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Polychlorinated biphenyls increase fatty acid desaturation in the proliferating endoplasmic reticulum of pigeon and rat livers

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1. Polychlorinated biphenyls (PCB) are abundant and persistent pollutants in the ecosystem. Commercial mixtures (e.g. Aroclor 1254) can contain up to 80 different isomers and congeners, many of which accumulate in biological systems by the ingestion of PCB-contaminated lipid components of food chains.

2. Commercial mixtures of PCB induce, in hepatic microsomal membranes *in vivo*, a variety of different forms of the cytochrome *P*-450 components of enzyme systems involved in the metabolism of drugs and other xenobiotics, and can also induce the proliferation of this membrane. Since these microsomal enzyme systems share a number of the requirements of microsomal fatty acid desaturases, we have investigated whether the induction by PCB *in vivo* of cytochrome-*P*-450-linked enzymes in the proliferating hepatic microsomal membrane of the pigeon and the rat is accompanied by increased proportions of polyunsaturated fatty acids in this membrane.

3. The most striking changes observed 120 h after treating pigeons and rats with 1.5 mmol Aroclor 1254/kg body mass were 2.2-fold and 1.6-fold increases, respectively, in the proportion of arachidonic acid in the hepatic microsomal membrane. When the effects of this treatment on the proliferation of this membrane and increase in liver mass are taken into account, the amount of arachidonic acid in the total microsomal membrane of pigeon and rat livers increased 6.7-fold and 1.9-fold, respectively.

4. These changes were accompanied by very significant increases in pigeons and rats of the concentration of hepatic microsomal cytochrome *P*-450, and in the activity in microsomal protein of a wide range of cytochrome *P*-450-dependent enzyme involved in the metabolism of drugs and other xenobiotics.

5. This effect of PCB, of increasing *in vivo* the degree of unsaturation of fatty acids of hepatic microsomal membrane, appears to be a novel finding, and does not seem to have been investigated for other drugs and xenobiotics. Preliminary results have shown that the effect is accompanied by substantial increases in the total activity of Δ_6 and Δ_5 microsomal fatty acid desaturases converting 18:2 (9, 12) (linoleic acid) to 20:4 (5, 8, 11, 14) (arachidonic acid) [Borlakoglu, J. T., Dils, R. R., Edwards-Webb, J. D. & Walker, C. H. (1988) *Biochem. Soc. Trans. 16*, 1072].

6. It is postulated that there is a significant link between increased fatty acid desaturation and the induction of cytochrome-*P*-450-linked enzymes, and this is discussed in terms of the mechanisms involved in the metabolism of foreign compounds.

Polychlorinated biphenyls are abundant and persistent lipid-soluble pollutants in the ecosystem (see [1–3] for reviews). Commercial mixtures (e.g. Aroclor 1254) may contain up to 80 different isomers and congeners, many of which accumulate in biological systems through ingestion of PCB-contaminated lipid components of food chains, and PCB are present in fatty tissues and blood of species at the apex of these chains [4, 5].

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Abbreviation. PCB, polychlorinated biphenyl.

Enzymes. Fatty acid desaturases (EC 1.14.99.5); NADH:ferricytochrome-b_5 oxidoreductase (EC 1.6.2.2); NADPH–cytochrome-*c* (*P*-450) reductase (EC 1.6.2.4); cytochrome *P*-450-dependent monooxygenases, aldrin epoxidase, ethoxycoumarin *O*-deethylase, ethoxyresorufin *O*-deethylase, lauric acid hydroxylase (EC 1.14.14.1).

Treatment of animals *in vivo* with commercial mixtures of PCB can induce a variety of different forms of the cytochrome *P*-450 components of the enzyme systems of hepatic microsomal membranes (i.e. endoplasmic reticulum) that are involved in the metabolism of drugs and other xenobiotics. That is, PCB produce a pattern of induction that is observed in rodents in response to treatment with 3-methylcholanthrene (i.e. the induction of cytochromes *P*-450c and *P*-450d), or with phenobarbitone (i.e. the induction of cytochromes *P*-450b and *P*-450e), or produce a pattern that is a combination of several different forms of this cytochrome (mixed-type induction). This effect of PCB can be accompanied by substantial proliferation of hepatic microsomal membrane *in vivo*. It is of interest that the induction by PCB of phenobarbitone-induced forms of cytochrome *P*-450 correlates well with this proliferation, whereas the induction by PCB of 3-methylcholanthrene-induced forms of this cytochrome does not [6]. This raises the

question of whether the induction of specific forms of cytochrome *P*-450 is directly linked to the proliferation of microsomal membrane.

The microsomal cytochrome-*P*-450-dependent components induced by PCB have similar relative molecular masses to microsomal fatty acid desaturases and have the same requirements for NADH:ferricytochrome-b_5 oxidoreductase, cytochrome b_5, NADH or NADPH and molecular oxygen. Furthermore, a number of dietary and other studies have shown that enhancing the proportion of polyunsaturated fatty acids in the phospholipids of the hepatic microsomal membrane increases the activities in this membrane of a number of cytochrome-*P*-450-linked enzymes involved in the metabolism of drugs and other xenobiotics [7–12].

These reports led us to investigate whether the induction of cytochrome-*P*-450-linked enzymes in the proliferating hepatic microsomal membrane of the pigeon and the rat in response to PCB *in vivo* is accompanied by increased proportions of polyunsaturated fatty acids in this membrane. A preliminary report of part of this investigation has been published [13].

MATERIALS AND METHODS

Materials

Aroclor 1254, a commercial mixture of PCB, was kindly provided by Dr J. P. G. Wilkens, Ministry of Agriculture, Food and Fisheries Harpenden Laboratory, Herts, England. This mixture of PCB contains approximately 80 different isomers and congeners with molecular masses ranging from 88 Da to 430 Da, and an average molecular mass of 326 Da. Dr Wilkens also provided the Clophen A50. Chrompak CP Sill 88 was obtained from Packard Instruments, Pangbourne, England, and standard mixtures of methyl esters of fatty acids from Sigma Chemicals Co., Poole, England, and from Supelco, Sawbridgeworth, England. Preparations of SE-52 and Epitoke 1001 for GC were also obtained from Supelco. Biochemicals were purchased from Sigma Chemicals Co., and chemicals from BDH, Poole, England.

Animals

Adult female feral pigeons (*Columba livia*) were obtained from Lincolnshire Pheasantries, Boston, England. They were fed a diet containing equal masses of barley, wheat and maize that was supplemented (10%, by mass) with a high protein concentrate (Chick Starter Crumbs; T. Allsop & Sons, Ltd., Crowthorne, England). Food and water were available *ad libitum*. Routine post-mortem examination at the end of each experiment showed that their ovaries were quiescent (i.e. they were not laying).

Adult female Sprague-Dawley rats were supplied by Charles River (UK) Ltd., Margate, with an average mass of 175–200 g. They were fed a diet of chow purchased from E. Dixon and Sons Ltd., Ware, England. Food and water were available *ad libitum*.

Methods

Pigeons of 300 ± 15 g body mass (mean value ± SD; n = 26) were given a single intraperitoneal injection (up to 1 ml total volume) of 0.2 g, 0.5 g, 0.8 g or 1.0 g (i.e. 0.6 mmol, 1.5 mmol, 2.5 mmol or 3.0 mmol, assuming an average molecular mass of 326 Da) of Aroclor 1254 in corn oil/kg body mass at 10 a.m., and killed 24 h, 48 h, 68 h or 120 h later. Control pigeons were injected with corn oil alone.

Table 1. *Fatty acid composition of hepatic microsomal lipids 120 h after treating pigeons with Aroclor 1254*
Pigeons were injected with 1.5 mmol Aroclor 1254/kg body mass, or with cornoil, and killed 120 h later. Details of the preparation of the microsomal fraction and analysis of the fatty acids are given under Materials and Methods. Values are the mean ± SD of control (n = 6) and treated (n = 8) pigeons

Fatty acid	Fatty acid composition	
	controls	treated
	mol/100 mol	
16:0	19.4 ± 3.7	16.1 ± 3.31
16:1 (9)	2.03 ± 0.51	0.63 ± 0.43[c]
18:0	23.5 ± 5.46	33.09 ± 2.98
18:1 (9)	25.7 ± 2.95	14.31 ± 6.59[b]
18:1 (11)	3.20 ± 1.25	2.2 ± 0.47
18:2 (9, 12)	16.7 ± 3.23	20.5 ± 2.55
18:3 (6, 9, 12)	0.14 ± 0.01	0.17 ± 0.09
20:3 (8, 11, 14)	0.43 ± 0.22	0.65 ± 0.05
20:4 (5, 8, 11, 14)	5.9 ± 1.2	12.7 ± 3.06[c]
20:5 (5, 8, 11, 14, 17)	0.61 ± 0.41	0.29 ± 0.23
22:4 (4, 7, 10, 13)	0.53 ± 0.23	0.57 ± 0.16
22:5 (4, 7, 10, 13, 16)	0.38 ± 0.06	0.30 ± 0.15
22:6 (4, 7, 10, 13, 16, 19)	0.82 ± 0.43	1.86 ± 0.59[a]
Unidentified	0.66	

[a] $P < 0.05$ compared with controls.
[b] $P < 0.02$ compared with controls.
[c] $P < 0.01$ compared with controls.

Rats of 175–200 g body mass were given a single intraperitoneal injection (made up to 1 ml total volume) of 0.2 g or 0.5 g (0.6 mmol or 1.5 mmol) Aroclor 1254 in corn oil/kg body mass at 10 a.m., and killed 48 h and 120 h later. Controls rats were injected with corn oil.

In one experiment (see Table 3), Aroclor 1254 was replaced by Clophen A50, which is similar in composition.

Subcellular fractionation of liver homogenates

The liver was removed, washed free from superficial blood with ice-cold 0.15 M KCl, homogenized in this medium and the $10500 \times g_{av}$, for 30 min was prepared. The microsomal fraction (i.e. predominantly endoplasmic reticulum) was also prepared [14]. The microsomal pellet was resuspended in 0.25 M sucrose, 5 mM EDTA and 25 mM Tris buffer, pH 7.4, at a concentration of approximately 20 mg protein/ml. Portions were stored at −20 °C and thawed once.

Extraction of lipids and analysis of their fatty acid composition

Samples of liver (2 g wet mass) and the $10500 \times g_{av}$ pellet were extracted by the method of Folch et al. [15] with the methyl ester of 21:0 (3 mmol in 0.25 ml methanol) added as internal standard.

Methanol (7.5 ml) was added to the microsomal suspension (4.2 ml adjusted to contain 10–20 mg protein) together with the methyl ester of 21:0 as internal standard. Chloroform (16 ml) containing 0.005% (by mass) butylated hydroxytoluene as antioxidant was added and the mixture vortexed. The aqueous phase was removed, the chloroform phase dried over anhydrous sodium sulphate and reduced in volume to about 0.5 ml.

Table 2. *Fatty acid composition of hepatic microsomal lipids 48 h and 120 h after treating rats with Aroclor 1254*
Rats were injected with 1.5 mmol Aroclor 1254/kg body mass, or with corn oil, and killed 48 h or 120 h later. Details of the preparation of the microsomal fraction and analysis of the fatty acids are given under Materials and Methods. Values are the mean ± SD of controls (n = 5) and of rats killed at 48 h (n = 4) and 120 h (n = 6)

Fatty acid	Fatty acid composition		
	controls	treated for	
		48 h	120 h
	mol/100 mol		
16:0	27.9 ± 4.2	25.1 ± 0.35	18.02 ± 0.17[d]
16:1 (9)	3.1 ± 0.45	1.41 ± 0.29[c]	1.07 ± 0.17[d]
18:0	23.4 ± 3.53	29.5 ± 5.5	32.7 ± 2.59[d]
18:1 (9)	10.0 ± 4.2	11.2 ± 0.07	12.03 ± 1.37
18:1 (11)	5.4 ± 0.6	4.27 ± 0.19[c]	3.54 ± 0.44[d]
18:2 (9, 12)	13.05 ± 4.3	18.83 ± 1.64[c]	15.21 ± 0.61
20:4 (5, 8, 11, 14)	9.75 ± 1.34	11.24 ± 1.13[a]	15.79 ± 0.62[d]
22:6 (4, 7, 10, 13, 16, 19)	4.7 ± 0.98	4.43 ± 0.60	4.15 ± 0.99
Unidentified	11.7		

[a] $P < 0.05$ compared with controls.
[c] $P < 0.01$ compared with controls.
[d] $P < 0.001$ compared with controls.

Methyl esters of fatty acids were prepared and separated by high-resolution capillary GC, as described previously [16]. A minimum of 230 pmol methyl linoleate could be measured and methyl esters of fatty acids were identified by comparison with authentic standards.

Microsomal enzyme activities and microsomal cytochromes P-*450 and* b_5

Aldrin epoxidase was measured using the incubation conditions described by Krieger and Wilkinson [17]. At the end of the reaction, the dieldrin formed was measured by GC using SE-52 (2%, by mass) and Epikote 1001 resin (0.2%, by mass) on AW-DCMS-treated Chromosorb W (80–100 mesh) with an injector temperature of 220 °C, a column temperature of 165 °C and a detector (Ni_{63} electron capture) temperature of 250–300 °C. The dieldrin formed was measured by comparison with the peak areas produced by known amounts of dieldrin.

Ethoxycoumarin *O*-deethylase and ethoxyresorufin *O*-deethylase were measured by a spectrofluorimetric method [18] using umbelliferone and resorufin respectively as standards; dimethylnitrosamine *N*-demethylase was measured by the method of Jannetti and Anderson [19] and NADPH–cytochrome-*c* reductase by the method of Peterson et al. [20]. Lauric acid 12-hydroxylase was measured by the formation of 12-hydroxylauric acid by capillary GC as described by Borlakoglu et al. [21].

All assays were carried out in triplicate; controls without added substrate or with microsomal suspension that had been heated at 100 °C for 5 min were used, and the rates of reaction measured were uniform during the incubation. Pigeon and rat hepatic microsomal preparations were incubated at 42 °C and 37 °C, respectively.

Cytochromes *P*-450 and b_5 were measured by the methods of Omura and Sato [22] and Gibson and Skeff [23], respectively, and the concentration of protein in microsomal suspensions by the method of Lowry et al. [24] using bovine serum albumin as the standard.

RESULTS

Changes in the fatty acid composition of hepatic membranes after treating pigeons with Aroclor 1254

Treating pigeons with Aroclor 1254 had little effect on the fatty acid composition of the lipids of the hepatic organelles isolated as the $10500 \times g_{av}$ fraction of pigeon liver homogenates, but had a profound effect on the fatty acid composition of the lipids of hepatic endoplasmic reticulum isolated as the microsomal fraction (Table 1). Compared to controls, there were very significant decreases in the proportions of the monounsaturated fatty acids palmitoleic, 16:1 (9) (3.2-fold) and oleic, 18:1 (9) (1.8-fold), and striking increases in the proportions of the polyunsaturated fatty acids 20:4 (5, 8, 11, 14) (2.2-fold) and 22:6 (4, 7, 10, 13, 16, 19) (2.3-fold). The fact that the ratio of 20:4-(5, 8, 11, 14)/18:2 (9, 12) increased significantly from 0.35 to 0.62 suggests enhanced Δ_5 and Δ_6 fatty acid desaturase activities in this membrane in response to PCB.

Substantial proliferation of the hepatic microsomal membrane occurred in response to Aroclor 1254, as shown by the 1.8-fold increase in microsomal protein/g wet mass of liver (see Table 3) and by examination of the livers of control and treated pigeons by electron microscopy [16]. The wet mass of liver also increased significantly ($p > 0.001$) from 6.92 ± 0.72 g (n = 6) to 11.70 ± 1.10 g (n = 8) (mean values ± SD). When both of these factors were taken into account, the total amount of 20:4 (5, 8, 11, 14), in the proliferating microsomal membrane of the enlarged pigeon livers increased 6.7-fold in response to Aroclor 1254, and the total amount of the three major polyunsaturated fatty acids 18:2 (9, 12), 20:4 (5, 8, 11, 14) and 22:6 (4, 7, 10, 13, 16, 19) increased 5.2-fold.

Changes in the fatty acid composition of hepatic microsomal membrane after treating rats with Aroclor 1254

Compared with controls, there were very significant decreases in the proportions of the fatty acids 16:0 (1.5-fold), 16:1 (9) (2.9-fold) and 18:1 (11) (1.5-fold) in the microsomal

Table 3. *Activity of drug-metabolizing enzymes and concentrations of cytochromes* P-*450 and* b_5 *in hepatic microsomes 20 h after treating pigeons and rats with Aroclor 1254 or Clophen A50*

Pigeons and rats were injected as follows: (a) 0.6 mmol or (b) 1.5 mmol Aroclor 1254 or Clophen A50 in corn oil/kg body mass, or with corn oil, and killed 120 h later. Details of the preparation of hepatic microsomes and measurement of enzyme activities and cytochromes *P*-450 and b_5 are given under Materials and Methods. The results are mean values ± SD, with the number of animals shown in parentheses. n.d. = not determined

Protein	Treatment	Dose	Species	Activity unit	Activity of			
					pigeon enzyme		rat enzyme	
					control	treated	control	treated
Microsomal protein	Aroclor 1254	b		mg protein/g wet liver	4.5 ± 0.3 (6)	8.05 ± 3.2 (6)[c]	23 ± 2.0 (6)	53 ± 6.5 (4)[c]
Cytochrome P-450	Aroclor 1254	b		nmol/mg microsomal protein	0.17 ± 0.04 (6)	1.94 ± 0.49 (6)[c]	0.80 ± 0.20 (6)	3.06 ± 0.49 (6)[c]
Cytochrome b_5	Clophen A50	b a	pigeon rat	nmol/mg microsomal protein	0.13 ± 0.09 (5)	1.0 ± 0.50 (4)[c]	0.50 ± 0.15 (3)	0.70 ± 0.25 (3)
NADPH-cytochrome-*c* (*P*-450) reductase	Aroclor 1254	b a	pigeon rat	nmol · min^{-1} · mg microsomal protein^{-1}	25 ± 8 (4)	130 ± 30 (4)[c]	55 ± 10 (4)	87 ± 9 (4)
Aldrin epoxidase	Aroclor 1254	b a	pigeon rat	nmol · min^{-1} · mg microsomal protein^{-1}	0.2 ± 0.1 (5)	2.4 ± 0.4 (8)[c]	3.1 ± 0.3 (5)	21.3 ± 2.5 (6)[c]
Ethoxycoumarin *O*-deethylase	Aroclor 1254	b		nmol · min^{-1} · mg microsomal protein^{-1}	0.41 ± 0.24 (6)	3.80 ± 1.30 (8)[c]	0.3 ± 0.1 (4)	n.d.
Ethoxyresorufin *O*-deethylase	Aroclor 1254	b		pmol · min^{-1} · mg microsomal protein^{-1}	52 ± 9 (3)	2520 ± 83 (6)[c]	26 ± 7 (4)	n.d.
Dimethyl-nitrosamine *N*-demethylase	Aroclor 1254	b a	pigeon rat	nmol · min^{-1} · mg microsomal protein^{-1}	3.4 ± 0.8 (4)	7.4 ± 1.2 (4)[b]	2.7 ± 0.8 (4)	6.1 ± 0.5 (4)[b]
Lauric acid 12-hydroxylase	Aroclor 1254	b a	pigeon rat	nmol · min^{-1} · mg microsomal protein^{-1}	0.12 ± 0.06 (4)	0.11 ± 0.03 (4)	0.36 ± 0.12 (3)	1.85 ± 1.0 (4)[b]

[a] $P < 0.05$ compared with controls.
[b] $P < 0.02$ compared with controls.
[c] $P < 0.01$ compared with controls.

membrane 120 h after treating rats with Aroclor 1254 (Table 2). These decreases were accompanied by equally significant increases in the proportions of 18:0 (1.4-fold) and 20:4 (5, 8, 11, 14) (1.6-fold), and the ratio of 20:4 (5, 8, 11, 14)/18:2 (9, 12) increased 1.3-fold. Many of these changes were already apparent 48 h after treatment with Aroclor 1254.

As with pigeons, substantial proliferation of hepatic microsomal membrane occurred in response to Aroclor 1254, as shown by examination of the livers of control and treated rats by electron microscopy and by the 2.3-fold increase in microsomal protein/g wet liver, even when a dose of 0.6 mmol/kg body mass was used (see Table 3). The mass of wet liver also increased significantly ($p < 0.001$) from 3.20 ± 0.26 g ($n = 4$) to 4.00 ± 0.30 g ($n = 4$) (mean values ± SD). These changes led to a 1.9-fold increase in the amount of 20:4 (5, 8, 11, 14) in the total microsomal membrane of rat liver in response to Aroclor 1254.

*Activities of cytochrome-*P*-450-dependent drug metabolizing enzymes and concentrations of cytochromes* P-*450 and* b_5 *in hepatic microsomes 120 h after treating pigeons and rats with Aroclor 1254*

Table 3 shows the changes, produced in pigeons and rats by Aroclor 1254, in the concentrations of hepatic microsomal cytochromes *P*-450 and b_5, and the activities/mg microsomal protein of a wide range of hepatic cytochrome-*P*-450-dependent microsomal enzymes involved in drug metabolism. These enzymes were chosen as markers for comparison with the induction by Aroclor 1254 of cytochrome-*P*-450-linked enzymes in the livers of pigeons and rats. That is, phenobarbitone characteristically induces aldrin epoxides and dimethylnitrosamine *N*-demethylase, and treatment with 3-methylcholanthrene and other polycyclic hydrocarbons characteristically induces ethoxyresorufin *O*-deethylase. The

induction of lauric acid 12-hydroxylase is characteristic of the induction of cytochrome-*P*-450-dependent enzymes [1, 21].

Pigeons. The higher dose of 1.5 mmol Aroclor 1254 (or of Clophen A50 which is similar in composition)/kg body mass produced very significant increases in the concentrations of cytochrome *P*-450 (11.4-fold) and cytochrome b_5 (7.7-fold) and in the activities of NADPH–cytochrome-*c* (*P*-450) reductase (5.2-fold), aldrin epoxidase (12.0-fold), ethoxycoumarin *O*-deethylase (9.3-fold) and ethoxyresorufin *O*-deethylase (48.5-fold). There was also a significant but smaller increase in the activity of dimethylnitrosamine *N*-demethylase (2.2-fold), but not in lauric acid 12-hydroxylase.

Rats. The higher dose of Aroclor 1254 caused a significant increase in the concentration of cytochrome *P*-450 (3.8-fold). The lower dose of Aroclor 1254 or Clophen A50 produced small increases in the concentration of cytochrome b_5 (1.4-fold) and in the activity of NADPH–cytochrome-*c* (*P*-450) reductase (1.6-fold), but significant increases in the activities of aldrin epoxidase (6.9-fold), dimethylnitrosamine *N*-demethylase (2.3-fold) and lauric acid 12-hydroxylase (5.1-fold).

DISCUSSION

The effect *in vivo* of PCB, of increasing the degree of unsaturation of the fatty acids of hepatic microsomal membranes, appears to be a novel finding, and this effect does not appear to have been investigated for drugs and other xenobiotics. Although part of the PCB induced changes in the composition of hepatic microsomal membranes reported here might have been due to increased incorporation in this membrane of polyunsaturated fatty acids derived from other sources, preliminary results [13] have shown increases of 40-fold and 10-fold (mean values, $n = 12$ for both species), respectively, in the total activity of the Δ_6 and Δ_5 microsomal desaturases converting 18:2 (9, 12) (linoleate) to 20:4 (5, 8, 11, 14) (arachidonate) in the whole liver of the pigeon and the rat in response to Aroclor 1254 *in vivo*.

The mechanism of desaturation of fatty acids is not fully understood, but involves the microsomal electron-transport chain in a series of redox reactions in which NADH–cytochrome-b_5 reductase catalyses the reduction by NADH of cytochrome b_5 and this, in turn, provides the two electrons required for the introduction of the double bond into the fatty acid [25]. It is of special interest that Enoch and Strittmatter [26] isolated an NADPH-dependent stearoyl-CoA desaturase from rat liver microsomal membrane, and found that its activity could be restored by its incorporation in lipid vesicles with purified NADPH–cytochrome-*P*-450 reductase. Holloway [27] subsequently proposed a mechanism for the desaturation of fatty acids that is analogous to that involved in the cytochrome-*P*-450-dependent oxidation of drugs and other xenobiotics by hepatic microsomal membranes. Briefly, this involves the reduction of the prosthetic group of cytochrome *P*-450 via the first electron transfer, the binding of oxygen, the oxidation of the prosthetic group and superoxide formation, the second electron transfer, cleavage of the oxygen-oxygen bond, binding of the substrate which probably produces an epoxy fatty acid, and removal of the hydrogen atoms to form the desaturated fatty acid.

There is also evidence from nutritional studies that increasing the proportion of polyunsaturated fatty acids in the phospholipids of the hepatic microsomal membrane increases the activities in this membrane of cytochrome-*P*-450-linked enzymes involved in the metabolism of drugs and other xenobiotics. For example, hepatic microsomal hydroxylation and glucuronidation activities decrease significantly when rats are fed a fat-free diet [7], whereas supplementing the diet with 3% (by mass) corn oil increases the activities of aniline hydroxylase and ethylmorphine *N*-demethylase [10]. Similarily, Agradi et al. [8] and Rowe and Wills [9] established that fasting increases the proportion of saturated fatty acids in rat hepatic microsomal membranes and decreases the activity in this membrane of *p*-nitroanisole *O*-demethylase. Becker et al. [12] reported a similar decrease during fasting in the hepatic microsomal metabolism of benzo(a)pyrene. Ingelman-Sundberg [10] has shown that, with microsomal preparations depleted of total lipids, increasing proportions of phosphatidylcholine, or of a mixture (10:1, by mass) of phosphatidylcholine and phosphatidylinositol, were needed to restore functional cytochrome *P*-450 in this membrane, whereas phosphatidylethanolamine or phosphatidylserine were less effective. It is interesting that the addition of cholesterol together with phosphatidylcholine (1:1, by mass) to microsomes depleted of lipids inhibited the regeneration of functional cytochrome *P*-450. Moreover, when cholesterol alone was added, functional cytochrome *P*-450 decreased to 35% of the original value. Unfortunately, the fatty acid composition of these phospholipids was not analysed, but the results of Ingelman-Sundberg [10] show the need for a correct phospholipid environment for cytochrome *P*-450 to function maximally.

This cumulative evidence linking the fatty acid desaturases of the microsomal membrane with the NADPH–cytochrome-*P*-450 reductase component of the microsomal electron-transport chain is supported strongly by the results shown in Table 3. With the same dose of PCB, the increase in the unsaturation of the fatty acids of pigeon hepatic microsomal membrane (Table 1) was accompanied by very large increases in the concentrations of the cytochrome b_5 and cytochrome *P*-450 components of the microsomal electron-transport chain and in the activities of a wide range of NADPH–cytochrome-*P*-450-dependent enzymes involved in drug and xenobiotic metabolism. Rats showed similar patterns of response to PCB (Table 3). These results showed that commercial mixture of PCB, such as Aroclor 1254, produced a pattern of induction in pigeons and rats of cytochrome-*P*-450-dependent enzymes that is observed with rodents in response to 3-methylcholanthrene (i.e. the induction of cytochrome *P*-450c and *P*-450d) and to phenobarbitone (i.e. the induction of cytochrome *P*-450b and *P*-450e), as well as the induction of lauric acid 12-hydroxylase which is characteristic of the induction of cytochrome-*P*-450-dependent enzymes [1, 21].

In view of this association, in response to PCB, between increases in the proportion of polyunsaturatd fatty acids and the activities of microsomal Δ_6 and Δ_5 fatty acid desaturases, it will be of considerable interest to discover whether these enzymes are induced by other drugs and xenobiotics and what molecular structures are required to produce these effects. If this turns out to be the case, it raises a number of important questions, the answers to which would greatly increase our understanding of the mechanisms involved in the metabolism of foreign compounds. For example, does this increase in the proportion of microsomal polyunsaturated fatty acids lead to increased activity of cytochrome-*P*-450-dependent enzymes? If so, is this due to increasing (a) the fluidity of the membrane so as to provide the optimal conditions for the active sites of these enzymes to interact with hydrophobic drugs and other

xenobiotics as substrates, (b) the flow of electrons between the cytochromes involved in the electron-transport chain, and (c) the interaction between cytochrome *P*-450 and NADPH–cytochrome-*P*-450 reductase? The microsomal cytochrome-*P*-450-dependent components induced by PCB have similar relative molecular masses to microsomal fatty acid desaturases and have the same requirements for NADH:ferricytochrome-b_5 oxidoreductase, cytochrome b_5, NADH or NADPH and molecular oxygen. This raises the intriguing question of whether specific forms of cytochrome *P*-450, in conjunction with NADPH–cytochrome-*P*-450 reductase, can desaturate fatty acids in addition to their well established role [21, 28] in the hydroxylation of fatty acids.

Finally, a number of drugs and other xenobiotics can cause subtantial proliferation of the microsomal membrane, which might enhance the metabolism of these compounds by increasing the area of membrane available to them for interaction with membrane-bound cytochrome-*P*-450-dependent enzymes. It is not known whether this proliferation is linked to changes in the fatty acid composition of the phospholipids of this membrane and whether specific classes of phospholipids are affected. Proof that the induction of fatty acid desaturases is identical to the induction of specific forms of cytochrome *P*-450, in conjunction with NADPH–cytochrome-*P*-450 reductase, might eventually help to explain why the proliferation of hepatic microsomal membranes correlates with the induction of phenobarbitone-induced forms of this cytochrome but not with the induction of 3-methylcholanthrene-induced forms [6].

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