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# Studies of dihydroxyacetone phosphate acyltransferase in rat small intestine

## Subcellular localization and effect of partially hydrogenated fish oil and clofibrate

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The subcellular localization of dihydroxyacetone phosphate acyltransferase (DHAPAT) activity in rat small intestine was investigated by Nycodenz-gradient centrifugation. We found that DHAPAT had a predominant peroxisomal distribution, with a separate enzyme activity located in the microsomal fraction, the same distribution as found in rat liver. The effect of feeding rats on a diet with 20% (w/w) partially hydrogenated fish oil (PHFO) or 0.3% clofibrate on the activity of DHAPAT in rat small intestine and liver was studied. Both 20% PHFO and 0.3% clofibrate gave a 1.8-fold stimulation of the specific activities of DHAPAT in peroxisomes of the small intestine, whereas in the liver 20% PHFO gave a 1.4-fold stimulation and 0.3% clofibrate a 1.6-fold stimulation of the total DHAPAT activities in the postnuclear supernatant. The specific activities of DHAPAT in liver were not affected.

## INTRODUCTION

The rat small intestine is an important site for lipid metabolism in the organism. The absorptive cells contain large numbers of peroxisomes, which are responsible for a significant part of the metabolic activity of these cells (Novikoff & Novikoff, 1972). Peroxisomes contain various enzymes participating in lipid catabolism and anabolism (Lazarow & de Duve, 1976; Horie *et al.*, 1990). Peroxisomal $\beta$-oxidation of fatty acids has been well characterized in rat small intestine, and the $\beta$-oxidation activity has been shown to increase after treatment with various compounds such as clofibrate and partially hydrogenated fish oil (PHFO) (Thomassen *et al.*, 1985). The intestine has also been shown actively to incorporate plasmalogen precursors into plasmalogens (Das & Hajra, 1988).

The first enzyme in plasmalogen synthesis, dihydroxyacetone phosphate (DHAP) acyltransferase (DHAPAT), is mainly localized in the peroxisomal fraction in rat liver (Hajra *et al.*, 1979; Hajra & Bishop, 1982), but there has been some controversy about possible microsomal and mitochondrial activities as well (Datta & Hajra, 1984; Declercq *et al.*, 1984; Hardeman & van den Bosch, 1988; Singh *et al.*, 1989).

In the present work we have studied DHAPAT in rat small intestine. By using Nycodenz-gradient centrifugation, we have compared the subcellular distribution of this enzyme in rat small intestine and liver. We have further investigated whether peroxisomal DHAPAT activities were stimulated by conditions previously known to increase peroxisomal $\beta$-oxidation in rat liver and intestine.

## MATERIALS AND METHODS

### Materials

Clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropionate] was obtained from Weiders Pharmaceutical Co. A/S, Oslo, Norway. PHFO, from capelin, was obtained from DeNoFa and Lilleborg Fabrikker A/S, Fredrikstad, Norway. The fish oil contained 30% $C_{20:1}$ and $C_{22:1}$ fatty acids. The proportion of *trans* double bonds was 0.55. For further details see Nilsson *et al.* (1984). [1-$^{14}$C]Palmitoyl-CoA and L-[U-$^{14}$C]glycerol 3-phosphate were obtained from Amersham International, Amersham, Bucks., U.K., and Nycodenz was from Nycomed A/S, Oslo, Norway. Other chemicals were commercially available products of high purity from Sigma Chemical Co., St. Louis, MO, U.S.A.

### Animals and diets

Male Wistar rats weighing about 100 g were purchased from Møllegaard Breeding Laboratory, Ejby, Denmark. Housing conditions were as described by Nilsson *et al.* (1984), and the composition of the standard pelleted diet, obtained from EWOS AB, Södertälje, Sweden, was as described by Christiansen *et al.* (1986). Clofibrate was dissolved in acetone and soaked into pellets (0.3%, w/w), and the acetone was then evaporated. The rats were fed on clofibrate-treated pellets or PHFO (20%, w/w)-containing semi-synthetic diet for 10 days; for further details see Nilsson *et al.* (1984).

### Preparation of crude subcellular fractions and Nycodenz-gradient centrifugation

Liver peroxisomes were prepared as described by Prydz *et al.* (1988), with modification introduced by Farrants *et al.* (1989). Intestinal peroxisomes were prepared as described by Thomassen *et al.* (1985), with some modifications. The homogenate was washed in 2 mM-potassium phosphate buffer, pH 7.4, and centrifuged at 160 *g* for 10 min. The post-nuclear supernatant (E fraction) obtained was then filtered through a double nylon cloth (pore size 100 $\mu$m), to remove mucus. The E fraction was further centrifuged at 64000 *g* for 60 min. The pellet obtained (particle fraction) was suspended in 2 mM-potassium phosphate buffer, pH 7.4. A 1 ml portion of this fraction was layered on top of a linear Nycodenz gradient from 12% (w/w) in 0.25 M-sucrose/

Abbreviations used: DHAP, dihydroxyacetone phosphate; DHAPAT, dihydroxyacetone phosphate acyltransferase; PHFO, partially hydrogenated fish oil.

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1 mM-EDTA/1 mM-Hepes, pH 7.4, to 40% in 1 mM-EDTA/1 mM-Hepes, pH 7.4, and with 3 ml of Maxidenz as a cushion at the bottom. Centrifugation was performed at 33000 *g* for 75 min in a vertical rotor, at 8 °C. Fractions (5 ml) were collected, diluted in 2 mM-potassium phosphate buffer, pH 7.4, and centrifuged at 65000 *g* for 45 min. The pellets were resuspended in a buffer containing 0.25 M-sucrose, 1 mM-EDTA and 5 mM-Hepes, pH 7.4.

### Enzyme assays and analytical procedures

To identify the organelle fractions, the following marker enzymes were used; catalase (peroxisomes), Baudhuin *et al.* (1964); esterase (microsomes), Beaufay *et al.* (1974); succinate dehydrogenase (mitochondria), Pennington (1961). Protein was determined by the method of Lowry *et al.* (1951). In fractions containing Nycodenz, a modified method described by Bensadoun & Weinstein (1976) was used. Peroxisomal $\beta$-oxidation was determined by conversion of acid-insoluble [$^{14}$C]-palmitoyl-CoA into acid-soluble radioactivity as described by Lazarow (1981).

DHAPAT activity was determined essentially as described by Schutgens *et al.* (1986). [U-$^{14}$C]DHAP was prepared from L-[U-$^{14}$C]glycerol 3-phosphate (10 $\mu$Ci). The reaction medium contained in addition 5 mM-pyruvate, 1 mM-$NAD^+$, 10 units of lactate dehydrogenase and 10 units of glycerol-3-phosphate dehydrogenase in 15 mM-triethanolamine/HCl buffer, pH 7.6, in a total volume of 1 ml. Reactions were carried out at 25 °C for 60 min, and stopped by addition of an equal volume of chloroform, and the resulting suspension was mixed vigorously for 5 min. The upper layer containing the labelled DHAP was pipetted off after centrifugation. A 20 $\mu$l portion of the $^{14}$C-labelled DHAP preparation (30000 c.p.m./nmol) was used in the standard DHAPAT assay, containing the following components: sodium acetate buffer 75 mM, pH 5.4, NaF 8 mM, $MgCl_2$ 8 mM, BSA 41.7 mg/ml, palmitoyl-CoA 0.4 mM (molar ratio palmitoyl-CoA/albumin 0.8), DHAP 0.1 mM and 20–80 $\mu$g of sample protein, in a total volume of 0.12 ml. The incubations were carried out at 37 °C and stopped with chloroform/methanol as described by Schutgens *et al.* (1984), after 40 min for the liver samples and 10 min for the intestinal samples.

### Statistics

The data were analysed by the Mann–Whitney non-parametric test, one-tailed (MINITAB statistical program, from Minitab Inc., State College, PA, U.S.A.).

## RESULTS

### Incubation conditions and fatty acid substrate specificity

Optimal incubation conditions for measuring the DHAPAT activity in crude peroxisomes isolated from small intestine and liver were found to be very similar, and essentially as described previously for the liver peroxisomal enzyme (Schutgens *et al.*, 1986). However, although DHAP acylation increased linearly for about 45 min, followed by a small decline for another 45 min, and finally a rapid decline when liver peroxisomes were used, intestinal peroxisomes revealed a rapid decline in the amount of acylated product after 20 min (Fig. 1*a*). Consequently, shorter incubation times (10 min in our standard assay procedure) were always used with preparations from small intestine. Slight differences were observed in the relationship between protein concentration and DHAPAT activity (results not shown), where linearity was obtained with the intestinal enzyme only at protein concentrations less than 0.4 mg/ml. For liver, linearity was obtained with protein concentrations less than 0.8 mg/ml.

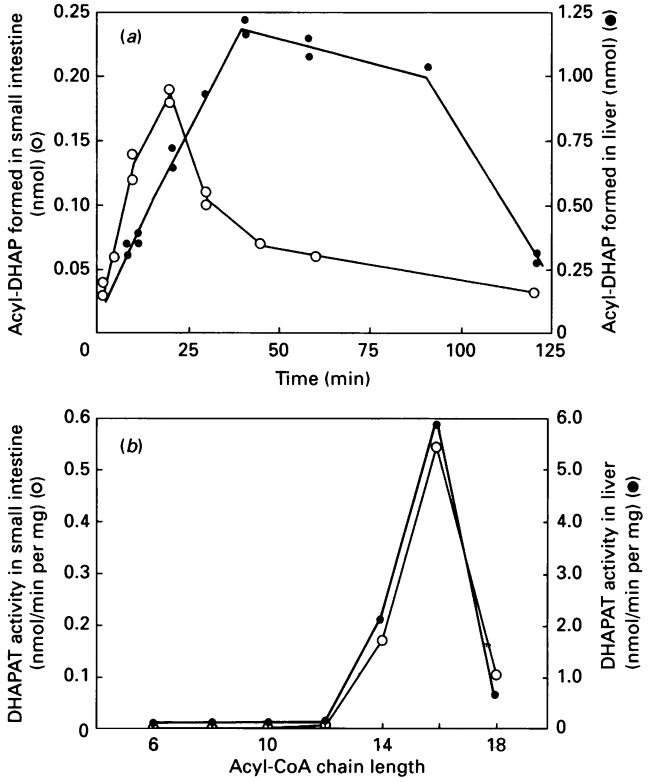


**Fig. 1. Incubation conditions and fatty acid substrate specificity of DHAPAT**

(*a*) Activity in crude peroxisomes as a function of time (protein concn. 0.3 mg/ml). (*b*) Activity in gradient-purified peroxisomes as a function of chain length of saturated fatty acids; values are means of three samples. Peroxisomes were prepared from rat small intestine and rat liver, and assayed for DHAPAT activity (formation of acyl-DHAP, as described in the Materials and methods section).

**Table 1. DHAPAT relative specific activity in peroxisomes from rat small intestine and liver with different unsaturated fatty acids as substrates**

In each experiment two animals were used. The experiments (1–3) were performed on separate days. The activity (in %) is calculated for each experiment ($C_{16:0}$ = 100%): 100% liver activity is 6.07 ± 0.50 nmol/min per mg; 100% intestinal activity is 0.54 ± 0.20 nmol/min per mg. Results are means ± S.D. ($n$ = 3).

| | DHAPAT relative specific activity | |
|---|---|---|
| Substrate | Small intestine | Liver |
| $C_{16:0}$ | 100 | 100 |
| $C_{18:0}$ | 13.4 (±8.1) | 14.3 (±5.8) |
| $C_{18:1,n-9}$ | 4.4 (±1.6) | 2.7 (±2.4) |
| $C_{18:2,n-6}$ | 0.3 (±0.3) | 0.7 (±1.0) |
| $C_{20:4,n-6}$ | 0.0 | 0.2 (±0.2) |

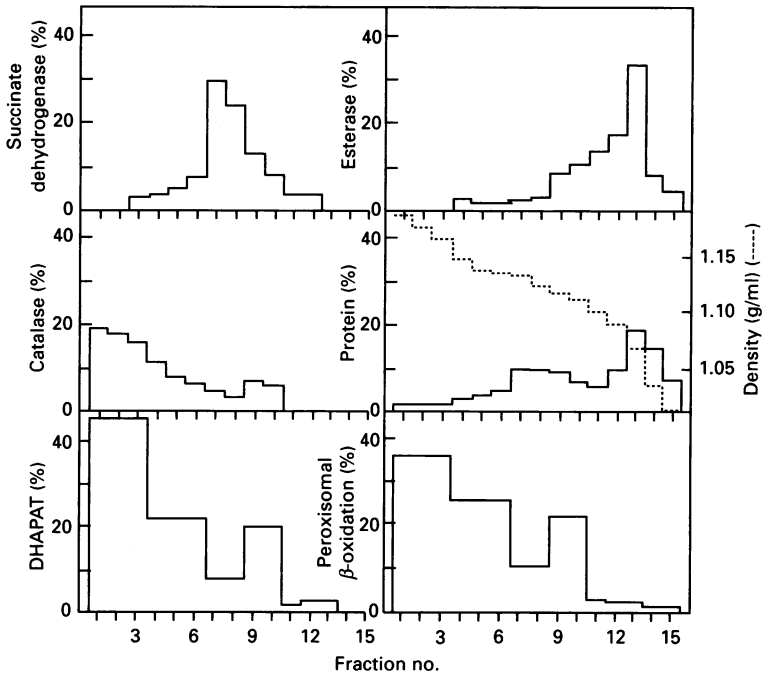


**Fig. 2. Subcellular distribution of DHAPAT activity in rat small intestine**

A particle fraction (obtained by centrifugation of the post-nuclear supernatant), which contained approx. 90% of the activity in the post-nuclear supernatant, was layered on top of a linear Nycodenz gradient from 12% (w/w) in 0.25 M-sucrose/1 mM-EDTA/1 mM-Hepes buffer, pH 7.4, to 40% in 1 mM-Hepes/1 mM-EDTA and centrifuged as described in the Materials and methods section. Protein distribution and enzyme activities are given as percentages of total amount and total activities respectively, and the numbers on the abscissa represent fraction numbers. Analytical procedures are described in the Materials and methods section.

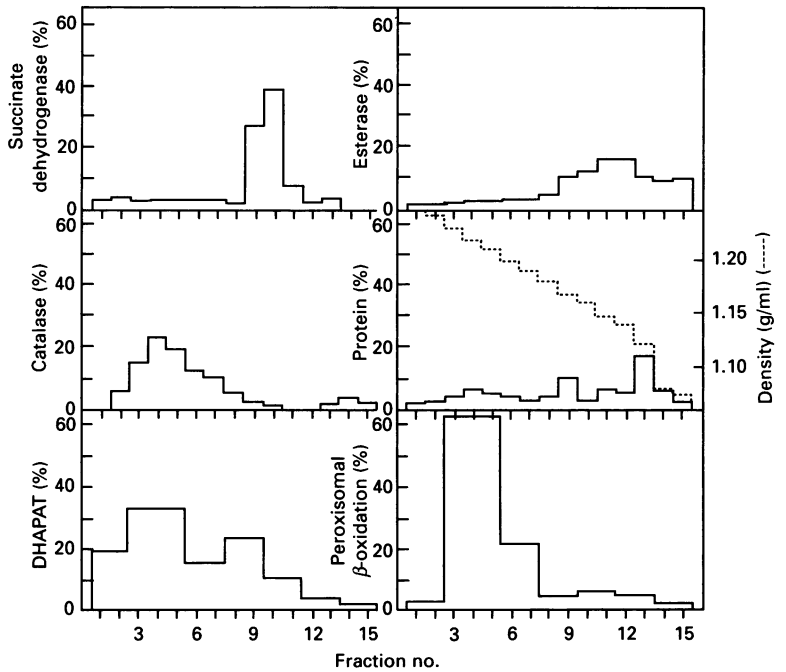


**Fig. 3. Subcellular distribution of DHAPAT activity in rat liver**

Light-mitochondrial fractions from rat liver were layered on top of a linear Nycodenz gradient from 12% (w/w) in 0.25 M-sucrose/1 mM-EDTA/1 mM-Hepes, pH 7.4, to 44% in 1 mM-EDTA/1 mM-Hepes and centrifuged as described in the Materials and methods section. Protein distribution and enzyme activities are given as percentages of total amount and total activities respectively, and the numbers on the abscissa represent fraction numbers. Analytical procedures are described in the Materials and methods section.

Palmitoyl-CoA was found to be the best substrate for the intestinal DHAPAT as well as for the liver enzyme, whereas the activity with longer or shorter chain-length fatty acids was much smaller (Fig. 1*b*). The unsaturated fatty acids were poor substrates for DHAPAT in intestine as well as in liver. Oleoyl-CoA initiated an activity less than one-third of that with stearic acid, and with

linoleic and arachidonic acids only minor activities were observed (Table 1).

### Subcellular distribution of DHAPAT in intestine and liver

Cell fractionation of the intestinal homogenate revealed that at least 85–95% of the DHAPAT activity of the post-nuclear supernatant (E fraction) could be recovered in a particle fraction. When this fraction was further fractionated on a Nycodenz gradient (Fig. 2), more than 70% of the DHAPAT activity was found to have a localization very similar to that of peroxisomal β-oxidation and catalase (fractions 1–6), demonstrating a predominantly peroxisomal localization of this enzyme in intestinal mucosal cells. About 25% of the activity was found in fractions exhibiting high succinate dehydrogenase activity (fractions 7–10), indicating a mitochondrial localization also. But the high activity of DHAPAT, catalase and peroxisomal β-oxidation in the last of these fractions, and the low activity of succinate dehydrogenase in the same fractions compared with the first mitochondrial fractions (7–8), suggests a secondary fraction of destroyed peroxisomes. Less than 5% of the total DHAPAT activity in intestinal homogenates were recovered in fractions exhibiting high esterase activity (fractions 11–14).

It has been reported that extra-peroxisomal DHAPAT activity in rat liver is inhibited by glycerol 3-phosphate, whereas the peroxisomal activity is not inhibited (Declercq *et al.*, 1984; van Veldhoven & Mannaerts, 1985). The presence of peroxisomal β-oxidation activity in the mitochondrial fraction, together with the fact that this activity was not inhibited by glycerol 3-phosphate, indicated that there were peroxisomal fragments in this fraction. The DHAPAT activity in the microsomal fraction was partially inhibited by glycerol 3-phosphate, indicating an enzyme different from the peroxisomal enzyme (results not shown).

The subcellular distribution of DHAPAT activity in the liver was also studied. In this case a peroxisome-enriched L-fraction was fractionated on the Nycodenz gradient (Fig. 3). The distribution of DHAPAT in subcellular fractions was approximately the same as for the intestine.

### Effects of the peroxisome proliferators PHFO and clofibrate

In the small intestine there was a significant increase in the specific activity of DHAPAT after treatment with both PHFO (1.8-fold) and with clofibrate (1.8-fold) (Table 2). There was also a significant increase in peroxisomal β-oxidation with PHFO (2.0-fold) and clofibrate (2.8-fold). The peroxisome proliferators did not affect DHAPAT specific activity in the liver to a great extent.

A comparison of total enzyme activities in the intestine was not meaningful, since there were great differences in the yield of mucosal cells from scrapings of the intestinal wall from preparation to preparation.

In the liver (Table 3) there was a significant increase in total

**Table 2. Effect of PHFO and clofibrate on the specific activity of DHAPAT and peroxisomal β-oxidation in peroxisomes from rat small intestine and liver**

Rats (150 g) were fed on pellets (control) or pellets with 0.3% clofibrate or 20% PHFO for 10 days as described in the Materials and methods section. The enzyme activities were measured in peroxisomes from small intestine and liver, purified by Nycodenz-gradient centrifugation as described in the Materials and methods section. Values are means ± S.D. ($n = 4$), and given in nmol/min per mg: *significantly different from control ($P \leq 0.05$).

| | Control | | PHFO | | Clofibrate | |
|---|---|---|---|---|---|---|
| | Intestine | Liver | Intestine | Liver | Intestine | Liver |
| DHAPAT | 0.28 (±0.09) | 1.31 (±0.10) | 0.50* (±0.13) | 1.49 (±0.08) | 0.52* (±0.15) | 0.89 (±0.18) |
| Peroxisomal β-oxidation | 2.88 (±0.42) | 13.01 (±4.16) | 5.83* (±1.65) | 50.83* (±0.47) | 8.07* (±1.65) | 61.44* (±0.95) |

**Table 3. Effect of PHFO and clofibrate on the activity of DHAPAT and peroxisomal β-oxidation in rat liver**

Animal treatments and measurement of enzyme activities were as described in Table 2 and the Materials and methods section. The enzyme activities of DHAPAT, β-oxidation and catalase, together with protein, were measured in post-nuclear supernatants from rat liver. The total activities for DHAPAT are given in nmol/min per g of liver, and the total activities for β-oxidation and catalase are given in μmol/min per g of liver. Protein content is given in mg/ml, and all the specific activities are in nmol/min per mg. Values are means ± S.D. ($n = 4$): *significantly different from control ($P \leq 0.05$).

| | Control | | PHFO | | Clofibrate | |
|---|---|---|---|---|---|---|
| Enzyme | Total activity | Specific activity | Total activity | Specific activity | Total activity | Specific activity |
| DHAPAT | 10.12 (±1.46) | 0.06 (±0.01) | 14.05* (±2.72) | 0.06 (±0.01) | 16.17* (±8.83) | 0.08 (±0.02) |
| β-Oxidation | 0.94 (±0.15) | 9.33 (±5.68) | 2.04* (±0.46) | 11.40 (±2.50) | 5.87* (±0.71) | 33.80* (±4.81) |
| Catalase | 87.22 (±26.77) | 0.49 (±0.21) | 68.32 (±25.67) | 0.44 (±0.13) | 107.36 (±26.68) | 0.58 (±0.19) |
| Protein | 184.20 (±25.62) | | 194.50 (±2.90) | | 195.83 (±22.85) | |

DHAPAT activity (E fractions) with PHFO (1.4-fold) and clofibrate (1.6-fold). The total peroxisomal $\beta$-oxidation increased to a higher extent, 2.2-fold with PHFO and 6.3-fold with clofibrate.

## DISCUSSION

The small intestine is of central importance in lipid metabolism, although its role in ether lipid synthesis is less known. The subcellular distribution of DHAPAT in the intestine from guinea pigs was recently investigated by Gitsman *et al.* (1989). It was shown that DHAPAT was located exclusively in the peroxisomal fraction, just as in the guinea-pig liver (Jones & Hajra, 1977).

Our present results suggest that DHAPAT in rat small intestine has the same localization as the corresponding activity in rat liver, i.e. a predominant peroxisomal activity (70%). This activity is not inhibited by glycerol 3-phosphate. The DHAPAT activity demonstrated in the mitochondrial fraction (25%) was presumably due to peroxisomal fragments, since the fraction also contained some peroxisomal $\beta$-oxidation activity which corresponded well to the DHAPAT activity. The DHAPAT activity found in the mitochondrial fraction was not inhibited by glycerol 3-phosphate.

Some activity was also found in the microsomal fraction (5%), but this activity was partially inhibited by glycerol 3-phosphate. This is in accordance with results of Declercq *et al.* (1984) for the liver enzyme, indicating an enzyme different from the peroxisomal one.

The subcellular localization of DHAPAT in rat liver has been investigated by several groups, and it is generally agreed that this activity is mainly located in the peroxisomal fraction (Hajra & Bishop, 1982). Some controversy still exists in the literature about the possibility of separate enzymes in mitochondrial and/or microsomal fractions (Datta & Hajra, 1984; Declercq *et al.*, 1984; Hardeman & van den Bosch, 1988; Singh *et al.*, 1989).

Results from this study confirm previous reports (Hajra & Bishop, 1982) of a predominant peroxisomal localization of DHAPAT in rat liver. However, as in several of the other investigations mentioned above, some activity was also demonstrated in the mitochondrial and the microsomal fractions. The activity of DHAPAT found in the microsomal fraction was partly inhibited by glycerol 3-phosphate, but there was no inhibition of DHAPAT in the peroxisomal and mitochondrial fractions. This indicates that the microsomal fraction contains a DHAPAT enzyme different from the peroxisomal enzyme, and that the mitochondrial enzyme activity may derive from peroxisomal fragments. This view is shared by Patel *et al.* (1987) and Skorve *et al.* (1990). Jones & Hajra (1977) found that in guinea-pig liver the DHAPAT activity is present only in peroxisomes, in contrast with rat liver, where it is present also in microsomes. This indicates that there may be differences in the organelle distribution of DHAPAT between different species.

The substrate specificity of DHAPAT from rat intestine and liver was very similar to what was previously found in guinea-pig liver (Hajra, 1968; Jones & Hajra, 1983) and in rat brain (Hajra & Burke, 1978). This means that DHAPAT exhibits a specificity for saturated acyl-CoAs (Fig. 1*b*) as substrates, with low activities for mono- and poly-unsaturated acyl-CoAs (Table 1).

Peroxisomal $\beta$-oxidation has been found to increase in rat liver, small intestine and several other organs after treatment with peroxisomal proliferators (Norseth, 1980; Norseth & Thomassen, 1983; Lock *et al.*, 1989; Reubsaet *et al.*, 1990). In liver, several studies have revealed an increase also in total DHAPAT activity (Burke & Hajra, 1980; Horie *et al.*, 1990; Hardeman *et al.*, 1990; Mikalsen *et al.*, 1990; Skorve *et al.*, 1990).

We also studied the activity of DHAPAT in rat liver and small intestine under conditions known to induce peroxisomal $\beta$-oxidation. We found that the high-fat diet containing PHFO, and the hypolipidaemic diet containing clofibrate, induced the total activity of DHAPAT in rat liver 1.3- and 1.5-fold respectively. Our results are consistent with the report by Horie *et al.* (1990).

The proliferators did not affect the specific activity of DHAPAT and catalase in the peroxisomal fraction in the liver. These data indicate that the increase in total activity of DHAPAT and catalase is a consequence of the increase in volume and/or number of peroxisomes, and is not caused by an increase in the amount of enzymes in the peroxisomes, as is the case for $\beta$-oxidation activity. This is in agreement with recent work by Hardeman *et al.* (1990).

Stimulation of DHAPAT in rat small intestine has, to our knowledge, not been demonstrated previously. We found a 1.8-fold increase in specific DHAPAT activity, with both PHFO and clofibrate. This increase was not observed in the liver (as discussed above). As exemplified by peroxisomal $\beta$-oxidation, there may be a relatively smaller increase in other enzymes compared with DHAPAT in the intestine than was observed in the liver.

In conclusion, the intestinal DHAPAT seems to have very much in common with the liver enzyme. This concerns optimal incubation conditions, substrate specificity and subcellular distribution. The only major difference is the higher specific activity of the hepatic enzyme (Table 1).

Most of the intestinal activity is located in the peroxisomal fraction, but there is also some activity present in the microsomal fraction.

A number of peroxisome proliferators are able to stimulate the activity of DHAPAT in the liver (Burke & Hajra, 1980; Horie *et al.* 1990; Hardeman *et al.*, 1990; Mikalsen *et al.*, 1990; Skorve *et al.*, 1990). In the present work we also found a significant increase in the DHAPAT activity in the small intestine after treatment with such compounds. This suggests that DHAPAT follows a general trend for a number of peroxisomally located enzymes in lipid metabolism, in being stimulated by peroxisomal proliferators.

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