,2″-nitrilotriethanol hydrochloride; Sigma Chemicals, St Louis , MO, USA, cat. no. T 1502)
- NAD (β-nicotinamide-adenine dinucleotide, free acid; Boehringer Mannheim, cat. no. 127302)
- Magnesium chloride ($MgCl_2 \cdot 6H_2O$; Merck, Darmstadt, Germany, art 5833)
- Pyruvate (pyruvate, monosodium salt; Boehringer Mannheim, cat. no. 128147)
- Palmitoyl-CoA (*S*-palmitoyl-coenzyme A, tetrapotassium salt; Boehringer Mannheim, cat. no. 663387)
- Glycerol-3-phosphate (L-glycerol-3-phosphate, dicyclohexylammonium salt dihydrate; Boehringer Mannheim, cat. no. 105961)

- Radiolabelled glycerol-3-phosphate (L-[^{14}C]glycerol-3-phosphate, ammonium salt, aqueous solution containing 2% (v/v) ethanol, >100 mCi/mmol; Amersham International, UK, cat. no. CFB 171)
- Sodium fluoride (NaF, purity ~99% (pfs); Sigma Chemicals, St Louis, MO, USA, cat. no. 51504)
- Bovine serum albumin (BSA, fatty acid free; Boehringer Mannheim, cat. no. 775827)
- Trichloroacetic acid (TCA, purity >99%; Sigma Chemicals, St Louis, MO, USA, cat. no. T 6399)
- Phosphoric acid (H_3PO_4; Sigma Chemicals, St Louis, MO, USA, cat. no. P 6560)
- Trizma hydrochloride (tris(hydroxymethyl)aminomethane hydrochloride; Sigma Chemicals, St Louis, MO, USA, cat. no. T 3253)
- KCl (potassium chloride; Sigma Chemicals; St Louis, MO, USA, cat no. P 4504)
- NaCl (sodium chloride; Sigma Chemicals, St Louis, MO, USA, cat no. S 7653)
- Chloroform and methanol, glass-redistilled, of highest purity
- Buffered saline: 154 mmol/L sodium chloride plus 10.5 mmol/L phosphate (pH 7.4)

3. Solutions necessary for the DHAPAT assay

A. Stock solutions required for the preincubation (synthesis of [^{14}C]DHAP from [^{14}C]G3P)

- 0.35 mol/L triethanolamine-HCl (pH 7.6). Dissolve 55.16 g in water, adjust pH to 7.6 and make up to 1 litre.
- 100 mmol/L sodium pyruvate. Dissolve 110 mg sodium pyruvate in 10 ml water. Prepare fresh each time.
- 20 mmol/L NAD. Dissolve 66.34 mg in 5 ml water. Store in 100 µl aliquots at −80°C.
- 12 mmol/L glycerol-3-phosphate. Dissolve 48.78 mg dicyclohexylammonium glycerol-3-phosphate dihydrate in 10 ml water. Store in aliquots at −80°C.

B. Stock solutions required for the actual DHAPAT assay

- 0.5 mol/L sodium acetate (pH 5.4). Dissolve 41.02 g sodium acetate in water, adjust pH and make up to 1 litre. Store at 4°C.
- 0.5 mol/L magnesium chloride. Dissolve 101.65 g magnesium chloride hexahydrate in 1 litre water. Store at 4°C.
- 0.5 mol/L sodium fluoride. Dissolve 1.05 g sodium fluoride in 50 ml water. Store at 4°C.
- 66.7 mg/ml BSA. Dissolve 66.7 mg in 1 ml water. Store deep-frozen (−20°C or −80°C).
- 1.0 mmol/L palmitoyl-CoA in 10 mmol/L sodium acetate buffer (pH 5.4). To 11.62 mg of palmitoyl-CoA add 10 ml of 10 mmol/L sodium acetate buffer prepared by diluting 50-fold the stock solution of 0.5 mol/L (see above). Store in portions of 1 ml at −80°C.
- 1 : 1 (v/v) chloroform–methanol
- 2 mol/L KCl plus 0.2 mol/L H_3PO_4. For 1 litre, weigh 149.1 g potassium chloride, add 11.63 ml H_3PO_4, and make up to 1 litre.

- TCA solutions. For 10%, 5% and 1% (w/v) solutions, weigh 100, 50 and 10 g TCA, respectively, and make up to 1 litre.
- 50 mmol/L sodium chloride plus 5 mmol/L Tris-HCl (pH 7.4). For 1 litre, weigh 2.92 g sodium chloride, add 1 litre 5 mmol/L Tris-HCl (pH 7.4) prepared by dilution from the 0.5 mol/L stock solution (see above).

EXPERIMENTAL PROCEDURE

1. Synthesis of [^{14}C]DHAP from [^{14}C]G3P (*preincubation step*)

(i) Add to a 5 ml glass tube: 145 μl 0.35 mol/L triethanolamine-HCl (pH 7.6), 50 μl 100 mmol/L Na-pyruvate, 50 μl 20 mmol/L NAD^+, 15 μl LDH (10 mg/ml), 100 μl *sn*-[^{14}C]G3P, 580 μl water, 15 μl glycerol-3-phosphate dehydrogenase, plus 45 μl 12 mmol/L G3P (total volume 1000 μl).

(ii) Close tube, mix gently and incubate at 25°C for 1 h. During this period, prepare the cell homogenates (see below) and mark tubes for the second step.

(iii) Stop the preincubation reaction by adding an equal volume of chloroform and mix thoroughly. Centrifuge at 2000 rpm for 5 min and transfer upper layer containing [^{14}C]DHAP to a clean tube.

Note: This quantity is enough for at least 40 incubations.

2. Preparation of chorionic villus biopsy specimens, blood platelets, cultured skin fibroblasts, cultured amniocytes and cultured chorionic villous fibroblasts

Chorionic villus biopsy specimens are usually split into two portions so that one can be used for direct analysis and the other cultured for future biochemical analysis if necessary. For direct analysis, the specimen is washed in physiological saline (see above) three times. After the final wash, the material is centrifuged (12000g_{av}, 5 min, 4°C) and stored at −80°C.

Blood platelets are prepared from venous blood samples using EDTA as anticoagulant (Wanders et al 1985). The final pellet is stored at −80°C.

Skin fibroblasts are cultured according to standard procedures and collected by trypsin treatment after the cells have reached confluency (see Wanders et al (1987) for full details). Cells are washed several times in physiological saline and the final pellet is stored at −80°C containing 1–2 mg protein.

Amniotic fluid cells and chorionic villus fibroblasts are cultured essentially as described for cultured skin fibroblasts.

3. Preparation of homogenates

On the day of the experiment the different cell pellets, prepared as described above and stored at −80°C, are taken followed by addition of 0.5 ml of a solution containing 50 mmol/l sodium chloride plus 5 mmol/l Tris-HCl (pH 7.4) and ultrasonic disruption by sonication for 3 periods of 15 s at 70–80 W. Homogenates should be kept in ice-water during sonication and there should be an interval of at least 45 s between two rounds of sonication.

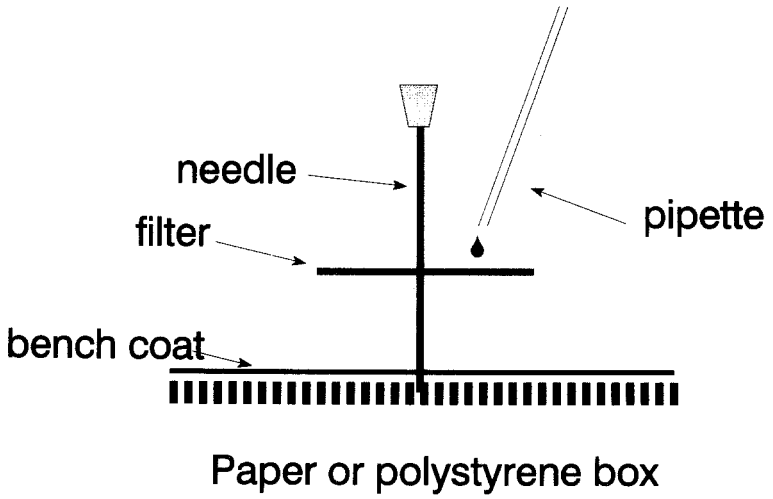

Figure 2 Schematic representation of the system used to prepare the filter papers

Protein concentrations can be measured using a variety of different methods using albumin as a standard. Protein content should be between 1 and 5 mg/ml.

4. DHAPAT assay

In our experience it is best first to prepare a reaction medium containing all necessary components at double their final concentrations with the exception of palmitoyl-CoA and radiolabelled [^{14}C]DHAP, which are added separately.

For preparation of the reaction medium, add in a 5 ml glass tube (total incubation volume 2.5 ml): 750 μl sodium acetate buffer (pH 5.4), 80 μl 0.5 mol/L $MgCl_2$, 80 μl 0.5 mol/L sodium fluoride, and 250 μl 66.7 mg/ml BSA. Make up to 2500 μl. Check pH. This quantity is enough for 40 assays.

Once the incubation medium has been prepared, the test tubes are prepared by adding the following constituents: 60 μl incubation medium prepared as described above, 20 μl 1 mmol/L palmitoyl-CoA, and 20 μl [^{14}C]DHAP as prepared enzymatically (upper phase obtained after addition of chloroform as described above). Reactions are then initiated by adding a 20 μl aliquot of cell homogenate (20–100 μg protein) or 20 μl 50 mmol/L sodium chloride plus 5 mmol/L Tris-HCl (blank). For all cell types analysed, enough acyl-[^{14}C]DHAP is formed under such conditions to ensure reliable results.

Reactions are allowed to proceed for 2 h except for platelets, in which case a 30 min incubation period has been selected. Reactions are terminated by adding 600 μl chloroform–methanol (1 : 1, v/v) plus 150 μl KCl/H_3PO_4 to each tube followed by thorough mixing.

At the end of the experiment, tubes are centrifuged (500g_{av}, 5 min, 4°C) and the upper phase is discarded using a Pasteur pipette. Subsequently, 200 μl of the lower chloroform layer is pipetted on to a filter paper by adding two 100 μl portions with a syringe or pipette with Teflon plunger. After applying the first aliquot, wait until the filter is dry before adding the second aliquot (see Figure 2).

The dried filters, which have been marked previously with a pencil, are then added to ice-cold 10% (w/v) TCA in a large beaker (0.5–1.0 litre). After 15 min the TCA is removed and fresh 10% (w/v) TCA is added. After an additional 15 min the filters are washed using 5% (w/v) TCA and 1% (w/v) TCA. Finally, the filters are collected and transferred to scintillation vials followed by addition of 10 ml scintillation cocktail. After thorough mixing, radioactivity is counted overnight.

5. Calculation of results

From the counts (in dpm), determine the specific activity of DHAPAT in the samples (nmoles of acyl-[^{14}C]DHAP formed/2 h per mg protein; for platelets, nmoles of acyl-[^{14}C]DHAP formed/30 min per mg protein).

$$\text{Activity} = (\text{counts} - \text{blank}) \times 12 \times 1.5 \times 1000 \times 1/\mu\text{g protein}$$

RESULTS AND DISCUSSION

Table 1 summarizes the results of DHAPAT activity measurements in a variety of different cell types including chorionic villus biopsy specimens, blood platelets, cultured skin fibroblasts, cultured chorionic villus fibroblasts and cultured amniocytes.

If the results in cultured skin fibroblasts are considered first, it is clear that the deficiency of DHAPAT is most pronounced in cells from patients suffering from a variant form of rhizomelic chondrodysplasia punctata due to isolated DHAPAT deficiency (compare Wanders et al 1992a; Barr et al 1993; Clayton et al 1994). In cells from patients suffering from classical Zellweger syndrome (ZS) DHAPAT is strongly deficient, although not completely deficient as in DHAPAT deficiency. In patients suffering from milder types of peroxisomal deficiency disorder including infantile Refsum disease (Poll-Thé et al 1987), neonatal adrenoleukodystrophy (Kelley et al 1986) and other phenotypes not easily assigned to either of these two entities (Bleeker-Wagemaker et al 1986; Barth et al 1987) residual DHAPAT activity is higher both in cultured skin fibroblasts and blood platelets. In some patients DHAPAT activity may even be near-normal, which can lead to erroneous conclusions in the absence of additional peroxisomal investigations (compare patient VII in Table IV of Wanders et al. (1993)).

As shown in Table 1, DHAPAT is also deficient in cells from patients suffering from the classical form of chondrodysplasia punctata. On the average, residual DHAPAT activity is higher in fibroblasts (and platelets) from RCDP patients than in Zellweger patients. It should be noted that biochemical abnormalities in RCDP are not restricted to the partial deficiency of DHAPAT (Heÿmans et al 1985; Hoefler et al 1988; Schutgens et al 1988). Indeed, alkyldihydroxyacetone-phosphate synthase and phytanic acid α-oxidation are also deficient. In addition, peroxisomal thiolase occurs in its precursor (44 kDa) rather than in its mature (41 kDa) form (Hoefler et al 1988).

Table 1 further shows normal activity of DHAPAT in fibroblasts from patients with defects in peroxisomal β-oxidation. This includes X-linked adrenoleukodystrophy, acyl-CoA oxidase deficiency (Poll-Thé et al 1988; Wanders et al 1990a; Suzuki et al 1994), bifunctional protein deficiency (Watkins et al 1989; Wanders et al 1992b, 1993; Suzuki et al 1994), peroxisomal thiolase deficiency (Goldfischer et al 1986; Schram et al 1987) and

Table 1 Dihydroxyacetone-phosphate acyltransferase activity measurements in blood platelets, cultured skin fibroblasts, chorionic villus biopsy specimens, cultured chorionic villus fibroblasts and amniotic fluid cells from peroxisomal disorder patients

	n	*DHAPAT-activity*[a]	*DHAPAT/GluDH ratio*[b]
Blood platelets			
Control	123	3.5±0.9 (2.2–4.9)	6.9±1.6 (4.5–9.1)
Classical ZS	11	0.24±0.29 (0–1.1)	0.57±0.60 (0–2.0)
IRD, NALD, variant ZS[c]	5	1.96±0.71 (1.3–3.3)	4.72±1.82 (2.6–8.0)
RCDP	6	0.65±0.37 (0.3–1.3)	1.42±0.88 (0.6–3.2)
X-ALD	5	3.7±0.7	7.2±0.8
DHAPAT deficiency	1	0.10	0.10
Cultured skin fibroblasts			
Control	78	8.1±2.5 (3.2–14.3)	6.8±3.4 (2.2–15.6)
Classical ZS	23	0.6±0.5 (0–1.9)	0.5±0.3 (0–1.2)
IRD, NALD, variant ZS[c]	12	2.4±1.5 (0.6–5.9)	1.9±1.7 (0.4–6.5)
RCDP	39	1.9±0.9 (0.4–4.4)	1.7±1.8 (0.2–3.3)
DHAPAT deficiency	6	0.0±0.0	0.0±0.0
X-ALD	5	8.7±1.3 (5.6–9.6)	7.0±0.9 (5.1–8.7)
AOX deficiency[d]	3	9.1±2.0 (8.1–10.1)	6.7±1.0 (6.0–7.8)
BP deficiency[e]	3	8.8±1.8 (7.4–11.1)	7.1±2.4 (5.9–8.2)
Chorionic villi			
Control	68	7.0±2.2 (3.0–10.8)	4.2±1.5 (1.8–9.4)
ZS	15	0.6±0.5 (0.0–1.7)	0.5±0.7 (0–0.8)
RCDP	2	0.2; 3.8	0.2; 1.1
Cultured chorionic villus fibroblasts			
Control	11	6.3±2.2 (3.7–10.3)	n.d.
ZS	8	0.1±0.2 (0.0–0.4)	n.d.
RCDP	2	0.2; 1.9	n.d.
Cultured amniotic fluid cells			
Control	21	6.5±2.5 (3.1–10.9)	n.d.
ZS	4	0.2±0.2 (0.1–0.5)	n.d.

Results are given as mean±SD with the range in parentheses. *n* denotes the number of different patients studied; n.d. = not determined

[a]DHAPAT-activity is expressed in nmol/2h per mg protein except for platelets in which case activity is in nmol/30 min per mg protein, or [b]relative to the activity of glutamate dehydrogenase (GluDH activity in nmol/min per mg protein) measured in the same sample

[c]This includes patients with infantile Refsum disease (IRD), neonatal adrenoleukodystrophy (NALD) and other patients with a deficiency of peroxisomes but with a phenotype different from ZS, IRD or NALD

[d]AOX deficiency = acyl-CoA oxidase deficiency

[e]BP deficiency = bifunctional protein deficiency

other disorders of peroxisomal β-oxidation of unknown aetiology (Clayton et al 1988; Naidu et al 1988; Barth et al 1990; Wanders et al 1990b; Espeel et al 1991; Mandel et al 1992; Van Maldergem et al 1992; Wanders et al 1992b; Santer et al 1993).

When the results obtained in blood platelets are considered, it is clear that these are in good agreement with the data on cultured skin fibroblasts. Finally, the data of Table 1 show that the activity of DHAPAT in chorionic villus biopsy specimens, cultured chorionic

villus fibroblasts and cultured amniocytes compares well with the activity in cultured skin fibroblasts and that the activity of DHAPAT is strongly deficient in cases of Zellweger syndrome and rhizomelic chondrodysplasia punctata. Based on these latter results, it is clear that DHAPAT activity measurement is of central importance in the prenatal diagnosis of Zellweger syndrome, other disorders of peroxisome biogenesis (infantile Refsum disease, neonatal adrenoleukodystrophy and other variant forms) rhizomelic chondrodysplasia punctata, and DHAPAT deficiency.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge The Princess Beatrix Fund, The Hague, The Netherlands for financial support through the years and Mrs Iet van der Gracht for preparation of the manuscript. Dr N. Leschot, Dr M. Verjaal and their co-workers, Department of Anthropogenetics, University of Amsterdam, are gratefully acknowledged for their generous help in culturing chorionic villus fibroblasts and amniocytes. Mrs E. de Jonge-Meijboom and P. Huizinga-Veltman are thanked for expert preparation of the cell cultures.

REFERENCES

Barr DGD, Kirk JM, Al Howasi M, Wanders RJA, Schutgens RBH (1993) Rhizomelic chondrodysplasia punctata with isolated DHAP-AT deficiency. *Arch Dis Child* **68**: 415–417.

Barth PG, Schutgens RBH, Wanders RJA, et al (1987) A sibship with a mild variant of Zellweger syndrome. *J Inher Metab Dis* **10**: 253–259.

Barth PG, Wanders RJA, Schutgens RBH, Bleeker-Wagemakers EM, Van Heemstra D (1990) β–Oxidation defect with detectable peroxisomes: a case with neonatal onset and progressive course. *Eur J Pediatr* **149**: 722–726.

Bleeker-Wagemaker EM, Oorthuys JWE, Wanders RJA, Schutgens RBH (1986) Long term survival of a patient with the cerebro-hepato-renal (Zellweger) syndrome. *Clin Genet* **29**: 160–164.

Chung KF (1992) Platelet-activating factor in inflammation and pulmonary disorders. *Clin Sci* **83**: 127–138.

Clayton PT, Lake BD, Hjelm M, et al (1988) Bile acid analyses in pseudo-Zellweger syndrome: clues to the defect in peroxisomal β-oxidation. *J Inher Metab Dis* **11**: 165–168.

Clayton PT, Eckhardt S, Wilson J, et al (1994) Isolated dihydroxyacetone-phosphate acyltransferase deficiency presenting with developmental delay. *J Inher Metab Dis* **17**: 533–540.

Davis PA, Hajra AK (1979) Stereochemical specificity of the biosynthesis of the alkyl ether bond in ether lipids. *J Biol Chem* **254**: 4760–4763.

De Clerq PE, Haagsman HP, Van Veldhoven PP, Debeer LJ, Van Golde LM, Mannaerts GP (1984) Rat liver dihydroxyacetone-phosphate acyltransferase and their contribution to glycerolipid synthesis. *J Biol Chem* **259**: 9064–9075.

Espeel M, Roels F, Van Maldergem L, et al (1991) Peroxisomal localization of the immunoreactive β-oxidation enzymes in a neonate with a β-oxidation defect: pathological observations in liver, adrenal cortex and kidney. *Virchows Arch A (Pathol Anat)* **419**: 339–347.

Goldfischer S, Collins J, Rapin I, et al (1986) Pseudo-Zellweger syndrome: deficiencies in several peroxisomal oxidative activities. *J Pediatr* **108**: 25–32.

Hajra AK, Bishop JE (1982) Glycerolipid biosynthesis in peroxisomes via the acyldihydroxyacetone phosphate pathway. *Ann NY Acad Sci* **386**: 170-182.

Hardeman D, Van den Bosch H (1988) Rat liver dihydroxyacetone-phosphate acyltransferase: enzyme characteristics and localization studies. *Biochim Biophys Acta* **963**: 1–9.

Heymans HSA, Oorthuys JWE, Nelck G, Wanders RJA, Schutgens RBH (1985) Rhizomelic chondrodysplasia punctata: another peroxisomal disorder. *N Engl J Med* **313**: 187–188.

Hoefler G, Hoefler S, Watkins PA, et al (1988) Biochemical abnormalities in rhizomelic chondrodysplasia punctata. *J Pediatr* **112**: 726–733.

Jones CL, Hajra AK (1980) Properties of guinea pig liver peroxisomal dihydroxyacetone phosphate acyltransferase. *J Biol Chem* **255**: 8289–8295.

Kelley RI, Datta NS, Dobijns WS, et al (1986) Neonatal adrenoleukodystrophy: new cases, biochemical studies, and differentiation from Zellweger syndrome and related peroxisomal polydystrophy syndromes. *Am J Med Genet* **23**: 869–901.

Mandel H, Berant M, Aizin A, et al (1992) Zellweger-like phenotype in two siblings: a defect in peroxisomal β-oxidation with elevated very-long-chain fatty acids but normal bile acids. *J Inher Metab Dis* **15**: 381–384.

Naidu S, Hoefler G, Watkins PA, et al (1988) Neonatal seizures and retardation in a girl with biochemical features of X-linked adrenoleukodystrophy. *Neurology* **38**: 1100–1107.

Ofman R, Wanders RJA (1994) Purification of peroxisomal acyl-CoA: dihydroxyacetonephosphate acyltransferase from human placenta. *Biochim Biophys Acta* **1206**: 27–34.

Poll-Thé BT, Saudubray JM, Ogier HAM, et al (1987) Infantile Refsum disease: an inherited peroxisomal disorder and comparison with Zellweger syndrome and neonatal adrenoleukodystrophy. *Eur J Pediatr* **146**: 477–483.

Poll-Thé BT, Roels F, Ogier HAM, et al (1988) A new peroxisomal disorder with enlarged peroxisomes and a specific deficiency of acyl-CoA oxidase (pseudo neonatal adrenoleukodystrophy). *Am J Hum Genet* **42**: 422–434.

Santer R, Claviez A, Oldigs HD, Schaub J, Schutgens RBH, Wanders RJA (1993) Isolated defect of peroxisomal β-oxidation in a 16-year-old patient. *Eur J Pediatr* **152**: 339–342.

Schram AW, Goldfischer S, Van Roermund CWT, et al (1987) Human peroxisomal 3-oxoacyl-coenzyme A thiolase deficiency. *Proc Natl Acad Sci USA* **84**: 2494–2496.

Schutgens RBH, Romeijn GJ, Wanders RJA, et al (1984) Deficiency of acyl-CoA: dihydroxyacetonephosphate acyltransferase in patients with Zellweger (cerebro-hepato-renal) syndrome. *Biochem Biophys Res Commun* **120**: 179–184.

Schutgens RBH, Romeijn GJ, Ofman R, Van den Bosch H, Tager JM, Wanders RJA (1986) Acyl-CoA: dihydroxyacetonephosphate acyltransferase: study of its properties using a new assay method. *Biochim Biophys Acta* **879**: 286–291.

Schutgens RBH, Heijmans HSA, Wanders RJA, et al (1988) Multiple peroxisomal enzyme deficiencies in rhizomelic chondrodysplasia punctata: comparison with Zellweger syndrome, Conradi–Hunermann syndrome and X-linked dominant chondrodysplasia punctata. *Adv Clin Enzymol* **6**: 57–65.

Schutgens RBH, Schrakamp G, Wanders RJA, Heijmans HSA, Tager JM, Van den Bosch H (1989) Pre- and perinatal diagnosis of peroxisomal disorders. *J Inher Metab Dis* **12**: 118–134.

Suzuki Y, Shimozawa N, Yajima S, et al (1994) Novel subtype of peroxisomal acyl-CoA oxidase deficiency and bifunctional enzyme deficiency with detectable enzyme proteins: identification by means of complementation analysis. *Am J Hum Genet* **54**: 36–43.

Van den Bosch H, Schutgens RBH, Wanders RJA, Tager JM (1992) Biochemistry of peroxisomes. *Annu Rev Biochem* **61**: 157–197.

Van Maldergem L, Espeel M, Wanders RJA, et al (1992) Neonatal seizures and severe hypotonia in a male infant suffering from a defect in peroxisomal β-oxidation. *Neuromusc Disord* **2**: 217–224.

Wanders RJA, Van Weringh G, Schrakamp G, Tager JM, Van den Bosch H, Schutgens RBH (1985) Deficiency of acyl-CoA: dihydroxyacetonephosphate acyltransferase in thrombocytes of Zellweger patients: a simple postnatal test. *Clin Chim Acta* **151**: 217–221.

Wanders RJA, Van Roermund CWT, Van Wijland MJA, et al (1987) Peroxisomal fatty acid β-oxidation in relation to the accumulation of very-long-chain fatty acids in cultured skin fibroblasts from patients with Zellweger syndrome and other peroxisomal disorders. *J Clin Invest* **80**: 1778–1783.

Wanders RJA, Heijmans HSA, Schutgens RBH, Barth PG, Van den Bosch H, Tager JM (1988) Peroxisomal disorders in neurology. *J Neurol Sci* **88**: 1–39.

Wanders RJA, Schelen A, Feller N, et al (1990a) First prenatal diagnosis of acyl-CoA oxidase deficiency. *J Inher Metab Dis* **13**: 371–374.

Wanders RJA, Van Roermund CWT, Schelen A, et al (1990b) Bifunctional protein with deficient activity: identification of a new peroxisomal disorder. *J Inher Metab Dis* **13**: 375–379.

Wanders RJA, Schumacher H, Heikoop J, Schutgens RBH, Tager JM (1992a) Human dihydroxyacetonephosphate acyltransferase deficiency: A new peroxisomal disorder. *J Inher Metab Dis* **15**: 389–391.

Wanders RJA, Van Roermund CWT, Brul S, Schutgens RBH, Tager JM (1992b) Bifunctional enzyme deficiency: identification of a new type of peroxisomal disorder in a patient with an impairment in peroxisomal β-oxidation of unknown aetiology by means of complementation analysis. *J Inher Metab Dis* **15**: 385–388.

Wanders RJA, Schutgens RBH, Barth PG, Tager JM, Van den Bosch H (1993) Postnatal diagnosis of peroxisomal disorders: a biochemical approach. *Biochimie* **75**: 269–279.

Watkins PA, Chen WW, Harris CJ, et al (1989) Peroxisomal bifunctional enzyme deficiency. *J Clin Invest* **83**: 771–777.

Webber KO, Hajra AK (1993) Purification of dihydroxyacetonephosphate acyltransferase from guinea pig liver peroxisomes. *Arch Biochem Biophys* **300**: 88–97.