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BIOCHEMICAL MECHANISMS OF INDUCTION OF HEPATIC PEROXISOME PROLIFERATION

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INTRODUCTION

The process of xenobiotic-induced proliferation of the cytoplasmic organelle, the peroxisome, in mammalian liver cells has received considerable attention because of the proposed relationship between the induction of hydrogen peroxide producing peroxisomal enzymes and the development of hepatocellular carcinomas in mice and rats (1–6). Several structurally dissimilar hypolipidemic drugs, certain phthalate esters used as plasticizers, and some halogenated hydrocarbon solvents are examples of important categories of chemicals that are capable of inducing proliferation of peroxisomes in hepatocytes (7–13).

The mechanism by which this diverse group of chemicals causes peroxisome proliferation and liver tumors is not understood, although it is currently thought that these chemicals are carcinogenic because of their ability to induce peroxisome proliferation. There are marked species differences in response to them; certain species, e.g. guinea pigs and monkeys, are relatively nonresponsive or resistant to the induction of hepatic peroxisomes (1, 2, 4, 6). It is therefore necessary to understand the mechanism by which they induce peroxisome proliferation to help in the safety evaluation of these chemicals to human beings.

This review briefly discusses the response, in biochemical and morphological terms, of hepatocytes to peroxisome proliferating chemicals and then focuses on possible biochemical mechanism(s) whereby these chemicals may produce peroxisome proliferation. For more detailed reviews on peroxisomes

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(14, 15), and the relationship between peroxisome proliferation and cancer (2, 3, 5), the readers are referred to reviews.

PEROXISOMES

Rhodin (16) first detected a single membrane-linked cytoplasmic organelle with a fine, granular matrix in the proximal convoluted tubular epithelium of the mouse kidney, and he called this organelle a microbody. Rouiller & Bernard (17) subsequently identified microbodies with crystalloid cores in liver parenchymal cells. Their chemical properties were ascertained by de Duve and co-workers (17, 18) who discovered that rat liver microbodies were enriched in urate oxidase (uricase), D-amino acid oxidase, and catalase. The oxidases present in microbodies produce hydrogen peroxide as a reaction product, and catalase reduces the hydrogen peroxide to water, either by a peroxidatic or catalatic mechanism. The term 'peroxisome' was introduced by de Duve to emphasize these properties.

Peroxisomes in most cells can be readily differentiated from mitochondria and lysosomes by routine transmission electron microscopy. Further identification of peroxisomes is possible by cytochemical techniques that use 3,3'-diaminobenzidine as a hydrogen donor in the peroxidatic reaction of catalase, this making it possible to stain these organelles for light and electron microscopy (19). Peroxisomes can also be identified by immunocytochemical techniques to localize catalase (20, 21) or peroxisomal enzymes such as enoyl-CoA hydratase (21). Peroxisomes of liver parenchymal cells in many species contain a crystalloid core or nucleoid that signifies the presence of urate oxidase. No crystalloid core is seen in the peroxisomes in nonhepatic cells of all species nor in hepatic peroxisomes of some species, like humans, that lack uricase. The biochemical composition of hepatic peroxisomes was characterized by de Duve and co-workers. Catalase is present in virtually all peroxisomes regardless of the source, whereas the presence of a number of the hydrogen peroxide-producing flaving oxidases and other enzymes is dependent on the source of peroxisomes. Peroxisomes also contain oxidases that reduce oxygen to hydrogen peroxide with the concomitant oxidation of an appropriate substrate. The catalase present within the peroxisomal matrix then degrades the hydrogen peroxide by either a catalatic or peroxidatic mechanism. In the peroxisomal β-oxidation pathway a fatty acid (usually C_8–C_{22} or more) is activated to an acyl-CoA derivative (22–24) by an ATP-dependent acyl-CoA synthetase located in the peroxisomal membrane. All subsequent reactions take place in the peroxisomal matrix. The fatty acyl-CoA is reduced with the utilization of oxygen to trans-2-enoyl-CoA, yielding hydrogen peroxide; this reaction is catalysed by acyl-CoA oxidase, the rate-limiting enzyme of peroxisomal β-oxidation. The next two reactions are catalysed by a bifunctional protein (25) possessing the activities of enoyl-CoA hydratase

and 3-hydroxy acyl-CoA dehydrogenase. This bifunctional protein is the same as the peroxisome proliferation-associated polypeptide (~Mr.80,000) reported by Reddy & Kumar (26) that can readily be detected by sodium dodecylsulphate-polyacrylamide gel electrophoresis. The final reaction of β-oxidation is carried out by 3-ketoacyl-CoA thiolase (27) which cleaves 3-ketoacyl-CoA into acetyl-CoA and a saturated acyl-CoA with two carbons less than the original molecule. The newly formed acyl-CoA then re-enters the β-oxidation pathway (22). Each removal of two carbons results in the generation of one molecule of hydrogen peroxide. The components of the peroxisomal β-oxidation system differ significantly from the enzymes of mitochondrial fatty acid β-oxidation (15, 23, 28) with respect to their molecular and catalytic properties. For example, peroxisomal β-oxidation does not require carnitine and is not inhibited by potassium cyanide.

The presence of fatty acid β-oxidation in peroxisomes is of major importance when considering the mechanism of xenobiotic induction of peroxisome proliferation and in examining the relationship between peroxisome proliferation and hepatocellular carcinoma.

PEROXISOME PROLIFERATION

Hess and co-workers in 1965 (7) and subsequently Svoboda & Azarnoff (29) made the first reports of peroxisome proliferation in the livers of rats and mice following the administration of clofibrate, a hypolipidemic drug. Since then, many hypolipidemic drugs have been shown to induce hepatic peroxisome proliferation (1, 2). These drugs appear to fall into two subgroups, clofibrate and structural analogues, and compounds structurally unrelated to clofibrate (see 2). This led to the hypothesis that all peroxisome proliferators possess a hypolipidemic property and that peroxisomes play a role in lipid metabolism.

Another important category of chemicals that cause hepatic peroxisome proliferation and hypolipidemia are the phthalate and adipate ester plasticizers, such as di(2-ethylhexyl)phthalate (DEHP) and di(2-ethylhexyl)adipate (2, 3, 8, 30–32).

A number of chlorinated hydrocarbons, such as trichloroethylene and perchloroethylene (4, 10–12), and branched-chained alkanes, such as 2,2,4-trimethylpentane (13), also induce hepatic peroxisome proliferation in rodents.

All these categories of chemicals induce hepatomegaly in rats and mice. The liver enlargement generally occurs within a few days of administration of the compound and reaches a steady-state level within 10–14 days. The hepatomegaly is maintained for as long as the chemical is administered and is dose-dependent (see 1, 2). The hepatic ultrastructure following the administration of any of these peroxisome proliferators is essentially the same except

for some differences in the magnitude of the increases in the numerical and volume densities of peroxisomes. These differences generally reflect the potency of the chemical and the dose administered. Clofibrate and other hypolipidemic drugs such as Wy-14643 ([4-chloro-6(2,3-xylidino)2-pyrimidinylthio] acetic acid) are several orders of magnitude more effective in inducing hepatomegaly and hepatic peroxisome proliferation than are phthalate esters or chlorinated hydrocarbons (2, 4).

Induction of Peroxisomal Enzymes

Induction of maximal peroxisome proliferation in the liver is associated with about a two-fold increase in the activity of the peroxisomal marker enzyme catalase, and up to a 30-fold increase in the activity of the peroxisomal fatty acid β-oxidation system as measured by CN-insensitive palmitoyl CoA oxidation (5, 28, 33). Although the catalase activity and its biosynthesis are not proportional to the increases in peroxisome volume density, the changes in the fatty acid β-oxidation system and the amount of Mr 80,000 polypeptide associated with peroxisomal proliferation appear to parallel the alterations in peroxisomal volume density. The bifunctional protein (Mr 80,000) becomes the most abundant protein in total liver homogenates of peroxisome proliferator–treated rats (34). Increases in the activities of other peroxisomal enzymes occur in livers with xenobiotic-induced peroxisome proliferation. These include carnitine acetyltransferase, carnitine octanoyltransferase, and acyl-CoA: dihydroxyacetone phosphate acyltransferase (2, 8). The existence of short chain-, medium chain-, and long chain-carnitine acyltransferases is now well documented (35). The short chain-carnitine acetyltransferase which is barely detectable in normal liver from rats or mice increases nearly 20-fold in animals treated with peroxisome proliferators (8). The activity of the peroxisomal enzyme urate oxidase, localised exclusively in the crystalloid core of the peroxisome is not, however, induced by peroxisome proliferators (36).

Immunoprecipitation and immunocytochemical studies with specific antibodies have shown that the protein content of catalase and the peroxisomal β-oxidation enzyme system is elevated in the livers of rats and mice treated with peroxisome proliferators. This increase in protein content is due to specific increases in messenger RNA in the liver as assayed by an in vitro reticulocyte translation system (37, 38). Analysis using cDNA probes has shown that two weeks treatment with ciprofibrate leads to about a 20-fold increase in the mRNAs encoding the peroxisomal acyl-CoA oxidase and bifunctional proteins in rat liver (39, 40). As anticipated, studies with a cDNA probe for rat catalase showed only about a two-fold increase in mRNA content. These large increases in mRNAs encoding for peroxisomal β-oxidation enzymes closely parallel the increase in protein content and

enzymatic activity of the β-oxidation system in the livers of rats that were fed peroxisome proliferators. Increases in hepatic mRNAs encoding for peroxisomal β-oxidation enzymes occur very rapidly; they are elevated within 4 hr of a single oral dose of the peroxisome proliferators ciprofibrate, clofibrate or DEHP (40), and methylclophenapate (41), suggesting that transcriptional activation of the genes of peroxisomal β-oxidation enzymes occurs. Similar in vivo studies in rats, using clofibrate only, demonstrated induction of mRNA for the peroxisomal bifunctional enzyme (enoyl CoA hydratase:3-hydroxyacyl-CoA dehydrogenase) at time periods of 12hr and greater (42). Studies using primary cultures of rat hepatocytes in the presence of Wy-14643 also showed an increase in the transcription rate of the 3-hydroxyacyl-CoA dehydrogenase gene (43). However, this did not occur until 15 hr after exposure, giving about a 50-fold increase by 48 hr. Similar in vitro studies in rat hepatocytes with nafenopin demonstrated induction of mRNA for acyl CoA oxidase and the bifunctional protein by one hour, increasing with time, although the response was small—about 5-fold at 20 hrs (44). There is thus a discrepancy in the time course of induction of mRNA in these various studies that may be related to the different potencies of the compounds, to in vitro versus in vivo differences, or to different mechanisms of induction (see later). More work is needed to examine the detailed time course of induction with different peroxisome proliferators.

Biogenesis of Peroxisomes

All peroxisomal proteins studied to date are synthesized on free polyribosomes and imported posttranslationally into existing peroxisomes. Some conflict as to the origin of peroxisomes exists. Early studies using electron microscopy suggested connections between the endoplasmic reticulum and the peroxisome, and it was suggested that peroxisomes arose by "budding" from the endoplasmic reticulum. However, biochemical and molecular biological studies have suggested that new peroxisomes are formed by division of preexisting peroxisomes. This topic has been thoroughly reviewed by Lazarow & Fujiki (45).

Induction of Cytochrome P-452

Induction of peroxisome proliferation in the liver is associated with proliferation of smooth endoplasmic reticulum (SER) (7–9, 46); this is generally associated with a concomitant increase in certain microsomal enzymes, in particular cytochrome P-450. Peroxisome proliferators appear to selectively induce liver cytochrome(s) P-450 that carry out ω-hydroxylation of lauric acid (13, 47–52). The cytochrome P-450 induced by clofibrate has been isolated and purified and designated cytochrome P-452 (53), cytochrome $P\text{-}450_{LA\omega}$ (54), or cytochrome P-450 IV (55). Cytochrome P-452 is induced by a

number of hypolipidemic agents that induce the ω-hydroxylation of both lauric acid and arachidonic acid (56). In general, long chain fatty acids are preferentially hydroxylated (57), but indirect evidence indicates that the side chain of mono(2-ethylhexyl)phthalate is also a substrate (58–59). Immunochemical studies with specific antibodies have shown that cytochrome P-452 protein is elevated 5- to 10-fold in the livers of rats treated with hypolipidemic agents (54, 60–61). The increase in this protein is due to specific increases in mRNA transcription. Analysis using cDNA probes has shown that a single dose of clofibrate leads to a three-fold increase in the mRNA encoding for cytochrome P-452 within 3 hr of administration, and 5–7 fold increase within 24 hr (54). This large increase in mRNA encoding for cytochrome P-452 closely parallels the increase in cytochrome P-452 protein and enzymatic activity for the ω-hydroxylation of lauric acid. Transcriptional activation of the cytochrome P-452 gene has been detected as early as one hour after clofibrate administration and has remained elevated for up to 24hr (54).

This rapid transcriptional activation of cytochrome P-452 is similar to the transcriptional activation of fatty acyl-CoA oxidase and bifunctional protein induced in rat liver by clofibrate (40). It appears that cytochrome P-452 is regulated by the same mechanism that regulates the peroxisomal enzymes. Indeed, a close link between the induction of microsomal and peroxisomal enzymes involved in the metabolism of lipids has been reported (49, 60), implicating a mechanistic interrelationship between changes in fatty acid metabolizing enzymes in the two hepatic subcellular compartments.

Hepatomegaly: Hyperplasia and Hypertrophy

Liver enlargement is a characteristic response of laboratory animals exposed to drugs or xenobiotics (62). All hypolipidemic drugs and chemicals such as DEHP that cause peroxisome proliferation produce significant hepatomegaly in rats, mice, and hamsters (for details see 2). The liver enlargement induced by these peroxisome proliferators results from both hyperplasia and hypertrophy. The hyperplastic response is seen within the first few days of peroxisome proliferator administration (10, 63, 64). Mitotic and ^{3}H-thymidine labeling indices were increased in mice treated with either nafenopin or trichloroethylene for 5 and 10 days respectively (10, 63). These chemicals are not necrogenic at doses stimulating peroxisome proliferation, so the wave of DNA replication and mitosis does not represent reparative hyperplasia. Recent studies with methylclofenapate and DEHP in the rat have shown that the initial hyperplastic response is due to the rapid conversion of binucleated cells to mononucleated tetraploid cells by amitotic cytokinesis following S-phase (65). Over a longer period of exposure there is an increase in the tetraploid:diploid ratio due to the conversion of newly formed binucleate cells

to tetraploid mononucleates (65–66). The implications of these cytological changes seen in the liver of rodents following peroxisome proliferation is unclear, but may be relevant to the carcinogenic process.

SPECIES DIFFERENCES IN RESPONSE TO PEROXISOME PROLIFERATORS

It is now well established that in rats and mice hypolipidemic agents and other chemicals such as DEHP induce hepatic peroxisome proliferation and peroxisomal and cytochrome P-452 enzyme induction (see 2, 6). Hamster liver is also responsive to these compounds, although to a lesser extent (67–70). However, several agents that are active in rats and mice have failed to elicit any peroxisome proliferation in dogs, marmosets, rhesus monkeys, or guinea pigs (33, 51, 70–76). In contrast, with the potent hypolipidemic drug ciprofibrate some hepatic peroxisome proliferation has been observed in both rhesus and cynomolgus monkeys (77). Examination of human liver biopsy material, obtained from patients receiving clofibrate, gemfibrozil, or fenofibrate, has demonstrated marginal or no increase in peroxisomal volume densities or numbers (78–83). All these studies show that there is a marked species difference in sensitivity to chemicals that cause hepatomegaly and peroxisome proliferation, with rats and mice being sensitive, hamsters intermediate, and guinea pigs, primates, and man insensitive or nonresponsive. However, in vivo studies of species differences in response to peroxisome proliferators are frequently confounded by variations in administered dose, target organ dose, or differences in routes and rates of biotransformation. Several laboratories have attempted to eliminate these confounding factors by using in vitro hepatocyte culture systems (84–89). Cultured hepatocytes from a number of species have been used to assess their response to peroxisome proliferators (4, 52, 90–92). These studies have shown a marked species difference in response to the proximate peroxisome proliferators: mouse and rat hepatocytes responded, while cultured guinea pig, and human hepatocytes revealed little effect upon peroxisomes or related enzyme activities, such as peroxisomal β-oxidation or microsomal ω-hydroxylation of lauric acid.

MECHANISM OF INDUCTION OF PEROXISOME PROLIFERATION

Two possible mechanisms for the induction of peroxisome proliferation have been considered: these are (*a*) activation of specific genes by the chemical or its metabolite, either directly or mediated by a specific receptor and (*b*) substrate overload, either as a result of lipolysis occurring outside the liver

and causing an influx of fatty acids into the liver, or as a consequence of the peroxisome proliferators or their metabolites perturbing lipid metabolism.

Receptor-mediated Mechanism

Reddy and co-workers have proposed that peroxisome proliferators exert their effects by a ligand-receptor mediated mechanism (2, 5, 93). A considerable amount of circumstantial evidence suggests that an interaction of a peroxisome proliferator-receptor complex with the nucleus is required for the induction of the peroxisomal β-oxidation and microsomal ω-oxidation gene transcription by peroxisome proliferators. This evidence includes (*a*) the tissue-specific biological response, particularly the information from liver cell transplantation studies (94–96); (*b*) the inducibility of peroxisome proliferation in hepatocytes in primary culture in defined medium (84–89); (*c*) the induction of similar specific changes in protein composition in the livers of rats exposed to structurally dissimilar peroxisome proliferators (34, 60); (*d*) the rapid and significant increase in the rate of synthesis of mRNAs for peroxisomal β-oxidation and microsomal ω-oxidation enzymes in liver (40, 54) and the rapidity of the transcriptional response of these genes; (*e*) the detection of a specific binding protein in liver cytosol for nafenopin, a peroxisome proliferator. All these findings imply that peroxisome proliferators act directly on hepatocytes to induce peroxisome proliferation. Lalwani et al (97) described a saturable pool of binding sites in the cytosol of rat liver that bound [^{3}H]-nafenopin; the level of this binding moiety correlated with the ability of this agent to induce peroxisome proliferation. Subsequent studies have isolated and partially characterized a 70 kD binding protein that binds nafenopin (98). When clofibric acid and ciprofibrate, two compounds closely related to nafenopin, were used as affinity ligands, they isolated the same 70 kD protein (98). However, recently other workers using high specific activity [^{3}H]-nafenopin or [^{3}H]-ciprofibrate have failed to detect any specific binding of these agents to hepatic microsomal or cytosolic fractions (99) and have thereby cast doubt on the existence of a specific cytosolic receptor for peroxisome proliferators. These latter authors did, however, show that these peroxisome proliferating hypolipidemic drugs reversibly bind to serum albumin, and they suggested that this may account for the binding observed by Lalwani and co-workers (97–98). Resolution of these conflicting views regarding binding proteins requires further investigation.

The structural requirements for chemicals that cause hepatic peroxisome proliferation have been examined (100–102). The only unifying theme with regard to the diverse chemical structures which cause peroxisome proliferation is that they all contain an acid function or are readily metabolized to a

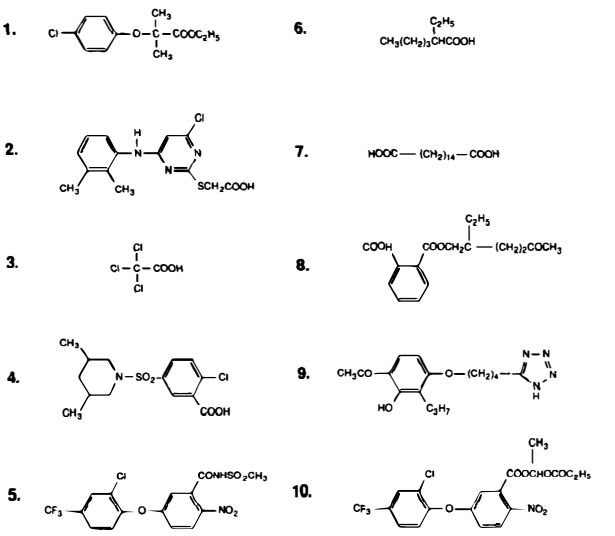

Figure 1 Structures of chemicals causing peroxisome proliferation in rodents.

For evidence of peroxisome proliferation in rodents see the following: 1, Clofibrate, α-p-chlorophenoxyisobutyrate (2); 2, Wy-14643, [4-chloro-6(2,3-xylidino)2-pyrimidinylthio] acetic acid (2); 3, Trichloroacetic acid (4); 4, Tibric acid, 2-chloro-5(3, 5-dimethylpiperidinosulphonyl) benzoic acid (9); 5, Fomesafen, 5(2-chloro-α,α,α-trifluoro-4-toyloxy)-N-methylsulphonyl-2-nitrobenzamide (91); 6, Ethylhexanoic acid, (8); 7, Hexadecan-1 : 16 dioic acid (107); 8, Metabolite VI of MEHP, mono(2-ethyl-5-oxohexyl)phthalate (52); 9, 4-THA, 2-hydroxy-3-propyl-4[6-(tetrazol-5-yl)hexyloxy] acetophenone (75); 10, Lactofen, 1'[carbethoxy] ethyl-5-[2-chloro-4 [trifluoromethyl] phenoxy] 2-nitrobenzoate (119).

chemical with an acid function. The acid function need not be a carboxylic acid, as shown by Fomesafen, which has a sulphonamide group (Figure 1), and 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone (4-THA), which has an acidic tetrazole moiety (Figure 1). The relative potencies of compounds to induce peroxisomal β-oxidation in isolated hepatocyte culture showed a good correlation with the electronic structural parameters of the molecules derived from molecular orbital calculations (101–102). These findings support a common intracellular site of action that may be a receptor protein or an enzyme binding site. However, the diverse nature of the chemical structures producing peroxisome proliferation (Figure 1) suggests that these agents do not act by interacting with a single type of binding protein with a single recognition site. It has been suggested (93) that a binding protein with multiple recognition sites exists or, alternatively, that several binding proteins with different ligand binding properties are involved.

Substrate Overload-Perturbation of Lipid Metabolism

The ability of structurally diverse chemicals to elicit peroxisome proliferation and the observation that this effect also occurs after a variety of physiological perturbations has led us to seek an alternative hypothesis to the receptor mechanism. Excess influx of fatty acids into the liver has been reported to cause small increases in peroxisomal β-oxidation (103). Feeding of high dietary fat can also produce increases in peroxisomal β-oxidation in the liver (104–106). The most striking effect of the chemicals that cause peroxisome proliferation in the liver is on lipid metabolism. For example, peroxisomal β-oxidation and microsomal ω-oxidation are increased along with the activities of carnitine acetyltransferase and carnitine octanoyltransferase, while the levels of CoA and carnitine are elevated. In contrast, other peroxisomal enzymes such as D-amino acid oxidase, urate oxidase and catalase are hardly affected. As a result of these findings, it has been proposed (90) that increased intrahepatic lipid may be an important factor in the genesis of peroxisome proliferation. The accumulation of hepatic lipid can occur in a number of ways, and the diverse chemical structures that produce peroxisome proliferation may act at many different loci to perturb lipid metabolism. Administration of DEHP results in a transient accumulation of small droplets of neutral lipid in the liver (31–32, 107) which disappear when the peroxisomes are induced. Studies in our laboratory (90, 107–109) have shown that the proximate peroxisome proliferator derived from DEHP, namely metabolite VI (mono(2-ethyl-5-oxo-hexyl)phthalate), causes a concentration-dependent decrease in the oxidation of palmitic acid by isolated rat hepatocytes and selectively inhibits medium chain octanoyl, but not palmitoyl, carnitine oxidation in isolated mitochondria (Figure 2). The precise site of action of metabolite VI is not known; it may be direct inhibition of a mitochondrial β-oxidation enzyme specific for medium chain fatty acids, or of carnitine octanoyl transferase responsible for the transport of medium chain acylcarnitines across the mitochondrial membrane (108–109). The inhibition by metabolite VI of long chain fatty acid metabolism (such as palmitic acid) in hepatocytes, but not in isolated mitochondria, may be explained by the depletion of essential CoA via metabolite VI per se, or by sequestration by medium chain fatty acids (Figure 2). Studies in our laboratory with isolated hepatocyte cultures have shown a rapid (within 30 min) depletion of CoA following the addition of mono(2-ethylhexyl)phthalate (107, 108). Inhibition of mitochondrial β-oxidation due to sequestration of CoA would lead to the accumulation of medium (C_6–C_{10}) and long chain (C_{12}–C_{20}) fatty acids or their CoA esters in the cell (Figure 2). Furthermore, it is possible that these fatty acids may induce microsomal cytochrome P-452 and peroxisome proliferation in order to maintain lipid homeostasis. In support of this hypothesis,

we have shown that medium chain (C_6–C_8) fatty acids induce cytochrome P-452 in cultured hepatocytes. It is also known that cytochrome P-452 preferentially metabolizes long chain fatty acids (57). Hence, the accumulated long chain fatty acids could be converted to long chain dicarboxylic acids (110) by the newly induced cytochrome P-452 (Figure 2). Furthermore, we have shown in cultured hepatocytes that long chain dicarboxylic acids (C_{14}–C_{18}) induce peroxisomal β-oxidation, but not microsomal ω-oxidation (107). These long chain dicarboxylic acids are also able to stimulate ^{3}[H]-thymidine incorporation into DNA in isolated rat hepatocytes in culture (107); this contributes to the hyperplasia seen following the administration of peroxisome proliferators (see page 150). The proposed mechanism for the induction of peroxisomes and stimulation of DNA synthesis via perturbation of lipid metabolism is outlined in Figure 2.

In support of this hypothesis are the findings by Foxworthy & Eacho (111)

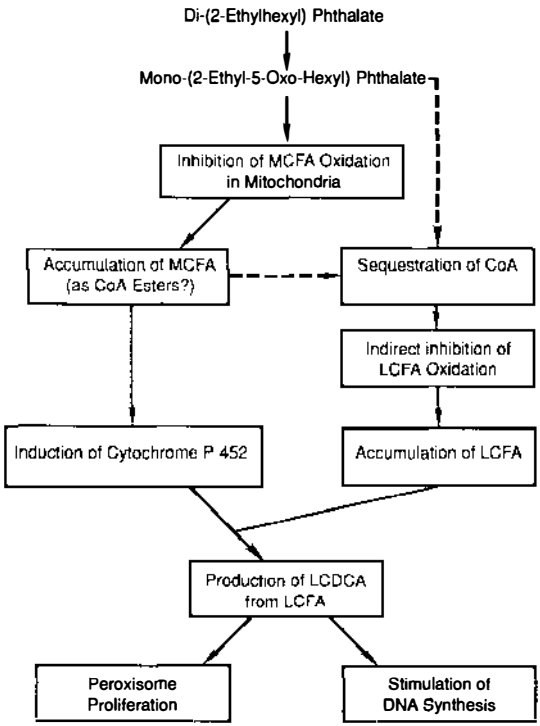

Figure 2 Proposed mechanisms of Di(2-ethylhexyl)phthalate (DEHP) elicited liver growth. MCFA, medium chain fatty acids C_6–C_{10}; LCFA, long chain fatty acids C_{12}–C_{20}; LCDCA, long chain dicarboxylic acids.

that the peroxisome proliferator, 4-THA, causes a concentration-dependent inhibition of fatty acid oxidation with oleate, but not with octanoate, in isolated rat hepatocytes (111). They also showed that 4-THA is a competitive inhibitor of carnitine palmitoyl transferase-1 in rat liver mitochondria (111). Other inhibitors of mitochondrial fatty acid oxidation, such as valproic acid and 2-[5-(4-chlorophenyl)pentyl] oxiran 2-carboxylate (112–114), also cause peroxisome proliferation in rodents (115–117).

Becker & Harris (112) reported that valproic acid caused a marked depletion in hepatic CoA and identified the formation of vaproyl-CoA plus an apparent CoA ester metabolite of vaproyl CoA, and proposed that either vaproyl CoA itself or the sequestration of CoA caused the inhibition of fatty acid metabolism. Bronfman and co-workers (118) also identified compounds that possessed the characteristics of CoA thio esters of clofibrate, nafenopin, and ciprofibrate in in vitro rat liver microsomal incubations. Bronfman (118) further suggested that acyl-CoA derivatives of hypolipidemic drugs could play a central role in peroxisomal proliferation. The substrate overload-perturbation of lipid metabolism hypothesis may explain the diversity of chemical structures and physiological conditions that can lead to peroxisome proliferation.

The two possible mechanisms of peroxisome proliferation discussed, i.e. the receptor hypothesis and substrate overload-perturbation of lipid metabolism, are not mutually exclusive, and a diagrammatic representation unifying the two is shown in Figure 3. In this scheme we are suggesting that specific lipid accumulation may be a key event in peroxisome proliferation, although inhibition of β-oxidation is not an essential requirement as other mechanisms may be operative. It is possible that certain peroxisome proliferating chemicals may coincidentally recognize an endogenous receptor involved in the regulation of lipid metabolism. The parallel mechanisms may account for the

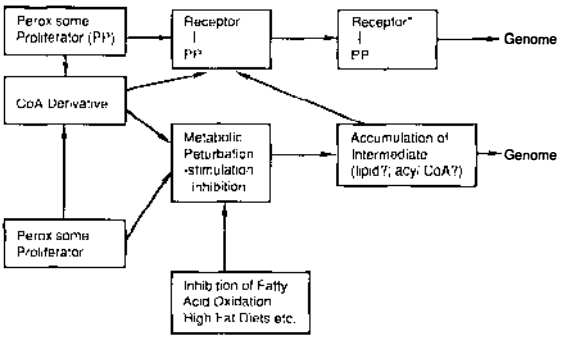

Figure 3 Unifying receptor-mediated mechanism of peroxisome proliferation.

The authors acknowledge valuable scientific discussion with Professor G. Mannaerts.

discrepancies in the time course of induction of key enzymes, as described earlier. Clearly, considerably more effort is needed to identify the precise biochemical mechanism whereby these structurally diverse chemicals perturb fatty acid metabolism and thereby induce peroxisomal β-oxidation and microsomal ω-oxidation, and the relationship between this event and the postulated receptor.

Further experiments are also required to determine whether the primary biochemical lesion, such as inhibition of β-oxidation by a DEHP metabolite, is operative in hepatocytes from species that are nonresponsive to peroxisome proliferators, or whether the actual lesion occurs but the cells do not respond to the accumulation of lipid. Preliminary findings in our laboratory have shown inhibition of mitochondrial β-oxidation, with the subsequent accumulation of lipid, in cultured guinea pig hepatocytes following exposure to metabolite VI of DHEP, although this required a higher concentration than in rat hepatocytes (C. R. Elcombe, A. M. Mitchell, unpublished observation). Inhibition of mitochondrial β-oxidation did not lead to the induction of either peroxisomal β-oxidation or microsomal ω-oxidation in guinea pig hepatocytes (52, and C. R. Elcombe, A. M. Mitchell, unpublished). These findings indicate a possible species difference in the metabolism of hepatic lipid.

CONCLUSIONS

Peroxisome proliferators are agents that cause a marked increase in the number of peroxisomes in liver cells. These agents produce marked changes in the profile of specific hepatic proteins and increase the levels of mRNAs encoding for peroxisomal β-oxidation and microsomal ω-oxidation enzymes. The regulation of expression of the peroxisomal and microsomal genes appear to be linked, and progress in understanding that regulation should be greatly facilitated by molecular biological techniques. The biochemical mechanism whereby the structurally diverse chemicals perturb fatty acid oxidation and the role of a cell-specific receptor(s) both require considerably more study. Only when a sound mechanistic basis has been established for the phenomenon of peroxisome proliferation will the species differences in sensitivity be elucidated and its association with liver cancer understood.

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