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The Effect of Tridiphane (2-(3,5-Dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane) on Hepatic Epoxide-Metabolizing Enzymes: Indications of Peroxisome Proliferation[1]

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The Effect of Tridiphane (2-(3,5-Dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane) on Hepatic Epoxide-Metabolizing Enzymes: Indications of Peroxisome Proliferation. MOODY, D. E., AND HAMMOCK, B. D. (1987). *Toxicol. Appl. Pharmacol.* **89,** 37–48. Administration of tridiphane (Tandem, DOWCO 356, 2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane) to male Swiss–Webster mice for 3 days at 100, 250, and 500 mg/kg (ip) resulted in increases in liver weight accompanied by an increase in mitotic index and increases in large particle and microsomal protein. Epoxide hydrolase (EH) activity towards *cis*-stilbene oxide (CSO, microsomal EH) was elevated in microsomes and cytosol, a decrease in microsomal cholesterol EH was found, and hydrolysis of *trans*-stilbene oxide (TSO, cytosolic EH) was elevated in the cytosol but not in the microsomes. Glutathione *S*-transferase (GST) activity was elevated in cytosol for CSO, TSO, and 1,2-dichloro-4-nitrobenzene (DCNB), with inconsistent responses found with 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP). Microsomal GST was not consistently effected by tridiphane. Clofibrate (500 mg/kg, 3 daily ip injections) treatment resulted in similar responses in liver size, microsomal protein, and the EHs. The increase in cytosolic EH activity previously has been noted only in animals treated with peroxisome proliferators. Examination of livers from mice treated with 250 mg/kg tridiphane revealed that an increase in hepatic peroxisomes was apparent after 3 days of treatment. This was accompanied by decreases in serum cholesterol and triglyceride levels and increases in liver carnitine acetyl transferase and cyanide-insensitive oxidation of palmitoyl-CoA. This study demonstrates that tridiphane does have *in vivo* effects on mammalian epoxide-metabolizing enzymes and extends the association of increased cytosolic epoxide hydrolase activity with peroxisome proliferation.

Increasing use of herbicides over the past few years has elevated them into the top class of pesticides with respect to volume produced and used (Storck, 1984). The limited data available on herbicides suggest that, as a class, they are relatively nontoxic. A paucity of data exists in the literature, however, concerning the short- and long-term effects of herbicides on mammalian systems. This is particularly so for many of the recently introduced herbicides.

Tridiphane has recently been registered as a postemergent herbicide to be used in conjunction with atrazine. Tridiphane and its glutathione conjugate have been found to inhibit the detoxification of atrazine by glutathione *S*-transferases (GST, EC 2.5.1.18) in several plants, and this has been proposed as

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one of the modes of action for tridiphane's synergistic effect on atrazine's herbicidal activity (Zorner and Olson, 1981; Lamoureux and Rusness, 1986). The conjugate has also been shown to inhibit GST purified from houseflies and equine liver (Lamoureux and Rusness, 1986).

Little is known concerning the effect of tridiphane on the mammalian GSTs or other xenobiotic metabolizing enzymes. As an epoxide, tridiphane would conceivably be metabolized by the epoxide hydrolases (EH, EC 3.3.2.3), as well as the GSTs. GSTs are a family of isozymes which are primarily found in cytosol, with minor activity also found in microsomes (Jakoby, 1978; Boyer and Kenney, 1985). Four different EHs have been identified in mammalian systems (Oesch, 1972; Lu and Miwa, 1980; Guengerich, 1982; Wixtrom and Hammock, 1985). The microsomal cholesterol EH (cholEH) (Watabe *et al.*, 1981; Levin *et al.*, 1983; Sevanian and McLeod, 1985) and a recently described leukotriene A4 epoxide hydrolase (McGee and Fitzpatrick, 1985) do not appear to interact with xenobiotics. Two of the epoxide hydrolases do have broad substrate specificities and appear to be involved in xenobiotic metabolism.

The xenobiotic-metabolizing epoxide hydrolases are historically designated according to their subcellular localization. One, primarily localized in microsomes with lesser activity in nuclei and cytosol, is referred to as microsomal EH (mEH). The other, primarily localized in the cytosol with lesser activity in the mitochondrial matrix and peroxisomes, is referred to as cytosolic EH (cEH). mEH and cEH have been found to differ, as well, by substrate specificity, physical and immunochemical criteria, and inducibility (Guenthner *et al.*, 1981; Hammock and Ota, 1983). mEH activity is induced by a wide number of xenobiotics (Oesch, 1972; Lu and Miwa, 1980; Guengerich, 1982). cEH activity and content, however, have been found to increase only in response to a limited number of compounds, all of which are peroxisome proliferators (Hammock and Ota, 1983; Waechter *et al.*, 1984; Loury *et al.*, 1985; Pichare and Gill, 1985). These latter compounds are nongenotoxic carcinogens which cause hepatocellular carcinomas in rats and mice (Reddy *et al.*, 1980; Reddy and Lalwani, 1983; Jackh *et al.*, 1984).

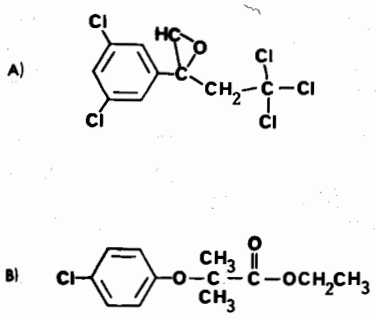

FIG. 1. The structure of (A) tridiphane and (B) clofibrate.

At this time we have studied the effect of tridiphane on epoxide hydrolase and GST activities in mouse liver microsomes and cytosol. These effects were compared to clofibrate which induces the activity of several enzymes involved in epoxide metabolism (Moody *et al.*, 1985) and is a known peroxisome proliferator (Svoboda and Azarnoff, 1966; Moody and Reddy, 1978). The structures of these two compounds are shown in Fig. 1. The ability of tridiphane treatment to increase cEH activity prompted additional studies on the response of hepatic peroxisomes to this herbicide.

MATERIALS AND METHODS

Materials. Tridiphane was provided by Dow Chemical Co. (Midland, MI). Clofibrate was provided by Ayerst Laboratories (New York, NY). *trans*-Stilbene oxide (TSO), *cis*-stilbene, 3,4-dichloronitrobenzene (DCNB), isooctane (99%), and hexanol (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Bovine serum albumin (fraction V), Lubrol PX, *p*-nitrophenyl phosphate, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP), NAD, dithi-

othreitol, FAD, CoA, acetyl-CoA, palmitoyl-CoA, carnitine, and reduced glutathione were purchased from Sigma Chemical Co. (St. Louis, MO). Bio-Rad protein reagent was purchased from Bio-Rad Laboratories (Richmond, CA). ACS scintillation fluid was purchased from Amersham (Arlington Heights, IL). Goat anti-rabbit IgG conjugated to alkaline phosphatase was purchased from Miles-Yeda Ltd. (Naperville, IL). *cis*-Stilbene oxide (CSO) and tritiated CSO and TSO were prepared as previously described (Gill *et al.*, 1983). Unlabeled and [^{14}C]cholesterol-α-epoxide were generously supplied by Alex Sevanian (School of Pharmacy, University of Southern California).

Animals and treatment. Male Swiss–Webster mice (Bantin-Kingman, Fremont, CA) weighing 25–30 g were housed in steel cages with kiln-dried pine shavings as bedding in an environmentally controlled room (12-hr light cycle, 22.5 to 24.0°C, constant humidity). Food (Purina Rodent Chow) and tap water were provided *ad libitum*. In the first set of experiments, tridiphane (100, 250, and 500 mg/kg) and clofibrate (500 mg/kg) were dissolved in corn oil and administered by ip injections given for 3 consecutive days between 9:00 and 10:00 AM. Control animals received corn oil alone (1.0 ml/kg). In the second set of experiments, treatments were as described above except that tridiphane was given only at a dose of 250 mg/kg. The second set of experiments was used for the preparation of liver section for morphologic examination and large particle fractions as described below.

Preparation of cell fractions. On the morning following the final injection (22–25 hr later) mice were killed by cervical dislocation, and livers were freed of gall bladders, perfused with 1.15% (w/v) KCl, and placed into tared beakers containing ice-cold 76 mM sodium phosphate (pH 7.4). Livers were then weighed and sections were taken for microscopy, and the remaining liver was minced and homogenized (20 sec, Polytron). Cytosol and washed microsomes were prepared from 10% (1 g liver/10 ml) homogenates using differential centrifugation as previously described (Moody *et al.*, 1985). In the second set of experiments, a large particle fraction was prepared. Livers were homogenized in 25 mM Tris (pH 7.4), 0.25 M sucrose, and 0.1% ethanol. The homogenate was centrifuged at 1000*g* for 10 min, the supernatant was collected and centrifuged at 18,000*g* for 20 min, and the pellet was resuspended in the above buffer. This pellet contains primarily mitochondria, peroxisomes, and lysosomes.

Morphology. Blocks of liver were cut from the medial lobe, covered with 2.5% glutaraldehyde in 0.1 M cacodylate (pH 7.5), and chopped into small (ca. 1-mm^3) blocks. These were fixed overnight at 4°C and then rinsed in buffer. Tissue was then embedded in Quetol 651, sectioned on a diamond knife with a Dupont Sorvall MT-2 ultramicrotome, stained with uranyl acetate and lead acetate, and examined with a Philips EM 400 electron microscope as previously described (Munn *et al.*, 1985). For light microscopy, sections were fixed in buffered formalin, and paraffin-embedded sections were prepared and stained with hematoxylin and eosin. Mitotic indices were determined as the number of mitotic figures observed per 1000 nuclei. Two slides of liver sections were used from each mouse with 20 fields observed (ca. 80–100 nuclei per field) per slide. Slides were prepared from four control and four treated mice.

Enzyme assays. cEH, mEH, and GST activities toward CSO and TSO were assayed using our previously described radiometric partition assays (Hammock *et al.*, 1985). In brief, protein was incubated with tritiated substrates (50 μM) as follows: mEH, CSO, pH 9.0; cEH, TSO, pH 7.4; GST, CSO, or TSO plus 5 mM glutathione, pH 7.4. Epoxides (EH) or epoxides and diols (GST) were then extracted with isooctane or hexanol, respectively. Aliquots of the aqueous phase were then taken for scintillation counting. CholEH activity was determined from the rate of hydrolysis of [^{14}C]cholesterol-α-epoxide to its diol using TLC as described by Sevanian and McLeod (1985). The rates of glutathione conjugation of CDNB, DCNB, and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP) were determined using the spectrophotometric methods described by Habig and Jakoby (1981). Catalase activity was determined as the rate of H_2O_2 disappearance at 240 nm as previously described (Moody and Reddy, 1978). Carnitine acetyltransferase and cyanide-insensitive oxidation of palmitoyl-CoA activities were determined spectrophotometrically using the methods previously described by Moody and Reddy (1974) and Lazarow (1981), respectively.

Other assays. Protein was determined using Bradford's assay modified for an enzyme-linked immunosorbent assay (ELISA) reader with computer readout as previously described (Moody *et al.*, 1985). Serum cholesterol and triglyceride levels were determined using a Gilford 3500 automatic analyzer. The immunochemical determination of mEH content was performed using our previously described indirect, double sandwich, noncompetitive ELISA (Moody *et al.*, 1987). Antibodies against mEH from *trans*-stilbene oxide-induced rats were prepared in rabbits and goats as previously described (Guenthner *et al.*, 1981) and were generously supplied by Tom Guenthner (University of Illinois, Chicago, IL) and Franz Oesch (University of Mainz, FRG). Briefly, ELISA plates were first coated with goat anti-mEH, rinsed, incubated with standards and microsomes in the presence of Lubrol PX, rinsed, incubated with rabbit anti-mEH, rinsed, incubated with goat anti-rabbit IgG conjugated to alkaline phosphatase, rinsed, and incubated with substrate, and the absorbance was read.

Calculations and statistics. Enzyme activities were routinely calculated as specific activity (nmol/min/mg protein), liver concentration (nmol/min/g liver), and total liver content (nmol/min/liver of 100-g mouse). Spe-

TABLE 1

THE EFFECT OF TRIDIPHANE ON MOUSE BODY AND LIVER WEIGHTS AND MICROSOMAL AND CYTOSOLIC PROTEINS

Treatment	Dose (mg/kg)	No.	Body weight (% initial)	Relative liver weight (g/100 g body wt)	Mitotic index (mitosis/ 1000 nuc)	Microsomal protein (mg/g liver)	Cytosolic protein (mg/g liver)	Mitochondrial protein (mg/g liver)
Control	—	5	101	5.3 ± 0.2	1.80	11.5 ± 1.6	89 ± 3	20.8 ± 0.4
Tridiphane	500	4	101	1.4 ± 1.1* (2.15)	—	21.3 ± 1.0* (1.85)	91 ± 10 (1.03)	—
	250	3	99	8.7 ± 0.6* (1.65)	5.54* (3.08)	18.8 ± 1.4* (1.64)	108 ± 5* (1.22)	25.8 ± 3.2* (1.24)
	100	3	97	6.6 ± 0.2* (1.25)	—	13.1 ± 1.3 (1.14)	98 ± 3* (1.10)	—
Clofibrate	500	4	99	6.7 ± 0.8* (1.27)	1.77 (0.98)	15.9 ± 0.8* (1.38)	111 ± 5* (1.26)	21.9 ± 5.6 (1.05)

Note. Male Swiss–Webster mice received 3 daily ip injections at the doses noted. Values are the means ± SD of the number of animals noted. Values in parentheses are the ratios to controls. Mitotic indices and large particle proteins were determined in the second experiment. Liver and body weight changes in these mice were comparable to those in the first experiment.

* Significantly different from controls, $p < 0.05$.

cific activities are reported, with the other values mentioned only when they lead to different interpretations. The significance of differences between treated and control mice was determined using Student's t test with $p < 0.05$ accepted as significant.

RESULTS

Treatment of male mice with 100, 250, or 500 mg/kg tridiphane and 500 mg/kg clofibrate for 3 consecutive days had no effect on their body weight gain as compared to controls receiving similar ip injections of corn oil (Table 1). The treatments did result in increases in liver weight which for tridiphane were dose dependent. An increase in liver weight was also seen in mice treated with clofibrate (Table 1). In liver sections from the second experiment, tridiphane, but not clofibrate, was found to increase the mitotic rate in hepatocytes. Microsomal protein was elevated in a dose-dependent fashion by tridiphane and also by 500 mg/kg clofibrate. Cytosolic protein was significantly increased after 100 and 250 mg/kg tridiphane but not after 500 mg/kg tridiphane and clofibrate treatment (Table 1). Tridiphane also increased the protein content of the large particle fraction in the second set of experiments.

The activity and content of mEH in microsomes were increased in a dose-dependent fashion by tridiphane. A slightly higher increase in immunochemically determined content than in activity occurred at each dose of tridiphane in contrast to the equivalent increases in immunochemically determined content and activity after clofibrate treatment. A similar increase in CSO hydrolysis was also found in the cytosol from mice treated with tridiphane and clofibrate (Table 2). A dose-dependent decrease in cholEH was found in mice treated with tridiphane while clofibrate treatment had no effect on this activity (Table 2). cEH activity was elevated in the cytosol, but not in microsomes, in a dose-dependent fashion by tridiphane, while treatment with clofibrate resulted in increases in both the cytosol and microsomes (Table 2).

TABLE 2

THE EFFECT OF TRIDIPHANE ON MOUSE LIVER MICROSOMAL AND CYTOSOLIC EPOXIDE HYDROLASE ACTIVITY

Treatment	Dose (mg/kg)	Microsomes				Cytosol	
		Anti-mEH	CSO	CholEH	TSO	CSO	TSO
		(ng/mg protein)	(nmol/min/mg protein)			(nmol/min/mg protein)	
Control	—	5.2 ± 0.5	3.15 ± 0.11	0.34 ± 0.02	0.074 ± 0.004	0.60 ± 0.07	5.8 ± 0.5
Tridiphane	500	10.2 ± 3.0*	5.12 ± 0.51*	0.21 ± 0.05*	0.067 ± 0.008	1.86 ± 0.04*	12.0 ± 1.0*
		(1.97)	(1.63)	(0.62)	(0.90)	(3.13)	(2.05)
	250	10.6 ± 1.5*	4.43 ± 0.21*	0.25 ± 0.01*	0.083 ± 0.008	0.99 ± 0.06*	8.5 ± 0.4*
		(2.04)	(1.40)	(0.73)	(1.12)	(1.66)	(1.14)
	100	8.6 ± 1.8*	3.28 ± 0.16	0.39 ± 0.09	0.081 ± 0.004	0.82 ± 0.04*	7.1 ± 0.3*
		(1.66)	(1.04)	(1.12)	(1.09)	(1.38)	(1.22)
Clofibrate	500	7.1 ± 0.4*	4.66 ± 0.50*	0.38 ± 0.03	0.116 ± 0.010*	0.99 ± 0.21*	11.2 ± 0.8*
		(1.37)	(1.48)	(1.10)	(1.57)	(1.67)	(1.92)

Note. Male Swiss–Webster mice received 3 daily ip injections at the doses noted. Values are the means ± SD of the number of animals shown in Table 1. Values in parentheses are the ratios to controls.

* Significantly different from controls, $p < 0.05$.

Treatment with tridiphane resulted in dose-dependent increases in cytosolic GST activities with CSO, TSO, and DCNB as substrates. Conjugation of ENPP in cytosol was elevated only at the highest dose of tridiphane, while no significant change in CDNB conjugation was seen in the cytosol or microsomes (Table 3). Treatment with tridiphane resulted in a decrease in microsomal GST toward CSO at 100 mg/kg, but with no significant changes resulting from higher doses. Clofibrate treatment resulted in significant decreases in microsomal GST for both CSO and CDNB and cytosolic GST for TSO and DCNP (Table 3).

Tridiphane's (250 mg/kg) effect on liver morphology was examined in mice from the second set of experiments. Upon gross examination the hepatomegalic effect of tridiphane was readily apparent, but livers were otherwise normal. Examination of liver sections under light microscopy revealed enlarged hepatocytes and a number of clear intracellular vacuoles primarily in the midzonal and centrilobular regions of the liver. The vacuoles were typical of fatty change, but no special stains were employed to confirm this observation.

The hepatic ultrastructures of control (Fig. 2) and tridiphane-treated (Figs. 3 and 4) mice were compared using tissue from mice receiving 250 mg/kg tridiphane. In control mice compact mitochondria with cristae, regular arrays of rough endoplasmic reticulum, scattered areas of smooth endoplasmic reticulum, a few peroxisomes, and glycogen deposits are readily apparent (Fig. 2). In liver tissue taken from tridiphane-treated mice, mitochondria are still compact with readily apparent cristae and the rough endoplasmic reticulum is still present in regular arrays. Increases in the number of peroxisomes are apparent, numerous lipid vacuoles are now present, and increases in smooth endoplasmic reticulum are evident even at low magnification (Fig. 3). Examination at higher magnification confirms the proliferation of the smooth endoplasmic reticulum and reveals that some peroxisomes still have a dense core as more commonly seen in controls (Fig. 4).

Additional evidence for the peroxisome-proliferating effect of tridiphane was found

TABLE 3

THE EFFECT OF TRIDIPHANE ON MOUSE LIVER MICROSOMAL AND CYTOSOLIC GLUTATIONE *S*-TRANSFERASE ACTIVITIES

Treatment	Dose (mg/kg)	Microsomes		Cytosol				
		CSO	CDNB	CSO	TSO	ENPP	DCNB	CDNB
		(nmol/min/mg protein)		(nmol/min/mg protein)				
Control	—	0.22 ± 0.06	93.0 ± 11.1	33 ± 2	6.1 ± 0.3	93 ± 11	32.9 ± 2.8	1920 ± 190
Tridiphane	500	0.27 ± 0.06	76.5 ± 18.1	102 ± 31*	15.4 ± 3.6*	130 ± 19*	80.5 ± 19.8*	2890 ± 1210
		(1.24)	(0.82)	(3.12)	(2.50)	(1.39)	(2.45)	(1.50)
	250	0.15 ± 0.04	92.7 ± 22.4	56 ± 8*	9.4 ± 1.2*	83 ± 21	46.0 ± 5.8*	1300 ± 420
		(0.71)	(0.99)	(1.72)	(1.52)	(0.89)	(1.40)	(0.68)
	100	0.13 ± 0.01*	85.0 ± 6.3	43 ± 3*	6.2 ± 1.0	82 ± 10	46.9 ± 4.5*	1630 ± 290
		(0.59)	(0.91)	(1.31)	(1.01)	(0.88)	(1.43)	(0.85)
Clofibrate	500	0.14 ± 0.06	74.3 ± 8.1*	34 ± 7	4.8 ± 0.6*	81 ± 12	25.0 ± 4.0*	1880 ± 80
		(0.65)	(0.80)	(1.04)	(0.79)	(0.87)	(0.76)	(0.98)

Note. Male Swiss–Webster mice received 3 daily ip injections at the doses noted. Values are the means ± SD of the number of animals noted in Table 1. Values in parentheses are the ratios to controls.

* Significantly different from controls, $p < 0.05$.

from studies on serum lipids and enzyme activities in the large particle fraction. Both tridiphane and clofibrate had a hypolipidemic effect after 3 days of treatment. Clofibrate caused a slightly greater decrease in serum cholesterol while tridiphane was more effective in decreasing serum triglycerides (Table 4). Both compounds caused significant increases in carnitine acetyltransferase and cyanide-insensitive β-oxidation of palmitoyl-CoA (Table 5). Tridiphane had no effect, while clofibrate decreased the catalase activity in these fractions. Neither compound had an effect on the hydrolysis of TSO and CSO in these fractions (Table 5). These results further strengthen the association that has been found between increases in cEH activity and peroxisome proliferation.

DISCUSSION

The initial purpose of these experiments was to test whether or not the newly registered herbicide tridiphane had any effects upon epoxide metabolism in mammalian systems. As tridiphane is an epoxide known to inhibit plant GST activity (Zorner and Olson, 1981; Lamoureux and Rusness, 1986), mouse liver GST and EH activities were chosen as potentially responsive indices of tridiphane's ability to perturb mammalian systems. It was found that tridiphane treatment did indeed result in numerous alterations in hepatic enzyme activities.

Increase in liver size is a common response to xenobiotic exposure (Schulte-Hermann, 1979). In animals treated with 250 mg/kg tridiphane the increase in liver weight was found to be associated with increased cell division, an increase in microsomal and large particle protein, and morphological evidence for peroxisome proliferation, smooth endoplasmic reticulum induction, and fatty infiltration of the liver. Peroxisome proliferation has consistently been associated with increases in the smooth endoplasmic reticulum (Svoboda and Azarnoff, 1966; Moody and Reddy, 1976; Staubli *et al.*, 1977). The appearance of lipid vacuoles in hepatocytes has been noted after treatment with other peroxisome proliferators (Reddy and Lalwani, 1983; Mitchell *et al.*, 1985).

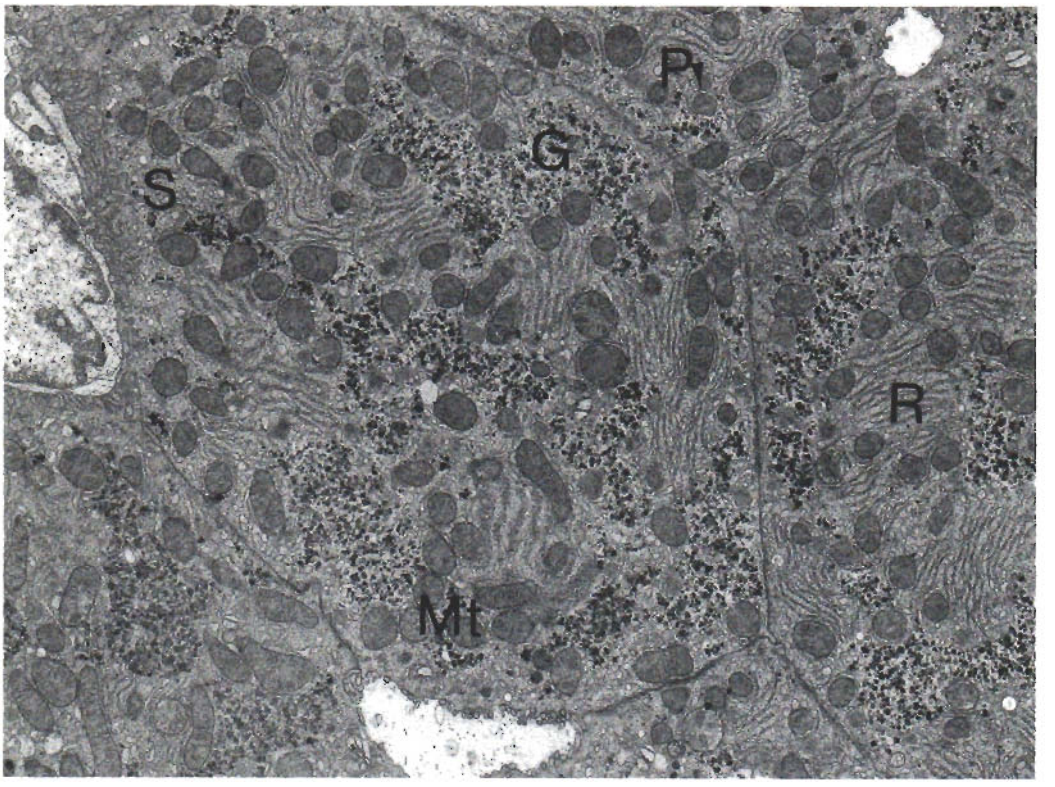

FIG. 2. Fine structure of liver from control male Swiss–Webster mouse. The common organelles are noted as Mt, mitochondria; S, smooth endoplasmic reticulum; R, rough endoplasmic reticulum; P, peroxisomes; and G, glycogen deposits. (6300×).

Certain consistent enzymatic changes have been associated with peroxisome proliferation (Moody and Reddy, 1974; Lazarow, 1977; Reddy and Lalwani, 1983). The increase in carnitine acetyltransferase and β-oxidation of palmitoyl-CoA in tridiphane-treated mice is characteristic of peroxisome proliferators. Maximal induction of these enzymes usually occurs after 14–21 days of treatment (Moody and Reddy, 1976, 1978; Lazarow, 1977). It is unlikely that the maximal increase in activities was achieved with the 3 days of tridiphane treatment used in this study, but significant changes could be detected within this time period. Catalase activity usually increases slightly (1.5- to 2-fold) in whole liver homogenates or cytosolic fractions, with a smaller or no response seen in large particle fractions after treatment with peroxisome proliferators (Moody and Reddy, 1976). The lack of change and decrease in catalase activity found in tridiphane and clofibrate-treated mice, respectively, may have been due to the brief treatment period and/or the choice of cell fraction examined.

Recently, the responses of epoxide-metabolizing enzymes, EHs and GSTs, have been studied in a number of laboratories. Perhaps the most unique response associated with these enzymes is the increase seen in cEH. At this time only peroxisome proliferators have been found to increase the activity of this enzyme (Hammock and Ota, 1983; Waechter *et al.*, 1984; Loury *et al.*, 1985). While both oral and intraperitoneal treatment with clofibrate increases the cEH-like activity in micro-

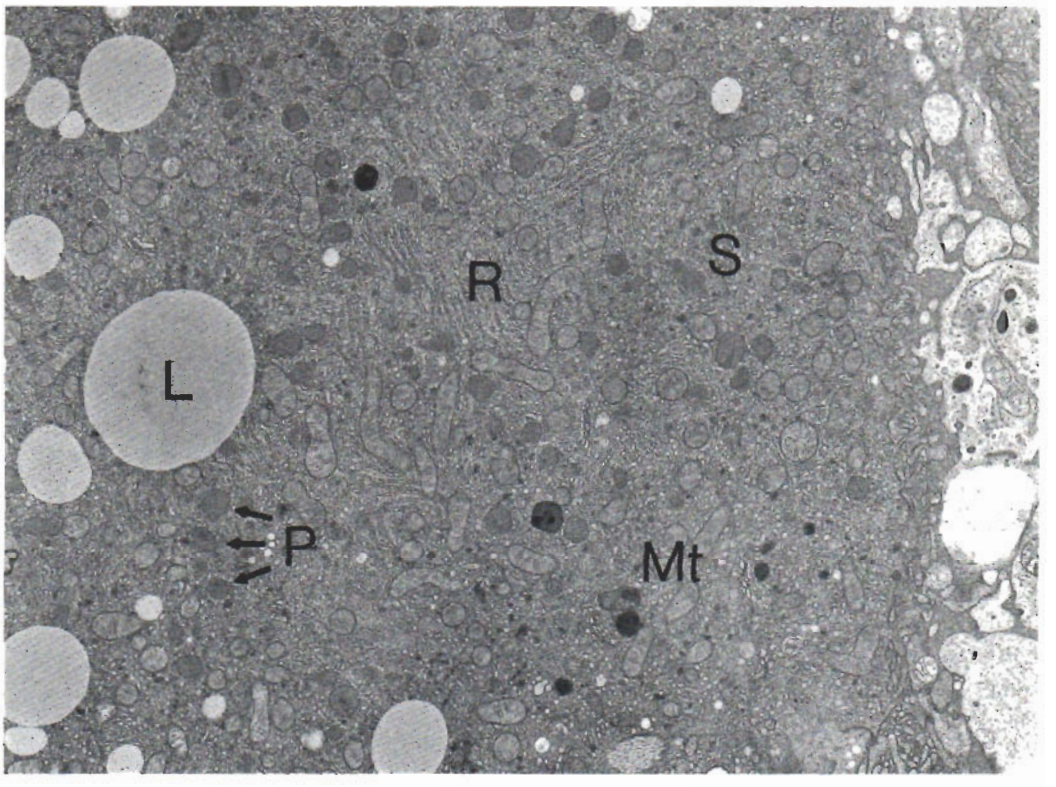

FIG. 3. Fine structure of liver from male Swiss–Webster mouse treated with tridiphane (250 mg/kg, ip) for 3 days. Abbreviations for organelles are the same as noted under Fig. 2. Note the abundance of peroxisomes and lipid vacuoles (L). (6300×).

somes, intraperitoneal treatment with tridiphane did not have this effect. While the cytosolic and microsomal enzymes acting on TSO have been found to share enzymatic and immunochemical properties (Guenthner and Oesch, 1983; Moody *et al.*, 1985), differential induction of the microsomal and cytosolic TSO hydrolase activity suggests that segregation of their regulation may occur.

The increases in mEH activity acting on arene oxides and CSO have also been consistently found after treatment with peroxisome proliferators (Levin *et al.*, 1983; Hammock and Ota, 1983; Waechter *et al.*, 1984). As previously found with clofibrate (Moody *et al.*, 1987), the increase in mEH activity after tridiphane treatment is accompanied by an increase in immunochemically determined content of the enzyme. As previously shown for rats (Levin *et al.*, 1983) no significant change occurred in microsomal cholEH in mice treated with clofibrate. A dose-dependent decrease in cholEH activity in mice treated with tridiphane is similar to the response seen after treatments with several other common inducers (Levin *et al.*, 1983).

Tridiphane treatment resulted in substrate-dependent responses in GSTs in both the cytosol and microsomes. GSTs exist as a number of isozymes in mouse liver (Lee *et al.*, 1981), and these findings may indicate isozyme-specific responses to tridiphane. Tridiphane and its glutathione conjugate are also known to inhibit certain *in vitro* and *in vivo* plant GST activities (Zorner and Olson, 1981; Lamoureux and Rusness, 1986), and

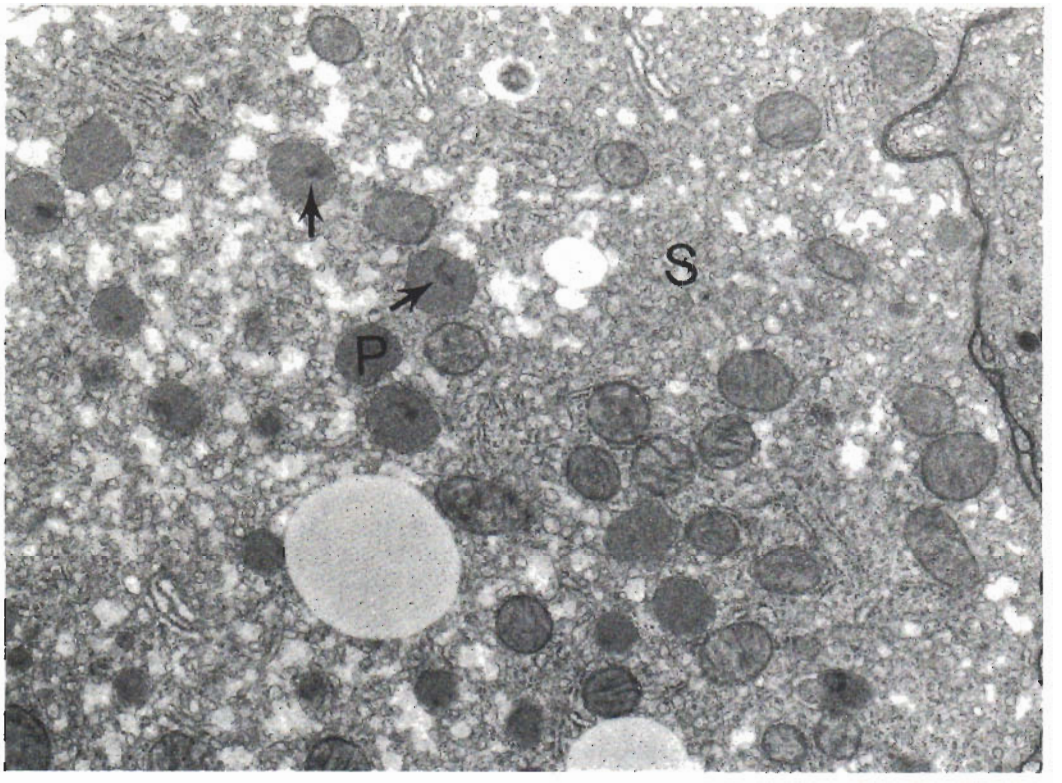

FIG. 4. Higher magnification photomicrograph of liver from tridiphane-treated mouse. Treatment and nomenclature are the same as in Fig. 3. Note that some peroxisomes have dense nuclei (arrows) and the abundance of smooth endoplasmic reticulum is readily apparent. (13,300×).

some other peroxisome proliferators are *in vitro* inhibitors of specific mammalian liver GST activities (Awasthi *et al.*, 1984; Vessey and Boyer, 1984; Foliot *et al.*, 1986). While limited data exist for the *in vitro* effect of tridiphane on mammalian GST activities, it may be possible that a mixed inductive–inhibition response occurs *in vivo*.

TABLE 4

EFFECT OF TRIDIPHANE ON SERUM LIPIDS

Treatment	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Control	137	408
Tridiphane	52	349
(250 mg/kg)	(0.38)	(0.86)
Clofibrate	88	321
(500 mg/kg)	(0.64)	(0.79)

Note. Assays were performed on sera pooled from four mice.

Peroxisome proliferation is a unique cellular response to a growing number of structurally diverse compounds. Recently, the chlorophenoxy herbicides 2,4-D, 2,4,5-T, and methylchlorophenoxyacetic acid (MCPA) have been identified as peroxisome proliferators (Vanio *et al.*, 1983; Kawashima *et al.*, 1984). These herbicides are structurally similar to clofibrate and it was not surprising that they also induce peroxisomes. Tridiphane shares a chlorinated phenyl ring with these compounds but lacks the phenolic ether as

TABLE 5

THE EFFECT OF TRIDIPHANE ON ENZYMES ASSOCIATED WITH PEROXISOME PROLIFERATION AND EPOXIDE HYDROLASES IN LARGE PARTICAL FRACTIONS

Treatment	Catalase (units/mg protein)	Carnitine acetyltransferase	Cyanide-insensitive β-oxidation of palmitoyl-CoA	Epoxide hydrolase	
				TSO, pH 7.4	CSO, pH 9.0
			(nmol/min/mg protein)		
Control	53.2 ± 6.7	8.2 ± 0.9	8.9 ± 1.6	3.72 ± 0.18	1.90 ± 0.24
Tridiphane (250 mg/kg)	41.7 ± 13.5 (0.78)	25.9 ± 5.0* (3.16)	20.4 ± 8.0* (2.28)	3.55 ± 0.51 (0.95)	1.96 ± 0.17 (1.03)
Clofibrate (500 mg/kg)	29.9 ± 9.2* (0.60)	37.8 ± 3.4* (4.62)	17.2 ± 3.8* (1.93)	3.46 ± 0.49 (0.93)	1.84 ± 0.35 (0.97)

Note. Assays were performed as described under Materials and Methods.
* Significantly different from controls, $p < 0.05$.

well as a free carboxylic acid or ester moiety. It therefore represents the first herbicide structurally unrelated to clofibrate and the chlorophenoxy herbicides to be a peroxisome proliferator. The demonstration that structurally diverse herbicides are also peroxisome proliferators suggests that other herbicides should also be screened for this activity. This is particularly so as it has been shown that peroxisome proliferators are nongenotoxic hepatic carcinogens in rats and mice (Reddy *et al.*, 1980; Jackh *et al.*, 1984). Preliminary reports on the long-term effects of tridiphane demonstrate that this compound causes liver tumors (Young *et al.*, 1986), strengthening this association. It is important to understand the mechanism by which these compounds exert their biological effects in order to more effectively regulate their use.

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