Specific changes in the protein composition of rat liver in response to the peroxisome proliferators ciprofibrate, Wy-14,643 and di-(2-ethylhexyl)phthalate

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The hypolipidaemic agents ciprofibrate and Wy-14,643 {[4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthio]acetic acid} and the phthalate-ester plasticizer di-(2-ethylhexyl)-phthalate (DEHP), like other peroxisome proliferators, produce a significant hepatomegaly and induce the peroxisomal fatty acid β -oxidation enzyme system together with profound proliferation of peroxisomes in hepatic parenchymal cells. Changes in the profile of liver proteins in rats following induction of peroxisome proliferation by ciprofibrate, Wy-14,643 and DEHP have been analysed by high-resolution two-dimensional gel electrophoresis. The proteins of whole liver homogenates from normal and peroxisome-proliferator-treated rats were separated by two-dimensional gel electrophoresis using isoelectric focusing for acidic proteins and nonequilibrium pH gradient electrophoresis for basic proteins. In the whole liver homogenates, the quantities of six proteins in acidic gels and six proteins in the basic gels increased following induction of peroxisome proliferation. Peroxisome proliferator administration caused a repression of three acidic proteins in the liver homogenates. By the immunoblot method using polyspecific antiserum against soluble peroxisomal proteins and monospecific antiserum against peroxisome proliferation associated M_r 80000 polypeptide (polypeptide PPA-80), the majority of basic proteins induced by these peroxisome proliferators appeared to be peroxisomal proteins. Polypeptide PPA-80 becomes the most abundant protein in the total liver homogenates of peroxisome-proliferator-treated rats. These results indicate that ciprofibrate, DEHP and Wy-14,643 induce marked changes in the profile of specific hepatic proteins and that some of these changes should serve as a baseline to identify a set of gene products that may assist in defining the specific 'peroxisome proliferator domain'.

Clofibrate, the ethyl ester of *p*-chlorophenoxyisobutyric acid, which was introduced as a hypocholesterolaemic drug in 1962 (Thorp & Waring, 1962), has served as a prototype for the development of additional highly potent hypolipidaemic agents (Kritchevsky, 1980; Reddy & Lalwani, 1983). Clofibrate has been used extensively to study the mechanism of drug-induced hypolipidaemia (Havel & Kane, 1982). Clofibrate and other hypolipidaemic compounds induce hepatomegaly

(Best & Duncan, 1964; Reddy *et al.*, 1982; Reddy & Lalwani, 1983) and a series of metabolic changes in rodents and some non-rodent species. The hepatomegaly is associated with extensive proliferation of the cytoplasmic organelle peroxisomes (Tolbert, 1981; de Duve, 1975) as well as increases in smooth endoplasmic reticulum (Hess *et al.*, 1965; Reddy & Krishnakantha, 1975; Moody & Reddy, 1976) and mitochondrial protein (Kurup *et al.*, 1970; Gear *et al.*, 1974) in liver parenchymal cells. These compounds, referred to as peroxisome proliferators (Reddy & Lalwani, 1983) because of their predictable effects on hepatic peroxisomes, also induce alteration in the activities of several enzymes in liver, especially those associated with mitochondria and peroxisomes. These include increases in the activities of glycerol phosphate dehydrogenase (Kurup *et al.*, 1970), cytochrome *c*

Abbreviations used: SDS, sodium dodecyl sulphate; polypeptide PPA-80, peroxisome proliferation associated M_r 80000 protein; NEPHGE, non-equilibrium pH gradient electrophoresis; IEF, isoelectric focusing; Wy-14,643, [4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthio]acetic acid; DEHP, di-(2-ethylhexyl)phthalate.

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oxidase (Gear *et al.*, 1974), carnitine palmitoyl-transferase (Solberg *et al.*, 1972), carnitine acetyl-transferase (Moody & Reddy, 1974), carnitine octanoyltransferase (Moody & Reddy, 1978), acyl-CoA : dihydroxyacetone phosphate acyltransferase (Hajra *et al.*, 1979), catalase (Hess *et al.*, 1965; Svoboda *et al.*, 1967; Reddy & Kumar, 1979) and the enzymes involved in the cyanide-insensitive peroxisomal fatty acid β -oxidation system (Lazarow & de Duve, 1976; Osumi & Hashimoto, 1979; Inestrosa *et al.*, 1980; Miyazawa *et al.*, 1980; Lalwani *et al.*, 1983b).

In recent years, peroxisome proliferation and induction of peroxisome-associated enzymes in the livers of rodents exposed to a variety of structurally dissimilar peroxisome proliferators have been extensively studied (reviewed recently by Reddy & Lalwani, 1983). Using SDS/polyacrylamide-gel electrophoresis, Reddy & Kumar (1977), demonstrated that peroxisome proliferators, regardless of their chemical structure, specifically increase the hepatic content of a protein with an M_r of about 80000. This polypeptide, PPA-80 (M. K. Reddy *et al.*, 1981), has been localized to the peroxisomes using immunocytochemical procedures (Bendayan & Reddy, 1982), and appears identical with the bi-functional protein (Osumi & Hashimoto, 1979) displaying the activities of enoyl-CoA hydratase and β -hydroxyacyl-CoA dehydrogenase, the two middle enzymes of the peroxisomal fatty acid β -oxidation system (Lazarow, 1978). The mechanism of induction of this polypeptide PPA-80 and of peroxisome proliferation by hypolipidaemic drugs and certain phthalate ester plasticizers is not understood (reviewed by Reddy & Lalwani, 1983). Short-term treatment of rats with these agents causes a marked increase in the concentrations of the mRNA species coding for polypeptide PPA-80 and other enzymes of the peroxisomal β -oxidation system (Furuta *et al.*, 1982; Chatterjee *et al.*, 1983). However, a major question is whether the induction process, mediated by these structurally dissimilar hypolipidaemic agents, specifically increases the expression of a limited set of genes, particularly those related to peroxisomes.

The present study was undertaken to investigate in greater detail whether there are significant alterations in the amount of specific proteins in rat liver following the induction of peroxisome proliferation by ciprofibrate {2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid}, a highly potent structural analogue of clofibrate (Arnold *et al.*, 1979; Lalwani *et al.*, 1983b,c), Wy-14,643, a hypolipidaemic agent structurally unrelated to clofibrate, and DEHP, a widely used phthalate-ester plasticizer. We have used high resolution two-dimensional polyacrylamide-gel electrophoresis (O'Farrell, 1975; Scheele, 1975; O'Farrell *et al.*,

1977) to document, for the first time, two-dimensional protein patterns observed after the induction of hepatic peroxisome proliferation.

Materials and methods

Chemicals

The hypolipidaemic compound ciprofibrate was obtained from Sterling-Winthrop Research Institute, Rensselaer, NY, U.S.A. DEHP was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Wy-14,643 was generously given by Wyeth Laboratories, Radnor, PA, U.S.A. Nonidet P-40 was obtained from Bethesda Research Laboratories, Rockville, MD, U.S.A. Acrylamide (enzyme grade), and NN' -bismethyleneacrylamide were purchased from Eastman Kodak Co., Rochester, NY, U.S.A. M_r standards for SDS gel electrophoresis, sucrose (electrophoresis purity), SDS (electrophoresis purity) and ammonium persulphate were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Ampholines were purchased from LKB. Metrizamide was obtained from Nyegaard & Co., Oslo, Norway. All other chemicals used in this study were of analytical reagent grade quality.

Animals

Inbred male F344 rats weighing 100–120 g were obtained from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. The hypolipidaemic peroxisome proliferators ciprofibrate, DEHP and Wy-14,643 were administered to these animals respectively at 0.025%, 2% and 0.1% (all w/w) in powdered rat chow *ad libitum* for 2 weeks. The animals were killed by cervical dislocation.

Subcellular fractionation

The livers were homogenized (10% homogenate, w/v), in ice-cold 0.25 M-sucrose containing 10 mM-EDTA (pH 7.5) using a Potter-Elvehjem homogenizer. These homogenates were fractionated into nuclear (700 g for 10 min), heavy mitochondrial (5500 g for 10 min), and light mitochondrial (15000 g for 15 min) fractions in a Beckman J-21C centrifuge at 4°C according to the procedure outlined by Baudhuin *et al.* (1964). The light mitochondrial fraction was washed with 0.25 M-sucrose, adjusted to pH 7.5, and finally suspended at the concentration of 3 g of original tissue/ml of the above solution.

Preparation of purified peroxisomes by metrizamide-density-gradient centrifugation

Peroxisomes from rat liver were isolated as outlined by Hajra & Bishop (1982). A 50% (w/v) stock solution of metrizamide was made by solubilizing metrizamide completely in alkaline water (pH 7.5).

The pH of this metrizamide solution was adjusted to 7.5 with 0.5M-NaOH. This solution was diluted to 44, 38, 32, 26 and 20% (w/v) with water at pH 7.5. Then, these solutions, 6.4ml each were layered in a Beckman quick-seal centrifuge tube (25mm $\times$ 89mm) from bottom (50%) to top (20%), sealed with Parafilm tightly and kept horizontally at 4°C overnight until use. The light mitochondrial fraction (2ml) was loaded on the gradient and centrifuged at 40000 rev./min for 1h using a Beckman VTi50 rotor in a Beckman L5-65 ultracentrifuge equipped with a slow-acceleration unit, and 2ml fractions were collected. After assaying the activity of the cyanide-insensitive fatty acid β -oxidation enzyme system as a marker enzyme of peroxisomes, peroxisome-rich fractions were com-

bined, diluted with 0.25M-sucrose and centrifuged at 15000 rev./min for 30 min at 4°C. The peroxisomal pellet was suspended in 0.25M-sucrose and kept at -20°C until use.

Extraction of soluble peroxisomal protein

Peroxisomal soluble extract was prepared by lysing the purified peroxisomes (Fig. 1) in ice-cold 100mM- Na_2CO_3 (pH 11.5) for 30 min (Fujiki *et al.*, 1982). A portion of peroxisome lysate was centrifuged at 48000 rev./min for 1h using a Beckman type 50 Ti rotor to separate soluble proteins from the peroxisomal membrane.

SDS/polyacrylamide-gel electrophoresis

Electrophoresis was performed on SDS/poly-

Fig. 1. *Electron micrograph of rat liver peroxisomes purified by metrizamide-gradient centrifugation*
This electron micrograph represents the overall purity of highly enriched peroxisome (p) population. Note the absence of mitochondria, lysosomes and smooth endoplasmic reticulum in these pellets. The arrow heads point to vesiculation or protrusion of peroxisomal membranes, some in cross section without any apparent contact with peroxisomes. Peroxisomal cores (c) are sometimes seen outside the peroxisomes due to rupture of membranes. Magnification $\times 22000$.

acrylamide-slab gels according to the method of Laemmli (1970).

Isoelectric focusing and two-dimensional gel electrophoresis

IEF/NEPHGE of the samples was performed in cylindrical gels (2.7 mm internal diameter, 10–11 cm long); the gels were subjected to SDS/polyacrylamide-gel electrophoresis in the second dimension essentially as described by O'Farrell (1975) and O'Farrell *et al.* (1977). In some experiments an acrylamide gradient gel (10–15%) was used for the second-dimensional gel as described by Scheele (1975).

Immunoblot analysis

Antibodies to soluble peroxisomal proteins obtained from ciprofibrate-fed rat livers were raised in New Zealand white rabbits. Peroxisomal soluble proteins were mixed 1:1 with complete Freund's adjuvant and injected subcutaneously into the back and foot pads of rabbits. The procedure was repeated three times, at weekly intervals. The polyspecific antiserum against peroxisomal proteins was collected 4 days after administering a booster dose. The antibodies against peroxisome proliferation-associated M_r 80000 protein polypeptide (PPA-80) were prepared as described elsewhere (Reddy *et al.*, 1981). For immunoblot analysis (Towbin *et al.*, 1979), proteins from unstained two-dimensional NEPHGE gels were transferred electrophoretically to nitrocellulose sheets. These sheets were treated either with antiserum against peroxisomal soluble proteins or with anti-(enoyl-CoA hydratase) serum and then with ^{125}I -labelled protein A and autoradiographed.

Analytical procedures

Protein concentrations were estimated by using the procedure of Lowry *et al.* (1951). The activity of the palmitoyl-CoA oxidation system was measured according to the method of Lazarow (1981).

Electron microscopy

Freshly prepared purified peroxisomes were suspended in 2.5% glutaraldehyde in 0.1 M-sodium cacodylate buffer, pH 7.4, and fixed for 30 min. They were then washed and postfixed in 1% OsO_4 in 0.1 M-s-collidine buffer, pH 7.4, for 1 h at 4°C, dehydrated and embedded in Epon. Thin sections were cut and examined in a JEOL JEM-100 cx II electron microscope at an accelerating voltage of 60 kV. The purity of the peroxisome pellets was assessed by examining at least 15 low- and high-magnification electron micrographs.

Results

One-dimensional electrophoresis of post-nuclear and light mitochondrial fractions

The one-dimensional SDS/polyacrylamide-gel electrophoretic patterns of total proteins in post-nuclear and light mitochondrial fractions of liver obtained from control rats and rats treated with ciprofibrate for 2 weeks are illustrated in Fig. 2. Ciprofibrate treatment caused the appearance *de novo* or a substantial increase in the amount of polypeptide PPA-80 in both fractions (Figs. 2c and 2d). This peptide has been shown previously to be associated with peroxisome proliferation in liver cells (Reddy & Kumar, 1977; Reddy *et al.*, 1981). In addition to this major protein band, five other

Fig. 2. One-dimensional SDS/polyacrylamide-gel electrophoresis profiles of proteins in the normal and ciprofibrate-treated rat liver

F344 male rats were fed a diet containing ciprofibrate (0.025%, w/w) for 2 weeks. Post-nuclear (lanes a and c) and light mitochondrial (lanes b and d) fractions were obtained from control (lanes a and b) and ciprofibrate-treated (lanes c and d) rat livers, and electrophoresed as described in the Materials and methods section. Ciprofibrate treatment resulted in increases in the quantities of six proteins, including polypeptide PPA-80 (M_r approx. 78000), in the post-nuclear and/or light mitochondrial fractions. Protein concentration was 50 µg/lane.

proteins in the M_r range 24000–65000 also increased to some extent following ciprofibrate treatment (Fig. 2). Similar patterns were also obtained with livers of rats after Wy-14,643 and DEHP treatment (results not shown).

Analysis of acidic and basic liver proteins by two-dimensional gel electrophoresis

Based on the one-dimensional gels described above, it was apparent that substantial changes in the profile of liver proteins appear in livers with ciprofibrate-induced peroxisome proliferation. To resolve these alterations further, proteins of whole liver homogenates were subjected to two-dimensional analysis using IEF for acidic proteins (O'Farrell, 1975) and NEPHGE for basic proteins (O'Farrell *et al.*, 1977).

Over 120 protein spots were visualized in Coomassie Blue-stained two dimensional gels of acidic proteins of whole liver (Figs. 3 and 4) of control, ciprofibrate-, DEHP- and Wy-14,643-treated rats. The acidic protein patterns of the total liver homogenates indicate that six proteins in the M_r 21000–94000 range (protein spots 2, 4, 5, 6, 8

and 9), were in relative abundance in ciprofibrate-, Wy-14,643- and DEHP-treated rats (Fig. 3b; Figs. 4a and 4b). In these gels, a decrease in the intensity of three proteins (protein spots 1, 3 and 7) was observed following peroxisome proliferator treatment (Fig. 3b; Figs. 4a and 4b).

The Coomassie Blue-stained two-dimensional gel electrophoretic patterns of homogenate proteins with basic isoelectric points revealed over 30 proteins (Figs. 3c and 3d; Figs. 4c and 4d). The composition of proteins with more basic isoelectric points was significantly different between control and peroxisome proliferator treated rat livers. For example, six basic proteins (proteins p1–p5 and p8; see Figs. 3c and 3d, 4c and 4d) were relatively abundant in the livers of rats treated with ciprofibrate, DEHP and Wy-14,643. Protein p1, which has an M_r of about 78000, was the most abundant protein in the livers of peroxisome-proliferator-treated animals, but it was present only as a faint spot in the profile of the control liver proteins (Fig. 3c). In contrast, the amount of proteins p6 and p7 was reduced in the treated animals (Figs. 3c and 3d; Figs. 4c and 4d).

Fig. 3. Two-dimensional gel electrophoretic profiles of total liver homogenate proteins of control and ciprofibrate-treated rats. Samples of whole liver lysates of whole liver homogenates of control (a and c) and ciprofibrate-treated (b and d) were analysed on two-dimensional gels with IEF (a and b) or NEPHGE (c and d) in the first dimension and 11% SDS/polyacrylamide-gel electrophoresis in the second dimension. In these Coomassie Blue-stained gels, seven acidic proteins (2, 4, 5, 6, 8 and 9) increased following ciprofibrate treatment (b). Ciprofibrate treatment also caused increases in basic proteins p1–p5 and p8 (d). Protein p1 is identified as polypeptide PPA-80. Protein concentration: (a) and (b), 1000 μ g/gel; (c) and (d), 500 μ g/gel.

Fig. 4. Two-dimensional gel electrophoretic profiles of total liver homogenate proteins of DEHP- and Wy-14,643-treated rats. Samples of whole liver lysates of whole liver homogenates of DEHP- (a and c) and Wy-14,643- (b and d) treated rats were analysed on two-dimensional gels with IEF (a and b) or NEPHGE (c and d) in the first dimension and 11% SDS/polyacrylamide-gel electrophoresis in the second dimension. These patterns are essentially similar to those of ciprofibrate-treated rat livers (Fig. 3). Protein concentration: (a) and (b), 1000 $\mu\text{g}/\text{gel}$; (c) and (d), 500 $\mu\text{g}/\text{gel}$.

Fig. 5. Western blots (autoradiographs) of basic proteins of liver homogenate of control and ciprofibrate-treated rats with polyspecific antiserum against peroxisomal proteins.

Samples of whole liver lysates (approx. 500 μg of protein) obtained from control (a) and ciprofibrate-treated (b) rat livers were subjected to NEPHGE/two-dimensional gel electrophoresis, transferred to nitrocellulose paper and immunoblotted using polyspecific antiserum against soluble peroxisomal proteins as described in the Materials and methods section. The autoradiograph shows that p1 and a few other basic proteins in the post-nuclear fraction are immunochemically identifiable as peroxisomal proteins. A marked increase in p1 content is evident (b).

Fig. 6. Western blots (autoradiographs) of basic proteins of whole liver lysates of normal and peroxisome proliferator-treated rats with anti-(polypeptide PPA-80) serum

Samples of whole liver lysates (approx. 200 μ g of protein) from control (a), ciprofibrate- (b), Wy-14,643- (c) and DEHP- (d) treated rats were subjected to NEPHGE/two-dimensional gel electrophoresis, transferred to nitrocellulose paper and immunoblotted with antibodies against polypeptide PPA-80. The autoradiograms identify p1 as polypeptide PPA-80 which is increased in treated livers.

The possibility that some of these 'extra' proteins with more basic isoelectric points appearing after ciprofibrate treatment represent peroxisomal soluble proteins was confirmed by Western blotting. Figs. 5(a) and 5(b) represent the autoradiograms of western blots of NEPHGE gels of control and ciprofibrate-treated liver homogenates using the polyspecific antiserum against peroxisomal soluble proteins induced by ciprofibrate. Furthermore, protein p1 was identified as polypeptide PPA-80 from the M_r (pI9.9) and by Western blotting using antiserum against enoyl-CoA hydratase-polypeptide PPA-80 (Fig. 6).

Discussion

The exposure of rats to ciprofibrate, a highly potent hypolipidaemic compound, results in a massive hepatomegaly with significant increases in the numerical and volume densities of peroxisomes in hepatocytes (Lalwani *et al.*, 1983c). Like other peroxisome proliferators (Reddy *et al.*, 1980), prolonged treatment of rats with this agent leads to the development of hepatocellular carcinomas (Reddy & Lalwani, 1983; Rao *et al.*, 1984). The

mechanism by which ciprofibrate and other peroxisome proliferators, all of which are non-mutagenic (Warren *et al.*, 1980), induce liver tumours is not understood (Reddy & Lalwani, 1983). However, it is proposed that endogenous generation of excessive amounts of H_2O_2 by peroxisomal oxidases, resulting from sustained increase in peroxisome population, leads to DNA damage and initiation of liver carcinogenesis (Reddy *et al.*, 1980). Treatment of rodents with peroxisome proliferators such as ciprofibrate, Wy-14,643 and DEHP, as used in the present study, also causes subtle increases in the content of smooth endoplasmic reticulum and mitochondria of hepatocytes; the predominant change however is confined to peroxisomes (Moody & Reddy, 1976) and rat liver parenchymal cells, even when transplanted to extrahepatic locations (i.e. in interscapular fat pads or anterior chamber of eye), respond to peroxisome proliferators in this predictable fashion (Reddy *et al.*, 1984). Accordingly, it is reasonable to assume that ciprofibrate, Wy-14,643, DEHP and other hepatic peroxisome proliferators induce changes in the production of specific liver proteins and that some of these alterations might be relevant in elucidating the molecular mech-

anisms of peroxisome proliferation and liver carcinogenesis.

The present studies demonstrate both increases and decreases in the content of specific proteins in rat liver in response to ciprofibrate, Wy-14,643 and DEHP. In the whole liver homogenates, the amounts of six proteins (proteins 2, 4, 5, 6, 8 and 9) in acidic gels and six proteins (proteins p1-p5 and p8) in the basic gels increased substantially following ciprofibrate administration. The amounts of three proteins (proteins 1, 3 and 7) in the acidic gels of whole liver homogenates was decreased. Evidence that the majority of the basic proteins increased in response to ciprofibrate, DEHP and Wy-14,643 are peroxisomal proteins includes the following. First, the most abundant increase occurred in the content of protein p1 detected in the basic gels. This protein has been identified as polypeptide PPA-80 on the basis of M_r , isoelectric point and immunoblotting with antibodies to enoyl-CoA hydratase. Second, the observed increases in basic proteins in the whole liver were similar to those that were most abundant in the peroxisomes purified from the livers of ciprofibrate-treated rats (results not shown). Third, when the basic proteins which were resolved in NEPHGE/two-dimensional gels were subjected to Western blot analyses with polypeptide-specific antibodies against peroxisomal proteins, proteins p1-p5 and p8 were immunochemically positive. Finally, these changes in the protein patterns were reversible following withdrawal of the inducer and correlated with the reversal of peroxisome proliferation (results not shown).

Even though proteins p1-p5 and p8 were tentatively identified as peroxisomal in origin by using the polyspecific antiserum against soluble peroxisomal proteins, the specific nature of the individual protein spots, with the exception of basic protein p1 (polypeptide PPA-80) remain to be determined. The many-fold increase in the intensity of protein p1 in these two-dimensional gels is consistent with the increases noted in one-dimensional SDS/polyacrylamide-gel electrophoresis (Reddy & Kumar, 1977; Lazarow *et al.*, 1982) and with the 15-20-fold increase in the mRNA species for polypeptide PPA-80 (Chatterjee *et al.*, 1983; Furuta *et al.*, 1982). Additional studies are necessary to identify the nature of the other induced basic proteins and to determine whether the changes in these proteins, as well as in p1, are due to increases in synthesis *de novo*.

In the present study we did not determine whether some or all of the changes induced by ciprofibrate, Wy-14,643 and DEHP in the acidic proteins in whole liver are in peroxisomal proteins. They could account for some of the observed alterations in liver following exposure of rats to

these agents. Detailed analysis of the subcellular distribution of these proteins appears necessary to delineate further the effect of the peroxisome proliferators on other cellular constituents. Additional studies are needed to establish which of the changes in liver proteins following peroxisome proliferator treatment are simply a consequence of massive hepatomegaly and therefore unrelated to changes in peroxisome-specific protein expression. Studies with non-peroxisome proliferator hepatomegalic agents such as phenobarbital, allylisonipropylacetamide, hexachlorocyclohexane, butylated hydroxyanisole etc., should enable such a distinction.

In summary, we undertook these experiments to establish the profile of changes in protein composition (i.e. gene expression) in the livers of rats exposed to the peroxisome proliferators ciprofibrate, DEHP and Wy-14,643. Peroxisome proliferators (Reddy & Lalwani, 1983) display a rank order of potency as inducers of hepatic peroxisome proliferation. The present studies with three structurally diverse peroxisome proliferators clearly suggest that they specifically induce a specific set of gene products. It would be worthwhile to determine whether all peroxisome proliferators specifically affect the same 'domain' of gene products (Ivarie & O'Farrell, 1978). If a specific set of polypeptides were induced in liver cells virtually every time in response to structurally unrelated peroxisome proliferators, such a set of gene products could be defined as the 'peroxisome proliferator domain'. The existence of such a highly specific 'peroxisome proliferator domain' would strongly support the contention that peroxisome proliferator action is mediated by a cell-specific cytosolic receptor (Lalwani *et al.*, 1983a).

Note added in proof (Received 20 February 1985)

During processing of this paper we repeated the experiments shown above with clofibrate (0.5%, w/w, in the chow). The results were very similar to those obtained with ciprofibrate.

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