EFFECT OF ENVIRONMENTAL POLLUTANTS ON HEPATOCELLULAR FUNCTION IN RATS: 3-METHYLCHOLANTHRENE AND AROCLOR-1254

M.S.I. Dhami[1], M. Menon[2], R. Aliling[2], M. Afzal[3], C.J. DiFonzo[4], B. Ali[5] and G. Feuer[6]

[1]*Department of Chemistry, Nuclear Science Programs, San Jose State University, CA, USA*
[2]*Dhamen Associates, San Jose, CA 95148, USA*
[3]*Department of Biochemistry, Faculty of Science, Kuwait University, Kuwait*
[4]*Parke-Davis, Division of Warner-Lambert Canada Inc., Toronto, Ontario, Canada*
[5]*Sisters of Charity Hospital, Buffalo, NY 14003, USA*
[6]*Departments of Clinical Biochemistry and Pharmacology, University of Toronto, Ontario, Canada*

SUMMARY

Environmental pollutants, Aroclor-1254 (PCB) and 3-methylcholanthrene (MC), were employed in this study to investigate some aspects of the induction of hepatic drug metabolism in rats. PCB and MC treatments increased 7-ethoxyresorufin and 7-ethoxycoumarin O-deethylase activities related to cytochrome P-448. Cytochrome P-450 reductase activity was increased by PCB while no effect was observed by MC treatment. Pretreatment with PCB resulted in approximately 50% increase in the phospholipid content of the microsomes whereas MC caused no change. Liver microsomal cholesterol content was decreased while triglycerides were increased by PCB. The ratio between saturated and unsaturated fatty acids (saturation index) decreased in the total microsomes and phospholipids with PCB treatment, whereas MC did not alter the ratio, except that

Author for correspondence:
M.S.I. Dhami, Ph.D., D.O.H.S.
Department of Chemistry
San Jose State University
One Washington square
San Jose, CA 95192, USA

the major effect of MC was observed in the acyl derivatives of microsomal phosphatidylethanolamine. It is proposed that the uniaxial rotation and mobility of hemoproteins may be restricted by an increase in the saturation index of the membrane, while a decreased index may facilitate contact with reductases for electron transfer by enhanced membrane fluidity. The decreased saturation index after treatment with MC may play a role in carcinogenicity by triggering induction of free radicals.

KEY WORDS

polychlorobiphenyl (PCB, Aroclor-1254), 3-methylcholanthrene, liver, drug metabolism, cytochrome P-450, cytochrome P-448, rat

INTRODUCTION

Increased activities of microsomal drug metabolizing enzymes following treatment of animals with complex environmental chemical mixtures or xenobiotic compounds are now well documented /1,2/. Environmental chemicals and pharmacological agents differ in their mechanisms of induction and inhibition of metabolic activities. The features they all seem to have in common are (a) lipid solubility which causes them to become localized in the ER of the liver, and (b) being substrates of, or becoming bound to the ER drug metabolizing enzymes. This latter property appears to be an important criterion for enzyme induction, at least at the translational level. Mixtures of chlorinated biphenyls (Aroclor-1254) are potent enzyme inducers, more potent than the same dose of PB and at least as effective as DDT /3/. Aroclor-1016 (16% chlorine) is a much less potent inducer of hepatic microsomal oxidation than Aroclor-1254 (54% chlorine) /4/.

It is generally accepted that the ability of the mammalian liver monoxygenase system to metabolize a remarkably wide range of xenobiotics and endogenous substrates results from the participation of multiple forms of cytochrome P-450. Cytochrome P-450 has been

---

Abbreviations: APDM-ase, aminopyrine N-demethylase; ER, endoplasmic reticulum; ECD-ase, 7-ethoxycoumarine O-deethylase; EROD-ase, 7-ethoxyresorufin O-deethylase; G6Pase, glucose-6-phosphatase; MC, 3-methylcholanthrene; PB, phenobarbitol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PCB, polychlorobiphenyl (Aroclor-1254).

solubilized and the various isozymes isolated and purified to apparent homogeneity, from rat, rabbit, mouse, pig, and human liver pretreated with inducing agents, or from untreated animals. These isozymes differ in their physicochemical properties, immunological characteristics amino acid sequences, substrate specificity, molecular weights, and enzyme kinetics /5/. Multiple forms of cytochrome P-450 have been isolated from tissues other than liver, and two major forms (P-450 I and II) (>70% tissue total hemoprotein content) have been isolated from rabbit lung microsomes /6,7/.

Certain environmental chemicals and drugs bring about proliferation of microsomal membranes and increase in enzyme activity, while other substances, independent of their chemical structure, fragment these membranes and inhibit enzymatic action /8/. The increase in microsomal phospholipid content by PCB administration is not caused by stimulation of synthesis, but rather due to the inhibition of catabolism of membrane phospholipids /9/. MC administration was shown to depress both the turnover rate of total phospholipids and the formation of PC in microsomes /10/. On the other hand, CCl_4 inhibits both the synthesis and the turnover rate of microsomal phospholipids /11/.

The precise mechanism of induction of microsomal enzymes by xenobiotics may involve enhanced synthesis of new enzyme protein /12/ or reduced degradation, or both /13/. In view of the dependence of the mixed function oxidase system on membrane phospholipids, two environmental pollutants, Aroclor-1254 (PCB) and 3-methyl-cholanthrene (MC), were employed to investigate the inhibition and/or induction of hepatic drug metabolizing enzymes.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (Woodlyn Farm, Guelph, Ontario) weighing 200-220 g were housed in individual cages and maintained on laboratory chow (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) and water *ad libitum*. Animal quarters were well aerated, and air conditioned rooms were kept at 20 ± 2°C and 50-60% relative ambient humidity with a 12 h light:dark cycle. All rats were starved

overnight to reduce liver glycogen before decapitation. There were six animals in each group.

Experimental compounds

MC and PCB, dissolved in arachis oil, were administered i.p. at a dose of 80 mg/kg body weight, for seven days. The animals received the last injection of these compounds 18 hours prior to sacrifice. All animals were sacrificed between 8 and 8:30 a.m. to minimize any possible differences in drug metabolism due to circadian rhythmic variations. Control animals received the appropriate vehicle only. Purina Laboratory Chow was analyzed to confirm the actual fat content and was found to be in agreement with the declared value of 2%.

All tissue preparations and lipid analysis (phospholipid and fatty acids) were performed as described previously /14/. Reproducibility and precision of all analyses were within 90-95%.

Enzyme assays

Assays of 7-ethoxyresorufin O-deethylase activity (EROD-ase) and 7-ethoxycoumarin O-deethylase activity (ECD-ase) were carried out by methods described previously /15,16/. The activities of APDM-ase, G6P-ase and NADPH-cytochrome *c* reductase were assayed using standard methods described previously /17,18/.

Other determinations

Protein, cytochrome P-450, and phosphate contents were determined according to previously published methods /19-21/. Phosphate content of samples was measured by the Bartlett method /22/. The recovery of lipid phosphorus from thin layer plates was consistently 80-85% for samples from control and treated animals.

Statistical analysis

Statistical tests included Student's t-test /23/. Each result represents the mean ± S.E.M. of six measurements. The percentage of recovery from gas chromatography was computed by a rapid method /24/. Significant differences were accepted either at $p<0.05$ or $p<0.005$.

RESULTS

Microsomal constituents

Treatment by either PCB or MC had essentially no effect on the amount of DNA/g liver. PCB treatment significantly increased the triglyceride content while MC treated animals did not show any change. However, the cholesterol content was decreased while triglycerides were increased by PCB treatment. Phospholipid, protein and RNA contents were increased by PCB, while with MC treatment only RNA showed an increase as compared to the control group (Table 1).

Enzyme activities

It is evident from the results shown in Table 2 that APDM-ase activity significantly increased in PCB ($p<0.005$) and MC ($p<0.05$) treated animals. The inductive actions of PCB and MC did not affect G6P-ase activity. MC profoundly increased EROD-ase activity, while PCB caused an induction of this enzyme to a lesser extent. PCB and MC treatments both induced ECD-ase activity equally. The EROD-ase activity is, apparently, a more specific and sensitive assay for P-448 induction, as observed under these experimental conditions. PCB and MC treatment both increased cytochrome P-450 or P-448, while NADPH cytochrome *c* reductase activity was increased only by PCB treatment.

Liver-microsomal preparation

The administration of the test compounds significantly altered the fatty acid composition of the endoplasmic reticulum. As shown in Table 3, PCB treatment significantly increased myristic, pentadecanoic, palmitic, stearic, eicosenoic and lignoceric acids among the saturated acyl derivatives, and palmitoloic, oleic, linoleic, eicosadienoic, eicosatrienoic, arachidonic, eicopentaenoic and docostrienoic acids among the unsaturated acids. Docosapentaenoic and docosohexaenoic acids were not modified. A three-fold increase was observed in linoleic, arachidonic and lignoceric acids. This resulted in a relative change (as a percentage of the control value) of approximately 90% increase in total fatty acids by PCB treatment. MC administration did not affect these acyl derivatives.

TABLE 1
Protein, RNA, DNA, phospholipid, cholesterol and triglyceride composition of liver microsomes from male rats

PARAMETER	TREATMENT GROUP		
	CONTROL	PCB	MC
Protein (mg/g)	20.0 ± 1.0	25.9 ± 1.5*	23.0 ± 1.2
RNA (mg/g)	43.2 ± 3.6	55.8 ± 3.8*	56.4 ± 3.1*
Phospholipid (μmol P/g)	8.5 ± 1.0	13.4 ± 1.3**	9.1 ± 0.7
Cholesterol (mg/g)	457 ± 18	389 ± 19*	486 ± 14
Triglycerides (mg/g)	310 ± 16	423 ± 25*	328 ± 17

Values are expressed as means ± S.E.M. of six rats in each group
Values differ significantly from control: *p<0.05; **p<0.005.

TABLE 2
Liver microsomal enzyme activities and cytochrome P-450 in male rats

PARAMETER	TREATMENT GROUP		
	CONTROL	PCB	MC
Aminopyrine N-demethylase (nmol/h/mg protein)	28.3 ± 1.6	69.7 ± 4.2**	40.5 ± 3.1*
Glucose 6-phosphatase (umol/h/mg protein)	11.2 ± 1.1	11.9 ± 2.3	9.4 ± 3.0
7-Ethoxyresorufin O-deethylase (pmol/min/mg protein)	28.0 ± 3.0	510 ± 90**	3200 ± 500**
7-Ethoxycoumarin O-deethylase (nmol/min/mg protein)	2.6 ± 0.4	31.7 ± 4.8**	39.9 ± 5.1**
NADPH-Cytochrome c reductase (umol/min/mg protein)	1.1 ± 0.1	21.9 ± 0.1**	0.9 ± 0.1
Cytochrome P-450 or P-448 (nmol/g liver)	20.4 ± 1.6	34.6 ± 1.9**	32.9 ± 1.4**

Values are expressed as means ± S.E.M. of six rats in each group.
Values differ significantly from control: *p<0.05; **p<0.005.

TABLE 3
Fatty acid content of rat liver microsomal preparation

FATTY ACID	TREATMENT GROUP					
	CONTROL		PCB		MC	
	ug/g	%	ug/g	%	ug/g	%
14:0	200 ± 15	4.1	400 ± 28**	4.2	200 ± 15	4.0
15:0	100 ± 7	2.1	180 ± 14**	1.9	90 ± 7	1.9
16:0	910 ± 83	18.5	1320 ± 118*	13. 7	1010 ± 88	20. 2
16:1	110 ± 8	2.1	190 ± 16*	2.0	90 ± 7	1.8
18:0	860 ± 72	17.5	1400 ± 140*	14. 6	840 ± 71	17. 0
18:1	540 ± 43	10.9	700 ± 64*	7.3	650 ± 53	13. 0
18:2	660 ± 56	13.3	1990 ± 143**	20. 7	650 ± 57	13. 0
20:0	20 ± 2	0.4	140 ± 12**	1.4	20 ± 1	0.4
20:1	30 ± 2	0.5	130 ± 10**	1.3	20 ± 1	0.4
20:2	50 ± 4	1.0	150 ± 12**	1.5	50 ± 3	1.0
20:3	74 ± 6	1.5	180 ± 16**	1.9	80 ± 7	1.6
20:4	690 ± 57	13.9	1860 ± 178**	19. 3	570 ± 51	11. 5
20:5	100 ± 7	2.1	140 ± 10*	1.4	110 ± 9	2.2
22.3	50 ± 4	1.1	160 ± 14**	1.7	50 ± 4	1.0
22:5	170 ± 14	3.5	160 ± 13	1.7	180 ± 16	3.6
22:6	340 ± 28	6.9	360 ± 31	3.7	350 ± 34	7.1
24	50 ± 3	1.1	160 ± 14**	1.7	60 ± 5	1.2
Sat.	2140 ± 186	43	3600 ± 244**	37	2200 ± 158	44
Unst.	2800 ± 241	57	6010 ± 503**	63	2780 ± 236	56
Total	4940 ± 411		9610 ± 736**		4980 ± 462	

Values are expressed in micrograms of fatty acid per gram of liver and given as means + S.E.M. of six rats in each group; % indicates the percentage value of the total fraction..
Values differing significantly from control: *p<0.05; **p<0.005.
Sat./Unst. indicate saturated and unsaturated fatty acids.

PCB treatment modified the percentage distribution of acyl derivatives of palmitic, stearic, oleic, linoleic, arachidic, eicosaenoic, arachidonic, eicosapentaenoic, docosapentaenoic and docosohexaenoic acids, while MC treatment altered the distribution of palmitic, oleic and arachidonic acids only. The percent ratio of saturated to unsaturated fatty acids was modified from 43/57 in the Control group to 37/63 by PCB and 44/56 by MC treatments.

Microsomal phosphatidylcholine

The fatty acids in the PC fractions of microsomes (Table 4) represented 26, 20 and 24% of the total microsomal fatty acids in control, PCB and MC treated rats, respectively. The experimental treatments caused similar trends as those seen in the composition of total microsomes. PCB treatment elevated the total amount of microsomal PC acyl derivatives by 51%, while MC treatment decreased acyl derivatives by 5% as compared to control values. PCB treatment caused a three-fold increase in palmitoleic acid content, while profound increases were observed in all other acyl derivatives

TABLE 4
Fatty acid content of rat liver microsomal phosphatidylcholine fractions

FATTY ACID	TREATMENT GROUP					
	CONTROL		PCB		MC	
	ug/g	%	ug/g	%	ug/g	%
14:0	4 ± 1	0.3	20 ± 3**	1.0	3 ± 1	0.2
15:0	3 ± 1	0.2	20 ± 3**	1.0	3 ± 1	0.2
16:0	320 ± 28	24.5	310 ± 27	15. 7	280 ± 20	23. 2
16:1	20 ± 2	1.6	60 ± 8**	3.0	20 ± 18	1.6
18:0	240 ± 21	18.4	320 ± 28*	16. 2	220 ± 21	18. 0
18:1	90 ± 7	7.2	140 ± 11*	7.1	120 ± 10	9.7
18:2	180 ± 17	14.0	370 ± 27**	18. 8	170 ± 14	14. 1
20:1	5 ± 1	0.4	20 ± 2**	1.0	4 ± 1	0.3
20:2	2 ± 1	0.1	20 ± 2**	1.0	3 ± 1	0.2
20:3	7 ± 1	0.5	20 ± 2**	1.0	7 ± 1	0.6
20:4	290 ± 24	22.2	390 ± 26*	19. 8	260 ± 20	21. 4
20:5	40 ± 3	2.9	70 ± 5**	3.5	30 ± 2	2.8
22:5	12 ± 1	0.9	50 ± 3**	2.5	11 ± 1	0.9
22:6	100 ± 8	7.5	160 ± 15**	8.1	90 ± 6	7.3
Sat.	560 ± 48	43	670 ± 46	33	510 ± 38	41
Unst.	740 ± 61	57	1300 ± 84**	66	720 ± 40	59
Total	1300 ± 110		1970 ± 139**		1230 ± 76	

Values are expressed in micrograms of fatty acid per gram of liver and given as means ± S.E.M. of six rats in each group; % indicates the percentage value of the total fraction..
Values differing significantly from control: *p<0.05; **p<0.005.
Sat./Unst. indicate saturated and unsaturated fatty acids.

except for palmitic acid. MC treatment did not elicit any significant effect on these fatty acids. The percentage composition of fatty acids was altered by PCB treatment in myristic, pentadecaenoic, palmitic, palmitoleic, stearic, linoleic, eicosaenoic, eicosadienoic, eicosatrienoic, arachidonic and docosohexaenoic acids. MC treatment altered oleic and eicosadienoic acid levels.

The percent ratios of saturated to unsaturated fatty acids were also modified by these experimental compounds (Control, 43/57; PCB, 34/66 and MC, 41/59).

Microsomal phosphatidylethanolamine

Table 5 shows the effects of PCB and MC on fatty acid concentrations in microsomal PE fractions. The inductive action of MC treatment modified various acyl derivatives only in this fraction. Those

TABLE 5
Fatty acid content of rat liver microsomal phosphatidylethanolamine fractions

FATTY ACID	TREATMENT GROUP					
	CONTROL		PCB		MC	
	ug/g	%	ug/g	%	ug/g	%
16:0	130 ± 12	20.9	240 ± 21**	21.4	200 ± 17**	23.8
16:1	7 ± 1	1.1	20 ± 2**	1.8	10 ± 1*	1.2
18:0	150 ± 9	23.8	240 ± 19*	21.4	210 ± 14*	25.0
18:1	80 ± 6	12.7	150 ± 12**	13.4	110 ± 9*	13.1
18:2	60 ± 4	9.5	170 ± 13**	15.2	70 ± 4	8.3
20:3	5 ± 1	0.8	20 ± 2**	1.8	8 ± 1*	0.9
20:4	140 ± 10	22.2	190 ± 11*	16.9	180 ± 15	21.4
22:5	5 ± 1	0.8	10 ± 2**	0.9	10 ± 2	1.2
22:6	40 ± 2	7.9	80 ± 3**	7.1	50 ± 3*	5.9
Sat.	280 ± 20	45	480 ± 40**	42	410 ± 31*	49
Unst.	340 ± 20	55	640 ± 51**	58	430 ± 35	51
Total	620 ± 47		1120 ± 89**		840 ± 64	

Values are expressed in micrograms of fatty acid per gram of liver and given as means ± S.E.M. of six rats in each group; % indicates the percentage value of the total fraction..
Values differing significantly from control: *p<0.05; **p<0.005.
Sat./Unst. indicate saturated and unsaturated fatty acids.

fatty acids altered by MC treatment were palmitic, palmitoleic, stearic, oleic, eicosatrienoic and docosohexenoic acids. PCB treatment caused a two-fold increase in the contents of palmitic, oleic, docosapentaenoic and docosohexaenoic acids, while a three-fold increase was observed in palmitoleic and linoleic acids. Stearic and arachidonic acid were also increased significantly.

In this fraction, fatty acids represented 13, 12 and 17% of the total microsomal fatty acids in control, PCB and MC treated rats, respectively. The percentage composition of fatty acids was modified by PCB treatment in palmitoleic, stearic, linoleic, eicosatrienoic and arachidonic acids, while MC treatment affected palmitic, stearic, linoleic, and docosahexaenoic acids. The total amount of both saturated and unsaturated fatty acids was increased by PCB treatment. However, both treatments increased the total amount of fatty acids in this fraction, thus modifying the percent ratios between saturated and unsaturated components (Control 45/55; PCB 42/58; MC 49/51).

DISCUSSION

Induction of drug metabolizing enzymes

As cytochrome P-450 and P-448 have widely different substrate binding sites and show markedly different substrate specificities (Table 2), they may be considered as two distinct enzyme proteins rather than isozymes of the same enzyme /6/. Hepatic microsomes from MC-pretreated rats incubated with biphenyl (which displaces ligand from heme) /25/ showed a 25% increase in the amount of cytochrome, whereas no such effect was observed in PB-pretreated rats. These observations indicate that MC or its metabolite is associated with the sixth ligand of the heme moiety of the hemoprotein. Electron spin state studies have shown that two forms of P-448 are formed when rats are treated with MC, one low-spin and one high-spin form /26/. Addition of MC to the low-spin form converted it to the high-spin form, demonstrating that the latter is a complex of the cytochrome with the carcinogenic activity. This association of MC with the sixth ligand of the heme could affect the conformation of the "heme-pocket" and of the substrate-binding site, and thus account for the different substrate specification. Cytochrome P-450 possesses one substrate-binding site of broad specificity which can accommodate a diversity of substrates,

while cytochrome P-448 possesses a different binding site exhibiting narrow specificity /27/. The results of the present work support this hypothesis (Table 2). MC and PCB, inducers of P-448, specifically enhance the ethoxyresorufin and ethoxycoumarin-O-deethylase activities. EROD activity along with liver weight have been suggested as biomarkers of PCB exposure in rodents /28/. The sensitivity and specificity of the ethoxyresorufin-O-deethylase assay for carcinogens reported previously /29/ agree with our results.

Undoubtedly, cytochrome P-448 is induced by *de novo* synthesis following treatment of animals with MC and other substrates. Furthermore, a cytosolic binding protein for MC and similar inducing agents has been identified /30,31/ and the intracellular appearance of an inducer-receptor complex has been shown to correlate with induction of cytochrome P-448 mRNA /32/. Induction of cytochrome P-448 appears to depend on this cytosolic receptor /33/. On the other hand, PB and PCB treatments led to increased levels of several other microsomal proteins, such as NADPH-cytochrome *c* reductase /34/ and epoxide hydrase /35/, keto aldehyde formation /36/ as well as cytosolic enzymes such as glutathione-S-transferase /29/ and aldehyde dehydrogenase /37/. Increased levels of translatable cytochrome P-450 mRNA in PB-treated rats /38,39/ up to 100-fold justifies further studies on the induction process to elucidate the involvement of transcriptional and/or post transcriptional mechanisms.

Hypertrophy of the endoplasmic reticulum

A modified bilayer may be required to accommodate the increased amounts of induced microsomal drug metabolizing enzymes. Thus, induction of these enzymes by PB and PCB should increase the protein content of the endoplasmic reticulum as well as RNA (Table 1). Indeed, induced microsomes do contain 26% more protein per g liver than do control microsomes /40/. The present values are in approximate agreement with the observed increases in protein (34%), RNA (30%) and phospholipid (57%) contents of the endoplasmic reticulum (Table 1).

Many compounds of widely diverse chemical structure induce the mixed function oxidase system in the liver and other tissues, with accompanying proliferation of the endoplasmic reticulum and cellular hypertrophy /2,6,29/. Since many of the xenobiotics used today are inducing agents, enzyme induction may have important clinical

implications /41,42/. Induction of the mixed function oxidase is associated with increased biosynthesis of the hemoproteins cytochrome P-450 and cytochrome *c* reductase (Table 2), which requires the synthesis of new protein, but not of heme, since the heme pool can usually meet the increased requirements /43/.

An increase of microsomal phosopholipid content was observed in the endoplasmic reticulum of rats treated with PCB (Table 1). Ishidate and Nakazawa /9/ suggested that the PCB-induced increase in the phospholipid content of microsomes, particularly phosphatidylcholine, is not due to stimulation of synthesis but is entirely due to a decrease in catabolism, thus differing from the effect of PB.

In the present study, MC treatment did not alter the phospholipid content of microsomes (Table 1). During induction with MC, alteration in phospholipid methylation is possibly the primary cause of the decrease in membrane viscosity /8/ and may decrease the turnover rate of the phospholipids in the ER.

Fatty acid composition of the endoplasmic reticulum

The properties of phospholipids are also greatly influenced by the nature of the fatty-acyl groups they contain. Relative to other subcellular fractions, microsomes are rich in essential fatty acids (i.e. the total contents of linoleic acid and polyenoic acids containing 20 to 22 carbon atoms). The polyenoic acid content of phospholipids is of special interest in several aspects of induction and inhibition /2/.

Since phospholipids compromise the majority of total lipids in the hepatic ER, changes in fatty acid composition in the present study under different treatments may be occurring mainly in the phospholipids of the membrane. *De novo* fatty acid synthesis is catalyzed by two enzyme systems (acetyl CoA carboxylase and fatty acid synthetase) for long-chain saturated fatty acids which function sequentially. Moreover, acyltransferase plays a role in the control of the fatty acid composition of the endoplasmic reticulum /44/. In addition, for the synthesis of mono- and polyunsaturated fatty acids, desaturation and chain elongation are supplementary processes /45/. Therefore, PB, PCB, and MC may influence these enzyme systems, resulting in changes of unsaturated and polyunsaturated fatty acid synthesis and their subsequent incorporation into the endoplasmic reticulum membrane.

A comparison of the levels of induced enzyme activities (Table 2) in hepatic endoplasmic reticulum with altered fatty acid composition (Table 3), with those membranes having normal fatty acid composition, gives an indication of typical concentrations of fatty acids required for maximal enzyme activity. The rats treated with PCB showed marked incorporation of both saturated and unsaturated fatty acids in the ER membranes, and a reduced ratio of saturated to unsaturated fatty acids. MC treatment did not affect the fatty acid content of the endoplasmic reticulum membrane. In rat liver microsomes, monooxygenase induction by PB and MC was associated with increases in membrane oleic acid and linoleic acid, respectively /2,46/. It may be suggested from the results of this study that the induction of cytochrome P-450 and P-448 dependent enzymes is associated in the membrane with different fatty acids. It is also proposed that cytochromes P-450 and P-448 may be located in different patches of non-uniformly distributed membrane lipids /47/ or may be on different sides of the endoplasmic reticulum membrane with an asymmetric lipid distribution /48/.

A three-fold increase in the content of linoleic and arachidonic acids was observed in microsomes of PCB-treated animals (Table 3). In the PC and PE fraction of microsomes, a major increase in these fatty acids was also observed (Tables 4,5). Palmitoleic acid was increased three-fold in both PC and PE fractions. A two-fold increase in the total fatty acid content in liver and microsomes may be attributed to these changes. On the other hand, MC treatment only altered the fatty acids in the PE fraction of the microsomes. Palmitic, palmitoleic, stearic, and oleic acids were increased in PE, which raised the total fatty acid content of this fraction.

In this study PCB was found to influence the microviscosity of the endoplasmic reticulum membrane by (a) changing the cholesterol/ phospholipid ratio, and (b) unsaturation of the fatty acid chains of phospholipids, especially linoleic, arachidonic and docosahexaenoic acids. It has been proposed that alterations in phospholipid methylation are possibly the primary cause for the decrease in microviscosity of liver microsomes of rats treated with MC /8/. For the newly induced cytochrome P-450 and NADPH-cytochrome *c* reductase (by PCB and MC to be effective) (Table 2), these enzymes must be inserted into the endoplasmic reticulum membrane in the optimal stereochemical relationship. Microviscosity or fluidity of the endoplasmic reticulum membrane may play an important role in bringing these enzymes into

the proper position to facilitate the effective topography of cytochrome P-450 and NADPH-cytochrome *c* reductase in the phospholipid matrix. Therefore, changes in phospholipid moieties attached to the endoplasmic reticulum membranes might help to reveal the mechanisms whereby xenobiotics act. Moreover, fatty acid changes might provide an explanation for the role of phospholipids in induction or inhibition of enzyme activities. Hence, exposure to certain environmental pollutants may intricately compromise drug metabolizing capabilities.

REFERENCES

1. Silkworth JB, Cutler DS, O'Keefe PW, Lipinskas T. Potentiation and antagonism of 2,3,7,8-tetrachlorodibenzo-p-dioxin effects in a complex environmental mixture. Toxicol Appl Pharmacol 1993; 119: 236-247.
2. Borlakoglu JT, Edwards-Webb JD, Dils RR. Polychlorinated biphenyls increase fatty acid desaturation in the proliferating endoplasmic reticulum of pigeon and rat livers. Eur J Biochem 1990; 188: 327-332.
3. Litterst CL, Van Loon EJ. Time course of induction of microsomal enzymes following treatment with polychlorinated biphenyls. Bull Environ Contam Toxicol 1974; 141: 206-212.
4. Alvares AP, Bickers DR, Kapps A. Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. Proc Natl Acad Sci USA 1973; 70: 1321-1325.
5. Kumar CC, Padmanaban G. Role of heme in the synthesis of cytochrome c oxidase in *Neospora crassa*. J Biol Chem 1980; 255: 11130-11134.
6. Ioannides C, Parke DV. The cytochromes P-448 – a unique family of enzymes involved in chemical toxicity and carcinogenesis. Biochem Pharmacol 1987; 36: 4197-4207.
7. Wolf CR, Szutowski MM, Ball LM, Philpot RM. The rabbit pulmonary monooxygenase system characteristics and activities of two forms of pulmonary cytochrome P-450. Chem Biol Interact 1978; 21: 29-43.
8. Sastry BV, Statham CN, Meeks RG, Axelrod J. Changes in phospholipid methyl transferases and membrane microviscosity during induction of rat liver microsomal cytochrome P-450 by phenobarbital and 3-methylcholanthrene. Pharmacology 1981; 23: 211-222.
9. Ishidate K, Nakazawa Y. Effect of polychlorinated biphenyls (PCB) administration on phospholipid biosynthesis in rat liver. Biochem Pharmacol 1976; 25: 1255-1260.
10. Davison SC, Willis ED. Studies on the lipid composition of the rat liver endoplasmic reticulum after induction with phenobarbitone and 20-methylcholanthrene. Biochem J 1974; 140: 461-468.
11. Purshottam T, Srivastava RK. Paration toxicity in relation to liver microsomal oxidases, lipid composition and fluidity. Pharmacology 1987; 35: 227-233.

12. Koga N, Beppu M, Yoshimora H. Metabolism in vivo of 3,4,5,3',4'-pentachlorobiphenyl and toxicological assessment of the metabolites in rats. J Pharmacobiodyn 1990; 13: 497-506.
13. Bar-Nun S, Kreibich G, Adesnik M, Alterman L, Negishi M, Sebatimi DD. Synthesis and insertion of cytochrome P-450 into endoplasmic reticulum membranes. Proc Natl Acad Sci USA 1980; 77: 965-969.
14. Dhami MSI. Inter-relationship of hepatic lipid moieties and drug metabolizing enzymes. Ph.D. Thesis, University of Surrey, Guildford, England, 1985
15. Burke MD, Mayer RT, Progh RA. Characteristic of a microsomal cytochrome P-448 mediated reaction. Ethoxyresorufin-O-deethylation. Drug Metabol Dispos 1974; 5: 1-8.
16. Wiebel FJ, Gelbom HV. Aryl hydrocarbon (benzo[a]pyrene) hydroxylases in liver from rats of different age, sex and nutritional status. Distinction of two types by 7,8-benzoflavone. Biochem Pharmacol 1975; 24: 1511-1515.
17. Kato R, Frontino G, Chiesara E. Induction of hepatic carisoprodal metabolizing enzyme by pretreatment with some neuropsychotropic drugs in rats. Jap J Pharmacol 1961; 11: 31-36.
18. Phillips AH, Langdon RG. Hepatic triphosphopyrinine nucleotide cytochrome reductase: Isolation, characterization and kinetic studies. J Biol Chem 1962; 237: 2652-2660.
19. Miller GL. Protein determination for large numbers of samples. Analyt Chem 1959; 31: 964.
20. Omura T, Sato R. The carbon monoxide binding pigment of microsomes. Evidence for its hemoprotein nature. J Biol Chem 1964; 239: 2370-2378.
21. Folch H, Lees M, Sloan-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226: 497-506.
22. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem 1959; 234: 466-472.
23. Snedecor GW, Chochran WG. Statistical Method, Sixth Edition. Ames, Iowa: Iowa State University Press, 1967.
24. Fross DA. A rapid method for calculation percentage recoveries of compounds from gas chromatograms. J Chromatog 1968; 34: 100-101.
25. Delaforge M, Ioannides C, Parke DV. Safrole: its metabolism, carcinogenicity and interactions with cytochrome P-450. Food Cosmet Toxicol 1981; 250: 657-666.
26. Ryan D, Lu AHY, West S, Levin W. Multiple forms of cytochrome P-450 in phenobarbitol and 3-methylcholanthrene treated rats. Separation and special properties. J Biol Chem 1975; 250: 2157-2163.
27. Dus KM. Insights into the active site of the cytochrome P-450 hemoprotein family. A unifying concept based on structural considerations. Xenobiotica 1982; 12: 745-772.
28. Simmons GJ, McKee MJ. Alkoxyresorufin metabolism in white-footed mice at relevant environmental concentrations of Aroclor-1254. Fundam Appl Toxicol 1992; 19: 446-452.

29. Ioannides C, Lum PY, Parke DV. Cytochrome P-448 and the activation of toxic chemicals and carcinogens. Xenobiotica 1984; 14: 119-137.
30. Kensler TW, Ketterer B, Guillouzo A. Preferential increase of glutathion S-transferase class alpha transcripts in cultured human hepatocytes by phenobarbital, 3-methylcholanthrene and dithiolethiones. Cancer Res 1993; 53: 231-234.
31. Linder MW, Prough RA. Developmental aspects of glucocorticoid regulation of polycyclic aromatic hydrocarbon-inducible enzymes in rat liver. Arch Biochem Biophys 1993; 30: 92-102.
32. Pan J, Hong JY, Ma BL, Ning SM, Paranawithana SR, Yang CS. Transcriptional activation of cytochrome P450 2B1/2 genes in rat liver by diallyl sulfide, a compound derived from garlic. Arch Biochem Biophys 1993; 302: 337-342.
33. Nebert DW, Eisen JJ, Negisha M, Lang MA, Hjelmeland LM, Okey AB. Genetic mechanisms controlling the induction of polysubstrate mono-oxygenase. Ann Rev Pharmacol Toxicol 1981; 21: 431-462.
34. Orrenius JEL, Eriksson L, Ernster J. Phenobarbitol induced synthesis of the microsomal drug metabolizing system and its relationship to the proliferation of endoplasmic membranes. A morphological and biochemical study. Cell Biol 1965; 25: 627-639.
35. Thomas PE, Karzenioski D, Levin W. Preparation of monospecific antibodies against two forms of rat liver cytochrome P-450 and quantitation of these antigens in microsomes. Arch Biochem Biophys 1979; 192: 524-532.
36. Guo Z, Smith TJ, Thomas PE, Yang CS. Metabolism of 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone by inducible constitutive cytochrome P450 enzymes in rats. Arch Biochem Biophys 1992; 298: 279-286.
37. Deitrich RA, Bludeau P, Stock T, Roper M. Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbitol and tetrachloro-dibenzo-p-dioxin. J Biol Chem 1977; 252: 6169-6176.
38. Bhat KS, Padmanabhan G. Site of synthesis of cytochrome P-450 in liver. Biochem Res Comm 1978; 198: 1-6.
39. Horbach GJ, Van Asten JG, Rietjens IM, Kremers P, Van Bezooijen CF. The effect of age on inducibility of various types of rat liver cytochrome P-450. Xenobiotica 1992; 22: 515-522.
40. Kraner JC, Lasker JM, Corcoran GB, Ray SD, Raucy JL. Induction of P4502E1 by acetone in rabbit hepatocytes. Role of increased protein and mRNA synthesis. Biochem Pharmacol 1993; 45: 1483-1492.
41. Ioannides C. In: Basu TK, ed. Clinical Implications of Drug Use. New York: CRC Press, 1980; 144.
42. Stchr PA, Miller DT, Steel G. Effects of polychlorinated biphenyls and lipemia on serum analytes. J Toxicol Environ Health 1986, 19: 369-381.
43. Ioannides C, Parke DV. The cytochrome P-450 I gene family of microsomal hemoproteins and their role in the metabolic activation of chemicals. Drug Metab Rev 1990; 22: 1-86.
44. Ellingson JS, Hill EE, Lands WEM. The control of fatty acid composition in glycerolipids of the endoplasmic reticulum. Biochim Biophys Acta 1970; 196: 176-192.

45. Sprecher H. In: Hawkins WW, ed, Essential Fatty Acids, Proc Miles Symp. Winnipeg, Canada: University of Manitoba, 1975; 411.
46. Becker JR, Meehan T, Bartholomew JC. Fatty acid requirements and temperature dependence of monooxygenase activity in rat liver microsomes. Biochim Biophys Acta 1978; 512: 136-146.
47. Dallner G, DePierre JW. In: Arias I, Popper H, Schachter D, Shafritz DA, eds, The liver: Biology and Pathobiology. New York: Raven Press, 1982; 41-
48. Nillson OS, Dallner G. Enzyme and phospholipid asymmetry in liver microsomal membranes. J Cell Biol 1977; 72: 568-583.