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Effect of "Drugs for Liver Disease" on Hepatotoxic Action of Carbon Tetrachloride. IV.[1] Relationship between Fatty Acid Composition of Phospholipids and Drug Monooxygenation Activity in Rat Liver Microsomes and Role of Phosphorylcholine[2]

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To clarify a correlation between the fatty acid composition and the drug-metabolizing enzyme activity in liver microsomes and the action of drug for liver disease, phosphorylcholine (PC), the fatty acid composition of microsomal lipids and aminopyrine N-demethylase activity were measured during PC administration to carbon tetrachloride (CCl_4)-poisoned rats. A dose of CCl_4 for 2 days produced a clear per cent decrease in stearic and arachidonic acids and an increase of palmitic and oleic acids of microsomal phospholipids. The phosphatidylcholine in the poisoned rats consisted of less arachidonic and stearic acids and more linoleic acid. Administration of PC resulted in the restoration of the fatty acid content to the control levels at relatively early stage. It was found that there was a good correlation between the arachidonic acid content of the phospholipids, especially phosphatidylcholine, and the aminopyrine N-demethylase activity. Electron transfer between endogenous P-450 and NADPH added significantly dropped and the percentage of P-450 reduced with NADPH added was much smaller in CCl_4-poisoned rats. These results suggest that the fatty acid composition of the phospholipids may regulate the structure of the endoplasmic membrane and phospholipids of specific structure may play an essential and functional role in oxidative demethylation in the particles.

Keywords——drugs for liver disease; phosphorylcholine; relationship between fatty acids and drug metabolism; liver microsomes; microsomal phospholipids; CCl_4 intoxication

An abundance of recent evidence indicates that the properties of many of tightly bound microsomal enzymes are intimately related to interactions with the microsomal phospholipids.[4] The important role that phospholipids play in microsomal drug hydroxylation has been demonstrated by preparation of solubilized components that will not function in a reconstituted system unless phospholipid is added[5] and the essential component of microsomal membrane was identified to be phosphatidylcholine.[5b] Further, treatment of microsomal suspensions with phospholipase causes a significant decrease in activity of N-demethylation of benzhetamine and aminopyrine, but aniline hydroxylation is unaffected.[6] Unsaturated fatty acids of the phospholipids of the endoplasmic reticulum may be destroyed by induction of peroxide formation, and this also causes a marked decrease in the rate of oxidative demethylation.[7] Kamataki and Kitagawa have also demonstrated the close inverse relationship between lipid

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peroxidation and drug-metabolizing enzyme activities in liver microsomes of rats.[8] The activity of membrane-bound enzymes may be affected in various ways by the lipid environment, such as the physical state of lipid phase,[9] phospholipid fluidity[10] which depends on the cholesterol content and the chain length and the degree of saturation of fatty acids.[11] Wilson *et al.*[12] and Overath *et al.*,[13] using *E. coli* auxotrophs, showed that alterations in the saturation of fatty acid supplements affected the membrane transport properties and enzyme activity of these auxotrophs.

In view of the dependence of drug monooxygenation activity on membrane phospholipids, the fatty acid composition of the lipids, especially phospholipids, of rat liver endoplasmic reticulum has been examined during administration of phosphorylcholine (PC), which is used as drugs for liver disease, to carbon tetrachloride (CCl_4)-poisoned rats in an attempt to clarify a correlation between the fatty acid composition and the drug-metabolizing enzyme activity and the action of PC.

Experimental

Materials——Phosphorylcholine chloride calcium salt was a gift from Dozin Iyakukako. Nicotinamide adenine dinucleotide phosphate (NADP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. Glucose 6-phosphate (G-6-P) dehydrogenase [EC 1.1.1.49] and G-6-P were obtained from Oriental Yeast Co., Ltd. and Boehringer Mannheim, respectively. Standard fatty acid methyl ester (FAME Mixtures) and methyl arachidate (GLC grade) were purchased from Nishio Kogyo Co., Ltd. and Gaschro Kogyo Co., Ltd., respectively. Kieselgel 60HR for thin-layer chromatography (TLC) was supplied by E. Merck.

Animals——Male Wistar rats weighing about 100 g were used throughout. The treatment of rats with CCl_4 and PC was carried out by the same method as described in the previous paper.[1]

Preparation of Liver Microsomal Fractions——The liver microsomal fractions were prepared according to the method of Omura and Sato.[14]

Assays of N-Demethylation of Aminopyrine and Cytochrome P-450 (P-450)——The demethylation of aminopyrine was assayed at 37° by the method of Ariyoshi and Takabatake,[15] and formaldehyde formed was determined by the method of Nash.[16] P-450 was routinely determined by the method of Omura and Sato.[14]

Protein Determination——Protein concentration was estimated by the procedure described by Lowry, *et al.*[17]

Quantitative Estimation of Lipid Peroxidation——To 2.0 ml (5 mg protein/ml) of microsomal fractions 0.2 ml of 0.22 mM $FeSO_4$ was added and incubated at 37° for 6 hr. The reaction was stopped by adding 2.0 ml of 10% trichloroacetic acid and after centrifugation the peroxide content in the supernatant was assayed by the thiobarbituric acid (TBA) reaction with the modification of Zalkin and Tappel.[18]

Separation of Lipids and Fatty Acid Analyses——Extraction of microsomal membrane lipids and separation of the neutral lipids and the phospholipids were done by the method of Colbeau *et al.*,[19] using a column of silicic acid–Celite 545 (2: 1, by weight).

Separation of phosphatidylcholine from phospholipids was carried out with TLC using a 0.25 mm-thick layer of Kieselgel 60HR and a solvent mixture composed of $CHCl_3$–MeOH–H_2O (65: 25: 4, by volume). The fatty acids of the lipids, following evaporation *in vacuo*, were methylated by incubating with 5% H_2SO_4 in MeOH at 40° for 72 hr.[20] The methyl esters were analyzed by gas-liquid chromatography (GLC) on Shimadzu

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gas chromatograph model GC-4BM apparatus with a hydrogen flame ionization detector. The samples were separated on a 3 mm × 2 m stainless steel column packed with 15% polyethylene glycol succinate polyester on 60—80 mesh Chromosorb W.

NADPH-dependent Reduction of P-450——The NADPH-dependent reduction of P-450 was measured in an anaerobic cuvette. Microsomes were suspended in 50 mM Tris–HCl buffer, pH 7.4, containing 150 mM KCl and 10 mM $MgCl_2$. After a preliminary deoxygenation with N_2, 2.5 ml of microsomal suspension was transferred to the anaerobic cuvette. Both sample and reference cells were gassed for 2 min with CO which had been previously deoxygenated with an alkaline solution of anthraquinone-2-sulfonate and dithionite. The reaction was initiated by adding 1.2 μmole of NADPH (in 10 μl) to the reaction mixture. The absorbance change at 450 nm was recorded with a Shimadzu MPS-50L spectrophotometer. Calculation was made based on the absorbance increments of 80.9 mM^{-1} cm^{-1} at 450 nm for the reduction of P-450.[21]

Results

Fatty Acid Composition of Microsomal Neutral Lipids

The fatty acid composition of the neutral lipids of the microsomal preparations, analyzed by GLC, is shown in Table I. A clear decrease in stearic and arachidonic acids was observed, while oleic and linoleic acids were increased in the CCl_4-treated rats at 2 days. PC-treated rats contained a relatively much stearic and arachidonic acids as compared with those of CCl_4-treated rats at the same days, and the altered levels of these fatty acids were almost restored to the control level at 5 days, indicating that PC contributes to recovery of the components of hepatic microsomes. At 8 days no deference in the fatty acid composition of the neutral lipids was found in all groups. The result suggests that the fatty acid composition of microsomal neutral lipids is modified at the early time after administration of CCl_4.

Table I. Fatty Acid Composition of Microsomal Neutral Lipids of Rat Liver treated with CCl_4 and PC

Fatty acid	Rats	Composition (μg/mg protein)		
		2 days	5 days	8 days
$C_{18=0}$	control-rats	3.22±0.31	3.27±0.46	3.54±0.38
	PC-rats	2.41±0.25[a]	2.79±0.23	3.22±0.03
	CCl_4-rats	1.86±0.25[b,c]	2.73±0.51	3.22±0.47
$C_{18=1}$	control-rats	6.27±0.04	6.34±0.68	6.46±0.85
	PC-rats	10.08±1.28[b]	6.77±1.05	6.11±0.70
	CCl_4-rats	10.72±0.46[b]	10.94±0.62[b,d]	5.89±0.72
$C_{18=2}$	control-rats	6.40±0.34	6.11±0.35	5.79±0.50
	PC-rats	9.95±0.74[b]	6.22±0.28	5.67±0.20
	CCl_4-rats	9.59±1.16[b]	9.63±1.47[c,e]	6.71±0.15
$C_{20=4}$	control-rats	1.34±0.19	1.22±0.19	1.28±0.30
	PC-rats	0.81±0.30	1.03±0.28[a]	1.06±0.28
	CCl_4-rats	0.27±0.09[d]	0.91±0.18[e]	0.91±0.12

Only the major acids are given. Each value represents the mean of 4 rats ± standard error.
a) $p<0.02$ in control-rats *vs.* PC-rats
b) $p<0.01$ in control-rats *vs.* PC or CCl_4-rats
c) $p<0.05$ in CCl_4-rats *vs.*PC-rats
d) $p<0.001$ in control-rats *vs.* CCl_4-rats
e) $p<0.05$ in control-rats *vs.* CCl_4-rats

Fatty Acid Composition of Microsomal Phospholipids

The changes in the fatty acid composition of microsomal phospholipids are shown in Table II. After a dose of CCl_4 for 2 days, a clear per cent decrease in arachidonic acid, which is the most unsaturated component of fatty acid spectrum of microsomal phospholipids, was observed, probably due to peroxidative decomposition of the acid. At 5 days after CCl_4

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poisoning, phospholipids from the treated rats consisted of more palmitic and oleic acids and less stearic and arachidonic acids. The percentage composition of linoleic and decosahexaenoic acids was not markedly changed. Administration of PC resulted in the restoration of the fatty acid content to the control levels approximately, indicating that PC incorporated into the membrane plays an important role to normalize the composition of the fatty acids which are involved in the membrane function.

Table II. Fatty Acid Composition of Microsomal Phospholipids of Rat Liver treated with CCl_4 and PC

Fatty acid	Rats	Composition (%)		
		2 days	5 days	8 days
$C_{16=0}$	control-rats	26.8±1.5	27.0±1.1	26.7±0.3
	PC-rats	25.5±0.4	28.1±1.7	26.8±0.6
	CCl_4-rats	26.3±1.9	33.5±2.1[a,b]	28.5±1.6
$C_{18=0}$	control-rats	25.9±0.9	26.3±1.8	27.6±0.3
	PC-rats	27.4±1.5	22.9±1.2	26.5±0.6
	CCl_4-rats	25.1±2.4	19.9±1.5[a]	27.0±0.6
$C_{18=1}$	control-rats	4.3±0.3	5.2±0.3	4.4±0.3
	PC-rats	4.2±0.3	6.1±0.5	4.7±0.7
	CCl_4-rats	4.5±0.6	7.3±0.5[a]	5.1±0.2[c]
$C_{18=2}$	control-rats	11.2±1.4	12.8±0.6	11.2±0.3
	PC-rats	12.3±0.7	12.0±0.7	11.8±0.4
	CCl_4-rats	13.4±0.5	13.5±0.9	11.2±0.5
$C_{20=4}$	control-rats	19.3±0.8	18.7±1.1	19.7±0.8
	PC-rats	17.4±0.5[c]	17.0±2.0	20.0±0.8
	CCl_4-rats	15.2±0.2[a,d]	14.3±1.2[a]	17.9±1.1[c]
$C_{22=6}$	control-rats	11.2±1.2	9.1±0.6	8.6±0.4
	PC-rats	12.2±1.1	10.2±1.4	9.5±1.0
	CCl_4-rats	11.6±0.2	10.1±0.9	9.9±0.3

Each value represents the mean of 4 rats ± standard error.
a) $p<0.01$ in control-rats *vs.* CCl_4-rats
b) $p<0.02$ in CCl_4-rats *vs.* PC-rats
c) $p<0.05$ in control-rats *vs.* PC or CCl_4-rats
d) $p<0.01$ in CCl_4-rats *vs.* PC-rats

Fatty Acid Composition of Phosphatidylcholine in Microsomes

The fatty acid changes in phosphatidylcholine are shown in Table III, as compared to the controls. A significant decrease in palmitic (at 2 days), arachidonic (at 2 and 5 days) and stearic acids (at 5 days) was found, while linoleic (at 2 days) and decosahexaenoic acids (at 2 and 5 days) were increased. Administration of PC to the CCl_4-poisoned rats caused the recovery of the fatty acid content to the control value at 5 days. This also indicates that PC has a strongly repairable capacity of the injured membrane component, probably due to increasing the synthesis of phosphatidylcholine which is an essential component of microsomes.

In Vitro Lipid Peroxidation with Fe^{2+}

To clarify whether or not the changes in fatty acid composition of phospholipids observed *in vivo* occur *in vitro*, lipid peroxidation with Fe^{2+} *in vitro* was examined in microsomal phospholipids. As shown in Table IV, the amount of the products of lipid peroxidation was enhanced about 10 times higher than the original value under the condition tested. The fatty acid composition of the microsomal phospholipids treated with Fe^{2+} significantly changed; a clear decrease in arachidonic and decosahexaenoic acids, while an increase in palmitic and oleic acids were observed in comparison with those of the control. The result approximately agreed with the data obtained *in vivo* experiment, except decosahexaenoic acid, which was not significantly altered. Thus, this indicates that the alterations observed in the fatty acid

Table III. Fatty Acid Composition of Microsomal Phosphatidylcholine of Rat Liver treated with CCl_4 and PC

Fatty acid	Rats	Composition (%) 2 days	5 days	8 days
$C_{16=0}$	control-rats	32.2±1.4	31.1±0.8	32.1±0.9
	PC-rats	29.7±1.5	28.6±1.1	32.2±2.4
	CCl_4-rats	28.2±2.1[a]	30.4±0.9	33.1±1.9
$C_{18=0}$	control-rats	23.3±0.9	23.7±0.6	25.0±0.8
	PC-rats	23.1±1.1	22.3±1.5	23.9±1.7
	CCl_4-rats	23.5±0.8	19.7±0.5[b,c]	23.4±0.7
$C_{18=1}$	control-rats	6.8±0.6	5.9±1.3	6.1±0.8
	PC-rats	6.5±0.8	6.4±0.6	7.0±1.9
	CCl_4-rats	7.0±0.5	6.9±0.6	6.3±0.3
$C_{18=2}$	control-rats	13.5±1.0	14.6±0.8	13.8±0.9
	PC-rats	17.1±1.3[d]	13.3±1.3	13.7±2.2
	CCl_4-rats	17.6±1.1[d]	14.6±1.0	13.5±1.2
$C_{20=4}$	control-rats	18.0±1.2	18.4±1.5	17.9±1.3
	PC-rats	12.9±1.0[d]	15.8±0.5[d]	16.7±3.5
	CCl_4-rats	12.5±0.9[d]	11.7±1.2[b,e]	17.2±3.3
$C_{22=6}$	control-rats	6.0±0.4	6.3±0.3	5.6±0.3
	PC-rats	8.5±0.2[b]	9.0±1.8[d]	5.9±1.5
	CCl_4-rats	7.4±0.4[d]	10.0±1.6[d]	7.3±1.0

Each value represents the mean of 4 rats ± standard error.
a) $p<0.05$ in control-rats *vs.* CCl_4-rats
b) $p<0.001$ in control-rats *vs.* PC or CCl_4-rat
c) $p<0.05$ in CCl_4-rats *vs.* PC-rats
d) $p<0.01$ in control-rats *vs.* PC or CCl_4-rats
e) $p<0.01$ in CCl_4-rats *vs.* PC-rats

Table IV. Fatty Acid Composition of Microsomal Phospholipids after Lipid Peroxidation with Fe^{2+} *in Vitro*

Fatty acid	Composition (%) Control	Peroxidized
$C_{16=0}$	28.4	33.8
$C_{18=0}$	27.0	26.4
$C_{18=1}$	5.2	6.0
$C_{18=2}$	11.5	11.5
Unknown	0.8	0.5
$C_{20=4}$	18.2	15.0
Unknown	1.2	0.7
$C_{22=6}$	7.8	6.2
Lipid peroxidation activity (OD_{530})	0.150	1.45

The values are means of values in 2 experiments.

composition of liver microsomal phospholipids were mainly dependent on *in vivo* peroxidative decomposition of the components.

A Correlation between Fatty Acid Composition of Phosphatidylcholine or Phospholipids and Aminopyrine N-Demethylation Activity

It is of interest to investigate whether or not a correlation exists between the fatty acid composition of phospholipids and drug monooxygenation activity in liver microsomes. The alterations in the fatty acid composition of total phospholipids and the aminopyrine N-demethylase activity are shown in Fig. 1. There was a good correlation between the arachidonic

acid content and the aminopyrine N-demethylase activity; the decrease proportion of arachidonic acid in phosphlipids after CCl_4 injection occurs simultaneously with decrease in the N-demethylase activity and the increase in the unsaturated fatty acid content induces the increased activity. The relationship between the fatty acid composition of phosphatidylcholine and the aminopyrine N-demethylase activity is shown in Fig. 2. The data also showed that there was a very good correlation between the arachidonic acid content and the aminopyrine N-demethylase activity, while no interrelationship was found between the stearic acid content and the activity. These results strongly suggest that the decrease in the arachidonic acid content of the phospholipids, especially phosphatidylcholine, in hepatic microsomes seems to cause the decline of drug-metabolizing enzyme activity by exerting changes in the microsomal membrane that may be ascribed to changes in the physical state of the membrane.

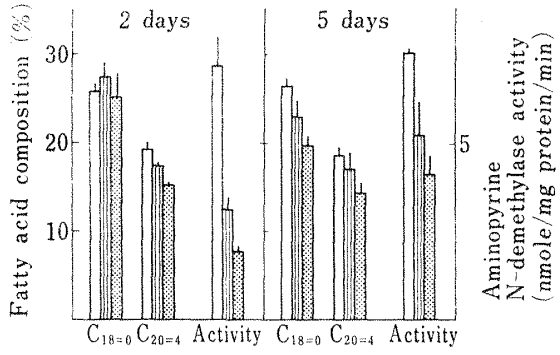

Fig. 1. Relationship between Aminopyrine N-Demethylase Activity and Some Fatty Acid Compositions of Microsomal Phospholipids of Rat Liver

The bar indicates the standard error.
□: control-rats, ▤: PC-rats, ▦: CCl_4-rats

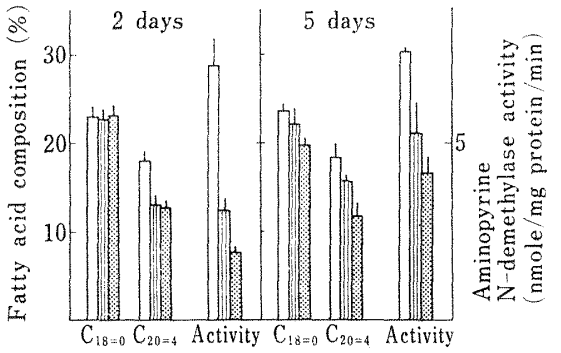

Fig. 2. Relationship between Aminopyrine N-Demethylase Activity and Some Fatty Acid Composition of Microsomal Phosphatidylcholine of Rat Liver

The bar indicates the standard error.
□: Control-rats, ▤: PC-rats, ▦: CCl_4-rats

NADPH-dependent Reduction of P-450

Electron transfer between endogenous P-450 in microsomes and NADPH added is shown in Fig. 3. In CCl_4-treated rats and PC-rats the electron transfer significantly dropped in comparison with that of the controls at 2 days. However, at 5 days the dropped electron transfer almost returned to the control level.

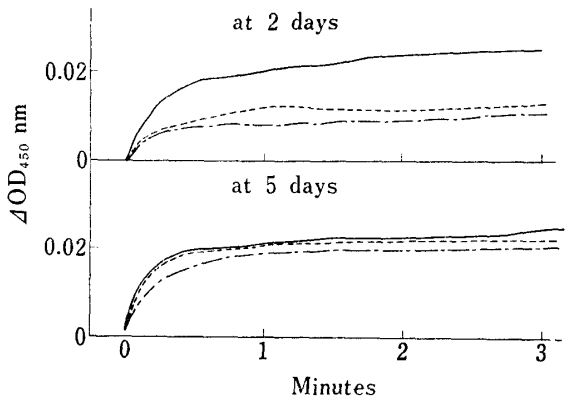

Fig. 3. Electron Transfer between Endogenous P-450 and NADPH added in Microsomes treated with CCl_4 or the Solvent and PC

The concentration of protein was 1.0—1.2 mg per ml.
———: control, - - - - - - - -: PC, ———: CCl_4

The amount of P-450 reduced with NADPH added is shown in Table V, together with total P-450 content calculated by the method of Omura and Sato,[14] although the amount of P-450 reduced with endogenous NADPH in the microsomal preparations is not calculated. This result indicates that the percentage of P-450 reduced with NADPH added for 3 min is much smaller in CCl_4-poisoned rats than in the controls. This may be attributed to the destruction of the membrane phospholipids, *i.e.* the unsaturated fatty acids as a consequence of lipid peroxidation. These results also suggest an importance of the intact phospholipid structure, including the unsaturated fatty acids, for the function of the microsomal membrane.

Table V. The Amount of P-450 reduced with NADPH added and Total P-450 in Microsomes treated with CCl_4 or the Solvent and PC

Groups	Days	Total[a] P-450	Reduced[a] P-450	%	Days	Total[a] P-450	Reduced[a] P-450	%
Control		0.61	0.37	60.7		0.70	0.42	60.0
PC	2	0.27	0.12	44.4	5	0.68	0.44	64.7
CCl_4		0.28	0.12	42.9		0.56	0.34	60.7

a) P-450 content is expressed as nmoles per mg of protein.

Discussion

We have been studying on the effects of some drugs for liver disease on the drug-metabolizing enzyme activities and the membrane structure of rat liver microsomes in CCl_4-poisoned rats, in an attempt to clarify the mechanism of their actions. As a result of the studies, it has been found that protoporphyrin administered protected lipid peroxidation and induced the microsomal drug-metabolizing enzymes,[22] while PC produced the increase in microsomal phospholipid content[22] and the recovery of the membrane phospholipid and the protein structures to native state.[1]

Many investigators have demonstrated that the phospholipids of hepatic endoplasmic reticulum play an important role in microsomal drug hydroxylation[5] and oxidative demethylation.[6] However, the interrelationship between the fatty acid composition of liver microsomes and the drug-metabolizing enzyme activity is not well understood. Thus, attention was given to fatty acid composition of microsomes in relation to the drug metabolism.

In the present study, it was found that the fatty acid composition of the neutral lipids of liver microsomes was modified at the early stage in comparison with that of the phospholipids after a dose of CCl_4 (Tables I and II), and the normalization of the fatty acid composition was induced at the reletively early time after administration of PC to CCl_4-poisoned rats. It is of interest to compare with the data obtained by Sugano, *et al.*[23] that liver triglycerides from rats sacrified 6 hr after CCl_4 treatment contained significantly more palmitic acid and less linoleic acid, however, by 24 hr the composition of the triglycerides resembled that of the controls. The significant alteration of the fatty acid composition, represented by a decrease in arachidonic acid, after CCl_4 administration appears to be due to peroxidative decomposition. Although no report that liver microsomal triglycerides are involved in the drug metabolizing enzyme activities has appeared, it may be suggested from the present result that the decrease in the amount of polyunsaturated fatty acids of the neutral lipids seems to be responsible for the parallel change in the polyunsaturated fatty acid-type 1,2-diglycerides, consequently it may decline the biosynthesis of tetraene-type phosphatidylcholine through the Kennedy's pathway.[24] Thus, the alteration of the fatty acids of microsomal neutral lipids may indirectly affect the content of the unsaturated fatty acids of liver microsomal phospholipids.

A dose of CCl_4 also resulted in the changes of the fatty acid composition of microsomal phospholipids and phosphatidylcholine; a clear per cent decrease in stearic and arachidonic acids in total phospholipids and phosphatidylcholine, an increase in palmitic and oleic acids in total phospholipids and an increase in linoleic and decosahexaenoic acids in phosphatidylcholine were seen. The decrease in arachidonic acid is interpreted on the basis of CCl_4-induced lipid peroxidation, indicating that the arachidonic acid in the microsomal phospholipids would be far more sensitive to damage induced by free radical and peroxide attack than other

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unsaturated fatty acids. This is strongly supported by the finding that the *in vitro* peroxidation of the microsomal phospholipids with Fe^{2+} produced a significant decrease in the arachidonic acid as compared with the controls (Table IV). The *in vivo* result is consistent with those of many reports.[25] May and McCay related malonaldehyde formation in microsomal suspensions mainly to the disappearance of the arachidonic acid and the decosahexaenoic acid from the β-position of membrane phospholipids as a result of peroxidation, and calculated that approximately 12% of the unsaturated fatty acids destroyed by peroxidation formed malonaldehyde.[26] Wills also reported that 50% of the inactivation of glucose 6-phosphatase activity resulted from a destruction of 10% arachidonic acid, in addition to the loss of oxidative demethylation and hydroxylation of drugs.[7] The decrease in stearic acid and the increase in linoleic and decosahexaenoic acids of phosphatidylcholine are difficult to explain. The changes, however, may be due to an enhancement of desaturation of fatty acids in order to supplement of the decreased arachidonic acid which is the most unsaturated component of the fatty acids of microsomal phospholipids. A decrease in stearic acid was observed in the phospholipids of erythrocytes peroxidized *in vitro*.[27]

Administration of PC to CCl_4-poisoned rats returned the fatty acid composition of phospholipids and phosphatidylcholine to normal levels at relatively early stage (at 5 days) (Tables II and III). A possible explanation for the restoring effects may be: 1) PC incorporated into the membrane enhances the biosyntheses of phospholipids, *e.g.* phosphatidylcholine which is an essential component of microsomal membrane, and consequently contributes the reconstruction and the rearrangement of the structure of the lipid phase in the membrane. 2) PC leads to normalize hydrophobic interactions, which are required to maintain the structure, between lipids and proteins, as reported previously.[1] This is also postulated by the fact that lipid removal from mitochondria profoundly affects the conformation of mitochondrial proteins, which is restored by phospholipid reincorporation.[28] 3) Normalizing the microsomal membrane with PC increases the synthesis and the desaturation of the fatty acids decreased in CCl_4 intoxication, by the enhancement of the appropriate enzyme activity.

The fact that there was a good correlation between the arachidonic acid content of phosphatidylcholine and the aminopyrine N-demethylase activity strongly suggests that the unsaturated component may be involved, at least partially, in microsomal drug metabolizing system. This is of interest in comparison with the data that the increased proportion of linoleic acid in phosphatidylcholine after phenobarbital injection occurs simultaneously with the increase of P-450 concentration, the rate of oxidative demethylation of aminopyrine and the rate of hydroxylation of biphenyl.[29] The alterations observed in the fatty acid composition of the membrane phospholipids may result in the changes in the physical state and the fluidity of the membrane, and accordingly induce the decline of the drug metabolizing enzyme activity.

Since NADPH is very unstable in solution when exposed to a radical producing system,[30] and the rapid drop in NADPH occurs after CCl_4 dosing,[31] the microsomes obtained from CCl_4-poisoned rats probably contain relatively small amount of NADPH. As a result, the amount of P-450 reduced with endogenous NADPH seems to be much less than that of the controls. Thus, the result obtained in the experiment on the electron transfer indicates that the decreased amount of P-450 reduced with NADPH added in CCl_4-poisoned rats might have

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some relevance to a significant destruction of the membrane phospholipid structure by free radicals and lipid peroxides. This is strongly supported by the results shown in Figures 1 and 2. Since P-450, the terminal oxidase in drug oxidation,[32] is located in the hydrophobic region of the membrane,[33] the activity probably depends on the lipid environment which is able to undergo a phase transition. Duppel and Ullrich concluded that phospholipid phase transitions of microsomal membrane affect the electron transfer from the NADPH cytochrome c reductase to P-450.[34] Therefore, the drug-metabolizing enzyme activity decreased by CCl_4 administered may be due to the alteration of the microsomal membrane structure in addition to decrease in either P-450 content or activities of NADPH-linked reductase.

These experiments on the fatty acid composition and aminopyrine N-demethylase of hepatic microsomes lead to the conclusion that administration of PC to CCl_4-poisoned rats induces the normalization of the fatty acid composition and enhances the N-demethylation, probably due to the increased reconstruction of the phospholipids. These findings, including that there was a good correletion between the arachidonic acid content of phosphatidylcholine and aminopyrine N-demethylase activity, indicate that the fatty acid composition of the phospholipids may regulate the structure of the endoplasmic membarne by influencing hydrophobic bonds between membrane proteins and phospholipids. In addition phospholipids of specific structure may play an essential and functional role in oxidative demethylation in the endoplasmic reticulum.

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