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# MICROSOMAL CYTOCHROME P-452 INDUCTION AND PEROXISOME PROLIFERATION BY HYPOLIPIDAEMIC AGENTS IN RAT LIVER

## A MECHANISTIC INTER-RELATIONSHIP

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**Abstract**—Eight structurally diverse hypolipidaemic agents have been examined for their ability to induce the microsomal cytochrome P-452-dependent fatty acid hydroxylase system and the enzymes of peroxisomal β-oxidation in rat liver. Using a specific ELISA method, we have shown that the cytochrome P-452 isoenzyme is induced up to ten fold by hypolipidaemic challenge, concomitant with a pronounced elevation of the peroxisomal β-oxidation enzymes, mirrored by an increase in peroxisomal volume as determined morphometrically. In addition, the induction of cytochrome P-452 is accompanied by a decrease in the activities of cytochromes P-450$_b$ and P-450$_e$ as measured by benzphetamine *N*-demethylase and ethoxyresorufin *O*-deethylase activities respectively, the latter being more extensively reduced by hypolipidaemic treatment. A hypothesis is presented whereby an early biological response is the hypolipidaemic induction of microsomal cytochrome P-452 resulting in ω-hydroxy fatty acids and their subsequent further oxidation to dicarboxylic acids, the latter providing the proximal stimulus for peroxisomal proliferation.

The administration of hypolipidaemic agents to rodents results in a significant proliferation of cellular organelles including peroxisomes and the smooth endoplasmic reticulum [1–4]. Certain of these agents have also been associated with the production of liver tumours in rodents [3–5]. Although the precise relationship between the early subcellular liver changes observed on exposure to hypolipidaemic agents and tumour formation still remains to be established, it is likely that the sustained proliferation of peroxisomes in rodents is frequently associated with the development of hepatocellular carcinomas [6].

The relationship, if any, between the hypolipidaemic-mediated induction of cytochrome P-452,‖ a unique microsomal fatty acid hydroxylase first isolated and characterised by Tamburini *et al.* [7], and peroxisome proliferation is at present unclear. Whether this co-induction is a result of interaction with a common cytosolic receptor protein [8], or a result of common or related organelle biogenesis remains to be established [9, 10]. Clofibrate has been shown to elevate the cytochrome P-452 driven ω-hydroxylation of medium-length fatty acids by 10–20-fold [2, 11]. The magnitude and specificity of this latter increase suggests that ω-hydroxylation may play an important role in the accelerated catabolism of fatty acids associated with the lipid-lowering action of this hypolipidaemic agent, although the relative contributions of both ω- and β-oxidation of fatty acids to the pharmacology of hypolipidaemic agents remains unclear at present.

The present study examines the short term effects of eight structurally diverse hypolipidaemic agents on rat liver and attempts to explore any possible correlation between the induction or depression of a spectrum of enzymes related to both drug and lipid metabolism. Additionally, quantitative morphometric analyses on hepatic peroxisome populations were performed and related to the latter observed enzyme changes.

### MATERIALS AND METHODS

*Chemicals.* The hypolipidaemic agents were obtained from the following sources: Wy-14,643

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‖ Abbreviations used: Wy, Wy-14,643; DEHP, di-(2-ethylhexyl)phthalate; MEHP, mono-(2-ethylhexyl)phthalate; ASP, aspirin; BEZA, bezafibrate; NAF, nafenopin; CLOF, clofibrate; CLOB, clobuzarit; cytochrome P-450$_b$, that isoenzyme of cytochrome P-450 induced by phenobarbital and active in the metabolism of benzphetamine; cytochrome P-450$_e$, that isoenzyme of cytochrome P-450 induced by β-naphthoflavone and active in the metabolism of ethoxyresorufin; cytochrome P-452, that isoenzyme of cytochrome P-450 that catalyses the ω-hydroxylation of fatty acids and whose comparative protein biochemistry is detailed in Ref. 7; LA 11-OH, lauric acid 11-hydroxylase activity; LA 12-OH, lauric acid 12-hydroxylase activity; PCoA, KCN-insensitive, palmitoyl-CoA oxidation; CAT, carnitine acetyl transferase; CPT, carnitine palmitoyl transferase; enoyl CoA, total or heat labile enoyl CoA hydratase activity; BZP, benzphetamine *N*-demethylase activity; EROD, ethoxyresorufin *O*-deethylase activity; ELISA, enzyme linked immunosorbent assay.

from Wyeth Laboratories (Radnor, PA); bezafibrate from Boehringer Ingelheim (F.R.G.); clofibrate and clobuzarit were gifts from I.C.I. plc, Pharmaceuticals Division (Macclesfield, U.K.); nafenopin (Ciba-Geigy, Basle, Switzerland); MEHP was synthesised as precisely described [12] and DEHP (98+% pure) was obtained from Lancaster Synthesis (Morecambe, U.K.; sodium salicylate from B.D.H. (U.K.).

Lauric acid and NADPH were purchased from the Sigma Chemical Co. (Poole, Dorset, U.K.). [1-$^{14}$C]Lauric acid was supplied by the Radiochemical Centre (Amersham, Bucks., U.K.). Benzphetamine was obtained from the Upjohn Co. (Kalamazoo, MI, U.S.A.).

All other chemicals were obtained from commercial sources and were of the highest purity available.

*Animals and drug pretreatment.* Male Wistar albino rats (150–200 g body weight, University of Surrey breeders) were pretreated by gastric intubation once daily for 3 days at the following dose-levels: Wy-14,643, 250 mg/kg; DEHP, 1200 mg/kg; MEHP, 100 mg/kg; aspirin, 500 mg/kg; bezafibrate, 200 mg/kg; nafenopin, 180 mg/kg; clofibrate, 250 mg/kg; clobuzarit, 50 mg/kg.

The above compounds were administered in peanut oil as the vehicle, control animals given peanut oil at 5 ml/kg by gastric intubation, and all the animals were killed at the start of the fourth day after commencement of treatment (i.e. 24 hr after the last dose). The livers were removed and slices taken and processed for electron microscopy as previously described [13]. Morphometric analyses were performed on ultrathin sections of centrilobular hepatocytes according to the procedure described by Weibel *et al.* [14].

The livers were then perfused with 0.9% (w/v) saline prior to homogenisation. Samples of whole liver homogenate were taken for the determination of peroxisomal and microsomal marker enzymes, the latter fraction prepared by a standard calcium precipitation and centrifugation method.

*Enzyme assays.* Cytochrome P-450 was determined according to Omura and Sato [15] using a difference absorption coefficient (450 to 490 nm) of 91 $mM^{-1}cm^{-1}$ for the sodium dithionite-reduced carbon monoxide adduct. The specific, hypolipidaemic-induced cytochrome P-452 enzyme was determined immunochemically by an ELISA method, developed from the basic method of Voller *et al.* [16] as follows. The cytochrome P-452 antigen in solubilised hepatic microsomes was coated to the wells of a micro-titre plate in 0.1 M sodium carbonate/bicarbonate buffer, pH 9.6 at 4° for a minimum of four hours. A polyclonal antibody raised in sheep to the highly purified, electrophoretically homogenous preparation of rat cytochrome P-452 was then added to the antigen coated wells. The binding of preimmune serum to the cytochrome P-452 antigen was employed as the level of non-specific binding observed in this assay and was subtracted from the test, immune data. Following a 2 hr incubation at 37° and washing with phosphate buffered saline (PBS) plus gelatin and Tween 20, donkey anti-sheep label was attached to the anti-cytochrome P-452 sheep IgG molecule. After a further incubation at 37° for 2 hr and a PBS wash, the substrate orthophenyldiamine was added to the wells. After 30 min at 37° the reaction was terminated by the addition of 2.5 M $H_2SO_4$. The plate was then read at 490 nm. A calibration curve using electrophoretically homogenous cytochrome P-452 was constructed and shown to be linear up to 0.02 pmol of authentic cytochrome P-452 per assay.

In order to demonstrate that the binding of the cytochrome P-452 antibody was specific only to the cytochrome P-452 antigen, we firstly cross reacted our antibody with various cytochrome P-450 isoenzymes as antigens under ELISA conditions. Secondly, Western Blotting analysis using the ELISA concentrations of antigen and antibody was performed. This would enable us to visualize any non-specific binding which may not be apparent by ELISA analysis. This technique involves the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets which are then subject to immunological analysis. The procedures employed were modifications of the basic procedures of Towbin *et al.* [17] and Burnett *et al.* [18].

The 11- and 12-hydroxy metabolites of lauric acid [11] were separated by reverse-phase HPLC using a Micropak MCH-10 column (30 × 0.4 cm, Varian Associates Ltd., Walton-on-Thames, Surrey, U.K.). The metabolites were resolved using a linear gradient of water:methanol (45:55 containing 0.1% acetic acid) to 100% methanol over a 35 min period at a flow rate of 1.5 ml/min. The HPLC eluate containing radioactive metabolites, was passed through a Berthold LB503 Radiodetector flow-cell (Lab-Impex, Twickenham), interfaced with a Commodore PET (Series 4000), enabling quantitative analyses of lauric acid metabolism.

Benzphetamine *N*-demethylase (BZP) activity was determined by the colorimetric procedure of Nash [19] and ethoxyresorufin *O*-deethylation (EROD) was measure spectrofluorimetrically according to Burke *et al.* [20].

Tissue whole homogenates were assayed for activity of KCN-insensitive palmitoyl-CoA oxidation as described previously [21], but with the addition of 10 $\mu$M FAD to each cuvette. Carnitine acetyl-CoA transferase, carnitine palmitoyl-CoA transferase, total and heat labile enoyl-CoA hydratase activities were determined as described by Gray *et al.* [21], Lake *et al.* [22]; and Lake *et al.* [13, 22]. Protein concentration was determined by the method of Lowry *et al.* [23] using crystalline bovine serum albumin as the standard.

## RESULTS

The effects of hypolipidaemic administration on liver/body weight ratio, total cytochrome P-450 content and the specific cytochrome P-452 isoenzyme levels are shown in Table 1. Significant hepatomegaly was observed in all the tests groups, with a less marked increase being seen with MEHP and aspirin. All compounds tested significantly increased the total cytochrome P-450 specific content of liver microsomes, except bezafibrate and nafenopin.

Specific cytochrome P-452 isoenzyme levels in control animals by ELISA were shown to be 3–4% of the

Table 1. The effect of hypolipidaemic agents on liver size and cytochrome P-450/P-452 content in the rat

| Treatment | Dose level (mg/kg) | Liver/body weight ratio (%) | Total cytochrome P-450 specific content (nmol/mg) | Specific cyt. P-452 quantitation | |
|---|---|---|---|---|---|
| | | | | Specific cyt. P-452 (nmol/mg) | % Total cyt. P-450 |
| Peanut oil control | — | 5.34 ± 0.23 | 1.07 ± 0.17 | 0.040 ± 0.013 | 3.73 ± 0.83 |
| WY-14,643 | 250 | 7.41 ± 0.38*** | 1.48 ± 0.31* | 0.297 ± 0.060*** | 20.20 ± 2.29*** |
| DEHP | 1200 | 7.10 ± 0.38*** | 1.54 ± 0.14*** | 0.280 ± 0.045*** | 18.33 ± 3.14*** |
| MEHP | 100 | 6.30 ± 0.54** | 1.27 ± 0.09* | 0.108 ± 0.030*** | 8.47 ± 2.15*** |
| Aspirin | 500 | 6.56 ± 0.61** | 1.54 ± 0.39* | 0.148 ± 0.058*** | 9.55 ± 2.54*** |
| Bezafibrate | 200 | 7.80 ± 0.70*** | 1.21 ± 0.25 | 0.347 ± 0.107*** | 30.30 ± 8.79*** |
| Nafenopin | 180 | 7.45 ± 0.59*** | 1.27 ± 0.14 | 0.340 ± 0.094*** | 26.53 ± 5.48*** |
| Clofibrate | 250 | 6.80 ± 0.40*** | 1.43 ± 0.16* | 0.317 ± 0.063*** | 22.20 ± 4.55*** |
| Clobuzarit | 50 | 6.95 ± 0.18*** | 1.55 ± 0.26* | 0.317 ± 0.053*** | 20.63 ± 2.26*** |

Compounds were administered by gavage for 3 days at the dose levels indicated. Values are means ± SD from six individual animals. P values for results significantly differed (Student's *t*-test) from control data at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

total cytochrome P-450 population, and induction by hypolipidaemic agents produced a 3–7-fold induction over constitutive levels at the dose levels studied (Table 1). The specificity of the ELISA assay is further illustrated in Fig. 1, which shows the reactivity of the cytochrome P-452 antibody with different microsomal preparations.

Reverse phase HPLC analysis showed that hypolipidaemic treatment preferentially induced the 12-hydroxylation of lauric acid over the 11-hydroxylation in rat liver microsomes (Fig. 2), as exemplified by the 12/11 metabolite ratio of 1 to 4 in control and approximately 5 to 6 in the treated groups. Total 11- and 12-hydroxylation of lauric acid has been shown to be induced by other workers [24], and our laboratory has shown lauric acid to be a suitable marker substrate for cytochrome P-452 [2, 7]. In addition to the induction of laurate 12-hydroxylase activity, the catalytic activities of hypolipidaemic treated liver microsomes show markedly reduced activities for the dealkylation of both benzphetamine and ethoxyresorufin, the latter being generally accepted as good markers for the cytochromes $P\text{-}450_b$ and $P\text{-}450_c$ isoenzymes respectively (Fig. 3).

Hypolipidaemic agent induced liver homogenates showed significant increases in cyanide-insensitive palmitoyl-CoA oxidation by the peroxisomal $\beta$-oxidation pathway (Fig. 4). This is matched by an induction of total (mitochondrial and peroxisomal) and peroxisomal enoyl-CoA hydratase (heat labile) activities (Fig. 5), the latter a specific peroxisomal enzyme involved in the second step of the $\beta$-oxidation pathway. Total carnitine acetyl-CoA transferase, involved in the transfer of fatty acyl-CoA derivatives through the membranes of mitochondria and peroxisomes, was significantly elevated subsequent to

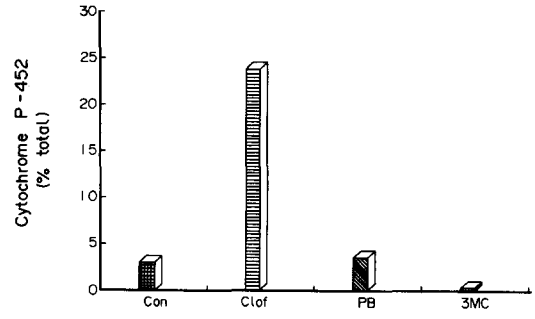


Fig. 1. Immunochemical detection of cytochrome P-452 in rat liver microsomes derived from livers pretreated with enzyme inducers. A polyclonal sheep antibody (1:4000 dilution) to electrophoretically homogeneous rat liver cytochrome P-452 was used in an ELISA procedure as described in Materials and Methods. Equivalent amounts of *total*, carbon monoxide-discernible cytochrome P-450 derived from each microsomal preparation were subjected to the ELISA analysis, and the cytochrome P-452 content expressed as a percentage of the total cytochrome P-450 population. The microsomal preparations were derived from either control (con), clofibrate (clof), phenobarbital (PB) or 3-methylcholanthrene (3MC)-pretreated animals and diluted appropriately to fall within the range of the authentic cytochrome P-452 standard curve.

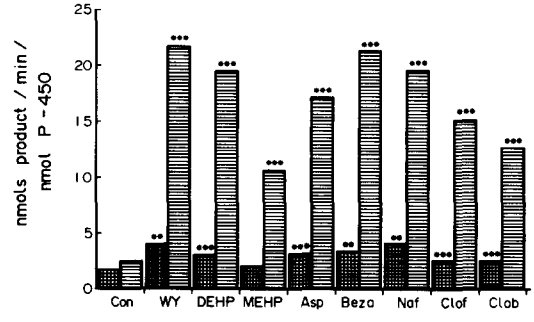


Fig. 2. Influence of hypolipidaemic pretreatment on the 11- and 12-hydroxylation of lauric acid by rat liver microsomes. Rats (6 animals per group) were pretreated with the hypolipidaemic agents as in Table 1, microsomal homogenates prepared and lauric acid 11- and 12-hydroxylase activities determined by reverse phase HPLC as described in Materials and Methods. ■, 11-hydroxylase and ▤, 12-hydroxylase. Abbreviations for the individual inducers are as previously defined. ** $P < 0.01$; *** $P < 0.001$.

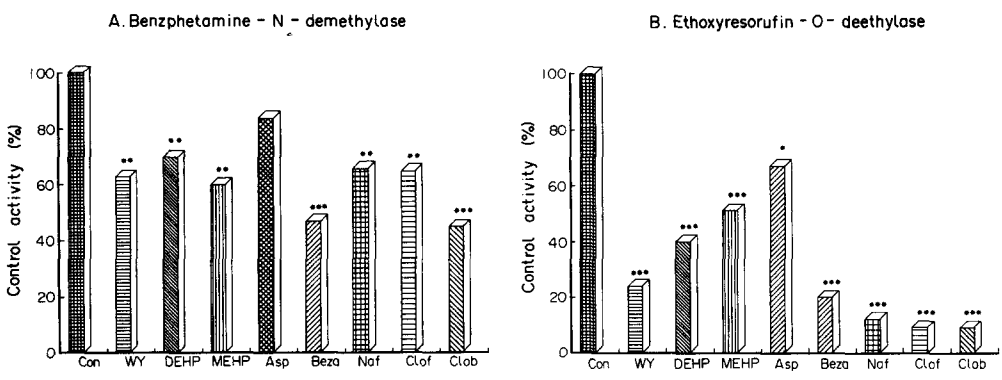


Fig. 3. Influence of hypolipidaemic pretreatment on rat hepatic drug-metabolising activity. Rats (6 per group) were pretreated with the hypolipidaemic agents as in the legend to Table 1. (A) Benzphetamine *N*-demethylase activities are expressed as nmol HCHO formed/min/mg microsomal protein where the 100% control turnover is 16. (B) Ethoxyresorufin *O*-deethylase activities are expressed as pmol resorufin formed/min/mg microsomal protein, where the 100% value is 77. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

hypolipidaemic challenge, as was carnitine palmitoyl-CoA transferase activity, a specific mitochondrial enzyme (Fig. 6).

Morphological studies performed on the livers of control and hypolipidaemic treated animals show marked subcellular changes between the two groups, as exemplified for control and clofibrate pretreated liver (Fig. 7). Although proliferation of the smooth endoplasmic reticulum, as indicated previously by the results in Table 1, was not clearly seen in the clofibrate treated liver, a large induction of the dark staining peroxisomes was clearly evident. These results are supported by measurements of peroxisomal volume (Table 2). Peroxisomal volume, measured as a percentage of the cytoplasmic volume, is induced between 2–5-fold after hypolipidaemic treatment.

In order to examine more fully the inter-relationship between the hypolipidaemic-dependent induction of microsomal and peroxisomal parameters, a correlation matrix was constructed in order to highlight positive and negative correlations which may exist between these various liver enzyme changes (Table 3). No correlation was obtained between the levels of total cytochrome P-450 and any of the other parameters studied. However, specific cytochrome P-452 isoenzyme levels correlated highly with all the other microsomal and peroxisomal parameters. A significant negative correlation was observed when cytochrome P-452 was compared with both benzphetamine and ethoxyresorufin dealkylation activities. The lauric acid 12-hydroxylase activity, attributed to cytochrome P-452 correlated particularly well with peroxisomal palmitoyl CoA oxidation, carnitine acetyl CoA transferase, carnitine palmitoyl-CoA transferase and peroxisomal volume.

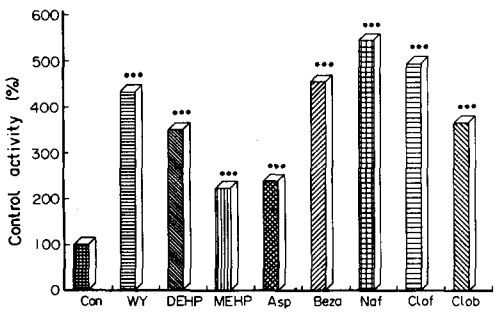


Fig. 4. Modulation of hepatic peroxisomal palmitoyl-CoA oxidase activity in the rat by hypolipidaemic agents. Animals (6 per group) were pretreated as in the legend to Table 1 and KCN-insensitive palmitoyl-CoA oxidase activity determined spectrophotometrically as described in Materials and Methods. 100% activity = 5.8 nmol product/min/mg protein. *** $P < 0.001$.

## DISCUSSION

The results of this study demonstrate the close relationship which exists between microsomal and peroxisomal enzyme parameters following the administration of a group of structurally diverse hypolipidaemic agents.

Administration of hypolipidaemic agents characteristically results in hepatomegaly which may be hypertropic and/or hyperplastic in nature [24–27] and the agents used in this study all produced significant increases in the liver/body weight ratio. Spectrophotometrically detectable cytochrome P-450 was also increased by hypolipidaemic challenge but it should be noted that this spectral method of analysis does not give a true indication of the extent of induction of the specific cytochrome P-452 isoenzyme. The true induction of cytochrome P-452 is more accurately reflected by the ELISA analysis where the constitutive level was increased up to 10-fold subsequent to hypolipidaemic challenge. Specific cytochrome P-452 isoenzyme quantitation in rat liver microsomes using the ELISA assay revealed significantly different basal and induced levels of the enzyme, to those previously reported in this laboratory using single radial immunodiffusion [28]. Previously, immunoquantitation via the single radial immunodiffusion method failed to quantitate the

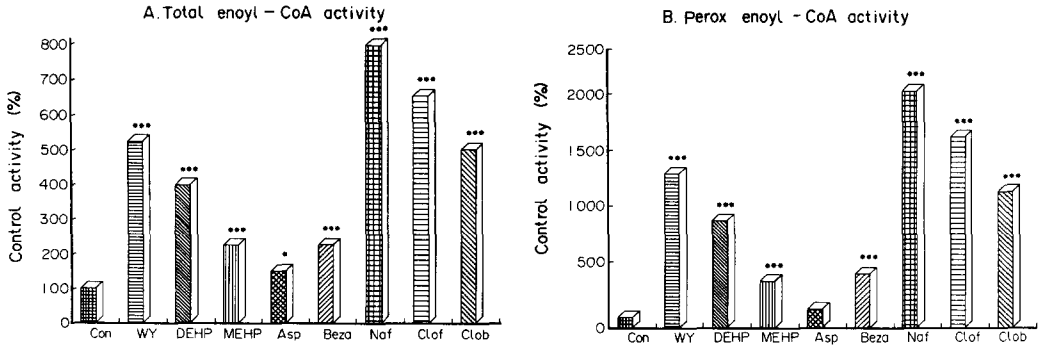


Fig. 5. Effect of hypolipidaemic agents on total and peroxisomal rat liver enoyl CoA hydratase activity. Rats (6 per group) were treated as described in Table 1 and activity determined as in Materials and Methods. (A) Total liver homogenate activity where 100% activity = 6.3 μmol product/min/mg protein. (B) Peroxisomal activity where 100% activity = 2.1 μmol product/min/mg protein. * $P < 0.05$; *** $P < 0.001$.

level of non-specific binding of the cytochrome P-452 antigen with preimmune serum. This led to the original over-estimation of cytochrome P-452 levels in both control and hypolipidaemic induced liver microsomes. Furthermore, the levels of cytochrome P-452 antigen used in the original single radial immunodiffusion assay (16 and 20 pmol) would, when visualised on a Western blot, exhibit a high degree of non-specificity, resulting in the appearance of several reactive bands. The much lower levels of antigen used in the current ELISA method (0.025 and 0.05 pmol of cytochrome P-452 antigen) cross-reacts on a Western blot to produce a single band with a molecular weight identical to the purified cytochrome P-452 isoenzyme (data not shown).

The low level of cross-reactivity seen with control, phenobarbitone and 3-methylcholanthrene-induced microsomes with the cytochrome P-452 immune serum (Fig. 1) can best be explained as follows. Firstly, the cytochrome P-452 antibody recognises similar haemoprotein epitopes in the microsomal preparations or secondly, that control, phenobarbitone and 3-methylcholanthrene microsomes possesses low but immunochemical quantifiable levels of cytochrome P-452. The evidence favours the latter explanation in that previous work has demonstrated a complete non-cross-reactivity between the cytochrome P-452 antibody and electrophoretically homogeneous preparations of cytochrome $P\text{-}450_b$ and $P\text{-}450_c$ by immunodiffusion analyses [7], coupled with the observation that both control microsomes (this study) and phenobarbitone- and 3-methylcholanthrene induced microsomes have low but detectable cytochrome P-450-dependent lauric acid 12-hydroxylase activity [11].

It should be emphasised that our data do not give an index of the potency of the various hypolipidaemic agents to induce cytochrome P-452 and the other enzyme activities described herein, but we have partially addressed this question of relative potencies elsewhere by examining the dose–response relationships of cytochrome P-452 induction and peroxisome proliferation by clofibrate and a phthalate ester plasticiser in the accompanying paper. Rather, our experiments were designed to use hypolipidaemic doses that have been reported in the literature to result in peroxisomal proliferation and it was our intention to determine if the induction of microsomal

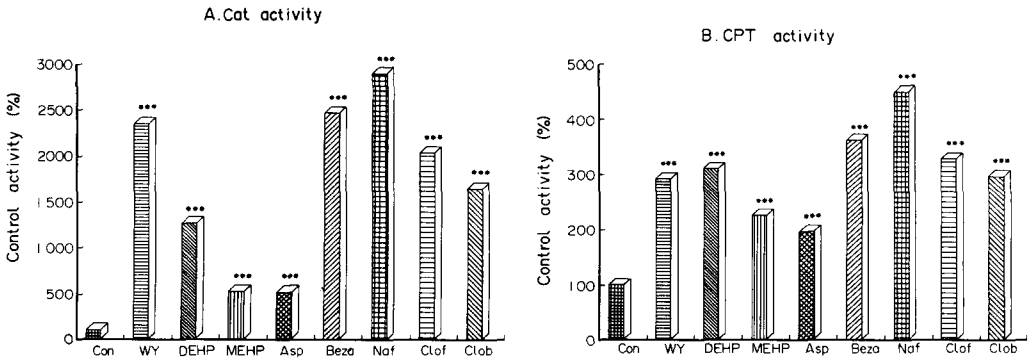


Fig. 6. Induction of carnitine acetyl- and palmitoyl-CoA transferase activities in rat liver by hypolipidaemic agents. Rats (6 per group) were treated as in Table 1 and activity determined as in Materials and Methods. (A) Carnitine acetyl-CoA transferase activity (CAT) where 100% activity = 3.4 nmol product/min/mg protein. (B) Carnitine palmitoyl-CoA transferase activity (CPT) where 100% activity = 9.3 nmol product/min/mg protein. *** $P < 0.001$.

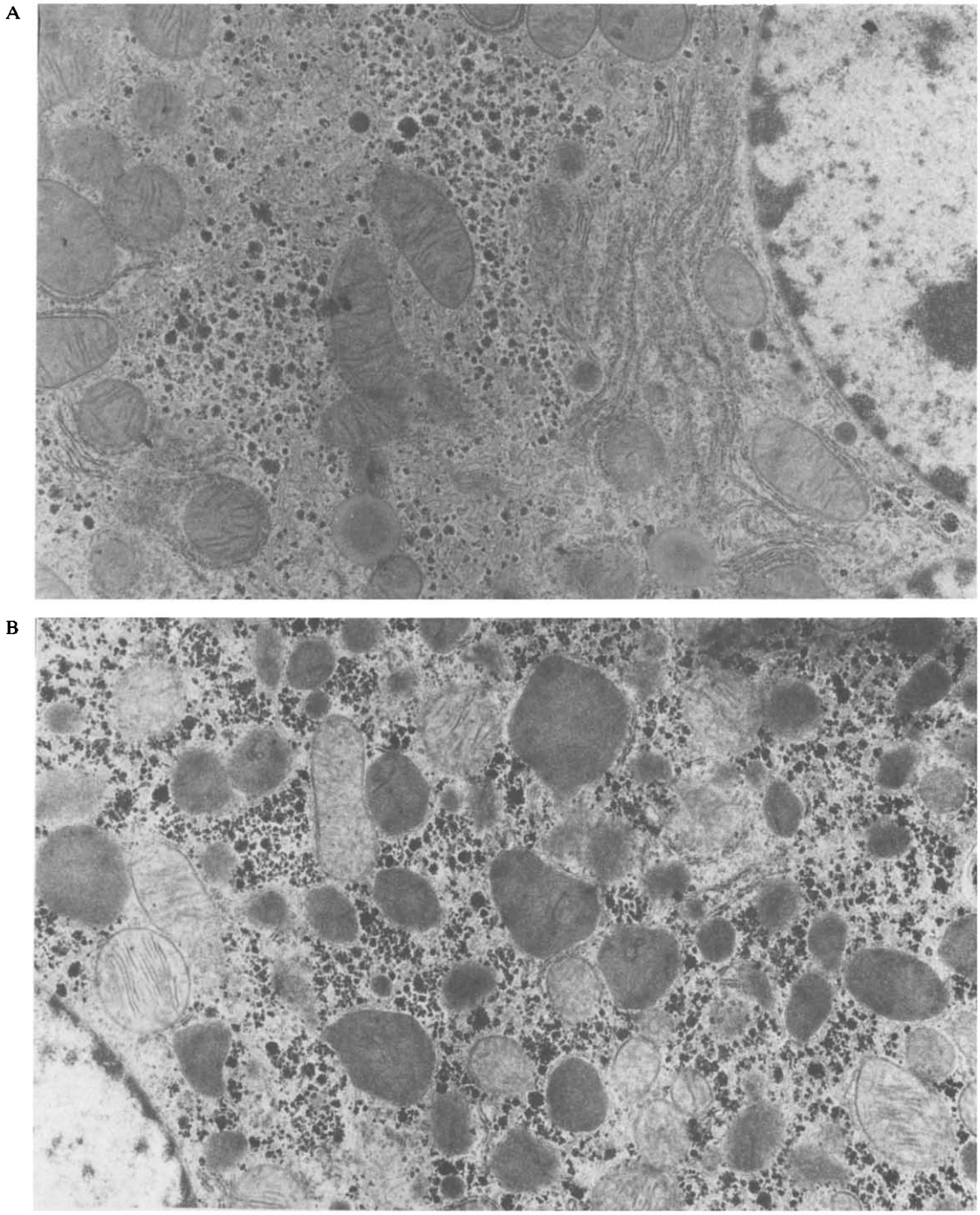


Fig. 7. Electron micrographs of control and clofibrate-treated rat liver. Sections for electron microscopy were prepared and processed as described in Materials and Methods. (A) Control, magnification ×16,600. (B) Clofibrate pretreated, magnification ×10,560.

cytochrome P-452 paralleled or was dissociated from proliferation of peroxisomes. From the data described herein, it is clear that there is a very close association between induction in the two liver organelles.

Immunochemically determined cytochrome P-452 (Table 3) correlates very significantly with the observed hepatomegaly (Table 1) and we can clearly state that agents which provide the greatest extent of hepatomegaly also induce the highest amounts of cytochrome P-452. Measurement of specific isoenzyme changes using changes in catalytic activities for cytochrome P-$450_b$ and cytochrome P-$450_c$ and their respective marker substrates benzphetamine-*N*-demethylase and ethoxyresorufin-*O*-deethylase (Figs. 3 and 4) indicated a marked depression of

Table 2. Effect of hypolipidaemic agents on hepatic peroxisomal volume

| Treatment | Peroxisome volume* (Percentage of cytoplasmic volume) |
|---|---|
| Peanut oil control | 1.8 ± 0.5 |
| WY-14,643 | 7.9 ± 1.8** |
| MEHP | 4.3 ± 1.2** |
| DEHP | N.D. |
| Aspirin | 3.5 ± 2.3* |
| Bezafibrate | 10.0 ± 1.5** |
| Nafenopin | 9.6 ± 1.3** |
| Clofibrate | 9.2 ± 3.2** |
| Clobuzarit | 7.6 ± 2.9** |

Peroxisomal morphometrics were determined as described in Materials and Methods and represent the mean ± SD from 4–6 animals.

* Significantly different from control at $P < 0.05$ (*) or $P < 0.001$ (**).

N.D., not determined.

these activities after hypolipidaemic treatment. Furthermore, these latter activities gave a high negative correlation when compared with both laurate hydroxylase activities and cytochrome P-452 levels. These results indicate a specific gene "switch on" for cytochrome P-452 with a concomitant gene "switch off" for cytochromes P-450$_b$ and P-450$_c$, and are matched by studies using the purified clofibrate induced cytochrome P-452 [7] which, in a reconstituted system, exhibits no detectable activity towards either of the substrates, benzphetamine and ethoxyresorufin. It should be noted in this context that this laboratory has successfully isolated a cDNA probe for cytochrome P-452 and Northern blot analysis has shown a substantial increase in the mRNA coding for this haemoprotein, subsequent to hypolipidaemic challenge (unpublished observations).

The induction of peroxisomal and mitochondrial $\beta$-oxidation enzymes in response to hypolipidaemic challenge agrees well with previous findings [29, 30]. Additionally, morphological examination and measurements of peroxisomal volume correlate highly with the enzymatic changes (Table 3).

To date, only limited studies in liver correlating the microsomal and peroxisomal parameters have been performed [24, 31]. Analysis of our results using a correlation matrix points to a strong inter-relationship between the coinduction of 11- and 12-microsomal fatty acid hydroxylation enzyme(s) and the $\beta$-oxidation enzymes of peroxisomes.

Whether the hypolipidaemic induction of the fatty acid hydroxylase is a primary event in lipid lowering is at present unclear. Previous studies have shown that a competition for fatty acids may exist between triglyceride synthesis on the one hand and $\omega$-oxidation of fatty acids on the other supported by the fact that the $\omega$- and $\omega$-1 products of fatty acids are not incorporated into triglycerides, but are further oxidised to dicarboxylic acids [32]. However, recent work disputing this evidence involved the specific inactivation of hepatic fatty acid hydroxylases by acetylenic fatty acids and the demonstration that the $\omega$-hydroxylation of medium chain fatty acids is

Table 3. Correlation matrix for the inter-relationship of enzyme changes induced by hypolipidaemic agents in rat liver*

| | Total P-450 (sp. content) | Cyt.P-452 | LA 11-OH | LA 12-OH | PCoA | CAT | CPT | Total enoyl CoA | Perox enoyl CoA | BZP | EROD | Peroxisomal volume |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total Cyt.P-450 | | 0.391 | 0.311 | 0.482 | 0.268 | 0.121 | 0.198 | 0.301 | 0.280 | −0.299 | −0.405 | 0.186 |
| Cyt. P-452 | | | 0.690 | 0.776 | 0.945 | 0.922 | 0.907 | 0.739 | 0.747 | −0.731 | −0.932 | 0.973 |
| LA 11-OH | | | | 0.894 | 0.736 | 0.778 | 0.725 | 0.544 | 0.559 | −0.309 | −0.549 | 0.646 |
| LA 12-OH | | | | | 0.768 | 0.741 | 0.752 | 0.450 | 0.464 | −0.512 | −0.656 | 0.750 |
| PCoA | | | | | | 0.964 | 0.949 | 0.837 | 0.845 | −0.630 | −0.920 | 0.970 |
| CAT | | | | | | | 0.919 | 0.779 | 0.795 | −0.624 | −0.867 | 0.962 |
| CPT | | | | | | | | 0.792 | 0.794 | −0.667 | −0.884 | 0.938 |
| Total enoyl CoA | | | | | | | | | 0.999 | −0.398 | −0.794 | 0.741 |
| Peroxisomal enoyl CoA | | | | | | | | | | −0.398 | −0.795 | 0.741 |
| BZP | | | | | | | | | | | −0.844 | −0.746 |
| EROD | | | | | | | | | | | | −0.937 |

* For 9 observations (i.e. 9 hypolipidaemic agents tested yielding 7 degrees of freedom), the correlation coefficient in a linear regression analysis is significant (95% probability) if the $r$ value is ≥0.666.

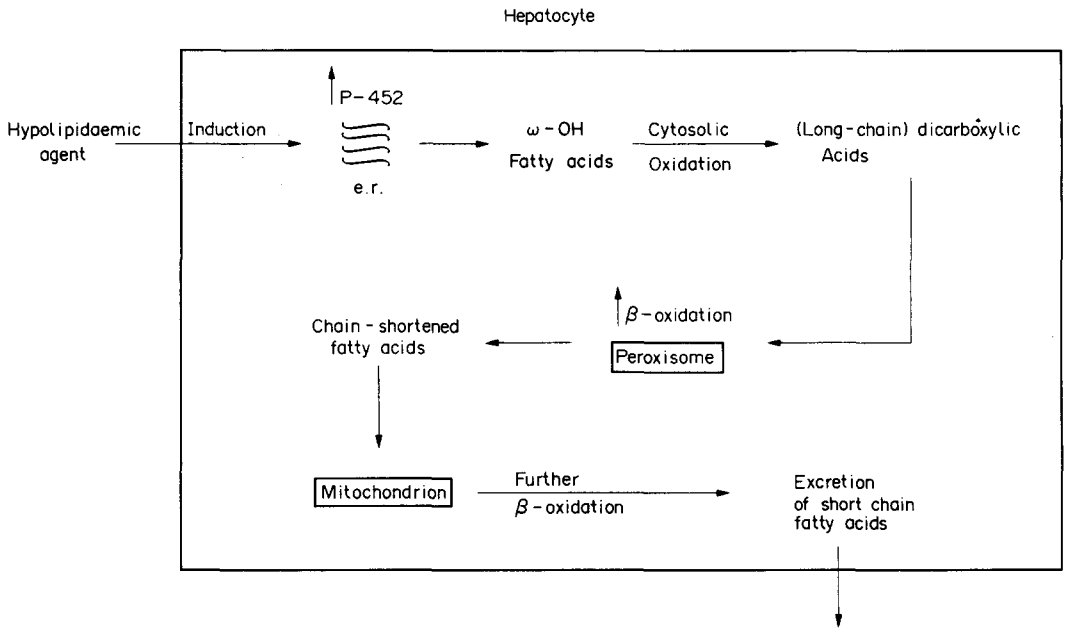


Fig. 8. Scheme to show the inter-relationship of microsomal and peroxisomal changes in fatty acid oxidation after hypolipidaemic challenge (abbreviation, e.r.; endoplasmic reticulum).

independent of the hypolipidaemic action of clofibrate [33]. The reason for the discrepancy is at present unclear.

Taken collectively, the information presented in this paper may be rationalised according to the scheme presented in Fig. 8, wherein a mechanism is proposed for the co-induction of hepatic microsomal cytochrome P-452 and peroxisomal β-oxidation enzymes by hypolipidaemic drugs. In this scheme, the hypolipidaemic agent is taken up by the hepatocyte and induces cytochrome P-452 synthesis. This step is clearly evidenced by an increase in both cytochrome P-452 level (Table 1) and activity (Fig. 2). It should be noted that the precise mechanism of cytochrome P-452 induction still remains speculative in that a cytosolic receptor mediating induction has been proposed [8], but largely unsubstantiated. Whether hypolipidaemic agents act via a cytosolic receptor or directly influence cytochrome P-452 gene regulation is still debatable, but it is abundantly clear that this class of compounds regulate the transcriptional activation of the cytochrome P-452 gene [34]. As shown in this study (Fig. 2), induction of cytochrome P-452 results in the increased ω-hydroxylation of fatty acids and our previous studies have shown that long chain fatty acids (including arachidonic acid) are also excellent substrates for this enzyme [28]. Thus, after ω-hydroxylation of fatty acids, further cytosolic metabolism results in the formation of long-chain dicarboxylic acids [35]. These are then taken up by the peroxisome facilitated by the co-induction of carnitine acetyl transferase (Fig. 6A), thus presenting this organelle with a substrate overload of one of its preferred substrates [36] because the mitochondrion cannot readily metabolise long chain fatty acids [37]. Therefore the well-documented hypolipidaemic induction of peroxisomal β-oxidation enzymes may then be considered an adaptive cellular response by the hepatocyte in an attempt to clear long chain fatty acids for subsequent chain shortening by the mitochondrion [37], thereby maintaining cellular lipid homeostasis. Accordingly this proposed mechanism is consistent with both our own studies and the literature evidence cited above, and is the subject of our continuing investigation.

Related to the above proposed mechanism derived from *in vivo* studies, the induction of peroxisomal enzyme activities in cultured rat hepatocytes by long chain fatty acids [38] or high fat diets *in vivo* [39, 40], additionally points to long chain fatty acids as putative endogenous peroxisomal proliferators. In addition, peroxisomal β-oxidation has also been reported to increase in the livers of starved or diabetic rats where there is an accumulation of long chain fatty acyl-CoA [41, 42]. Although this scheme remains to be fully developed, the time course of induction of the two organelles by hypolipidaemics would shed light on the proposed sequence of events and this aspect is currently being addressed in this laboratory.

In conclusion, the results of this study have demonstrated that using a range of structurally diverse hypolipidaemic agents, a close relationship exists between the induction of microsomal and peroxisomal fatty acid metabolising enzymes with a corresponding decrease in some drug metabolising enzymes. Although the inter-relationship and significance of these acute changes in enzyme composition in the pharmacology and toxicology of these compounds remains to be fully elucidated, they do appear to be a prerequisite to the more pronounced and toxicologically significant subcellular derangements in rodents that subsequently follow on chronic exposure [3, 6].

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