EFFECT OF "DRUGS FOR LIVER DISEASE" ON HEPATOTOXIC ACTION OF CARBON TETRACHLORIDE

II. EFFECT OF PROTOPORPHYRIN AND PHOSPHORYLCHOLINE ON MICROSOMAL DRUG-METABOLIZING ENZYME ACTIVITIES AND THE COMPONENTS IN INJURED LIVER

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Abstract—The effect of "drugs for liver disease", protoporphyrin (PP) and phosphorylcholine (PC), on CCl4-induced liver injury was studied. Attention was given to the levels of microsomal drug-metabolizing enzyme and lipolytic enzyme activities and of some microsomal components such as phospholipid and peroxides. Administration of PP to CCl4-poisoned rats was found to increase the decreased microsomal drug-metabolizing enzyme activities, aminopyrine N-demethylase, aniline p-hydroxylase, cytochrome P-450 and b5 and lipolytic enzyme activity in CCl4-poisoned liver (12-20% increase as compared with those of the poisoned rats), and returned to control levels earlier than in CCl4-poisoned rats. Furthermore, administration of PP to CCl4-poisoned rats caused a decrease in the lipid peroxidation. A single dose of PP to normal rats was shown to increase these parameters, to a small extent. One of the mechanisms may be attributed to the fact that PP increases the biosynthesis of the hemoproteins by means of the incorporation of PP into the pigments and protects the membranes from lipid peroxides and the free radicals. On the other hand, administration of PC to the poisoned rats did not enhance the levels of the drug-metabolizing enzyme activities except for aminopyrine N-demethylase. Phospholipid phosphorous content, however, increased by 13-14% when PC was given. Thus, it is considered that PC may enhance the reconstitution of phospholipids in the injured membrane.

It is known that liver endoplasmic reticulum or its fragments, the microsomes, contain an enzyme system capable of converting various drugs and aromatic substances to more polar compounds (1, 2), and changes in the activities of this enzyme system affect the pharmacological action and toxicity of drugs (3). The microsomal enzyme system which is responsible for the oxidation of steroids (4), fatty acids (5), drug and several xenobiotics (6), consists of NADPH-cytochrome c reductase, cytochrome P-450 (P-450) and phospholipid identified as phosphatidylycholine (7).

Numerous investigators have shown that carbon tetrachloride (CCl4) primarily affects the membrane of the endoplasmic reticulum (8, 9), this process being accompanied by inactivation of many proteins (10, 11), including P-450 (12, 13), changes in the composition of microsomal phospholipids (14), and defective protein synthesis (15). Ingestion of CCl4 also results in distinct and reproducible histological alterations of the liver.
(16), a severe centrilobular necrosis and fatty degeneration of the cells (17).

At present, many drugs are clinically prescribed for liver diseases. However, little is known of the effects of these drugs on the injured liver, especially recovery of drug-metabolizing enzymes and on lipid peroxidation in the endoplasmic reticulum.

In this study, protoporphyrin (PP) and phosphorylcholine (PC) which are used as drugs for liver disease, were chosen and their effects on decreased microsomal drug-metabolizing enzymes, lipolytic enzyme activity, phospholipid phosphorous contents and increased lipid peroxidation in rat liver microsomes during CCl₄ intoxication were studied.

MATERIALS AND METHODS

Materials: Protoporphyrin disodium salt and phosphorylcholine chloride calcium salt were a gift from Dozin Iyakukako. Nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide phosphate (NADPH), reduced nicotinamide adenine dinucleotide (NADH) and cytochrome c were purchased from Sigma Chemical Co. Glucose 6-phosphate (G-6-P) dehydrogenase [EC 1.1.1.49] from Oriental Yeast Co., Ltd. and G-6-P from Boehringer Mannheim were also used in this experiment. Acetylacetone, aniline and aminopyrine were used following purification by redistillation or recrystallization.

Animals: Male Wistar rats weighing about 100 g, maintained on MF diets (Oriental Yeast Co., Ltd.) for 3-4 days prior to the experiment, were used. The animals were divided at random into 3 or 4 groups, each consisting of 4 rats, and fasted for 12 hr prior to the experiment. (A) Controls, treated for 2 days with daily s.c. injections of 0.1 ml of olive oil per 100 g of body weight followed by a daily oral administration of water (Control rats). (B) Animals, treated for 2 days with daily s.c. injections of a mixture of 0.1 ml of CCl₄ and 0.1 nil of olive oil per 100 g of body weight followed by a daily oral administration of PP (0.3 mg/100 g body weight) or PC (5 mg/100 g body weight) solution (PP-rats or PC-rats). (C) Animals, treated as described in B), except that water was given instead of the drug solution (CCl₄-rats).

The administration of water or each drug solution for the first two days was given 2 hr after the injection. The animals were sacrificed by decapitation 2 hr after the final administration of water or the drug at 2, 6 (or 5) and 8 days. The doses of the drugs to rats were double the clinically effective daily doses.

Preparation of liver microsomal fractions: The liver microsomal fractions were prepared according to the method of Omura and Sato (18).

Assays of enzymes: NADPH-cytochrome c reductase activity was assayed at 20 C by observing the change in absorbance at 550 m,u resulting from cytochrome reduction, according to the procedure described by Slater and Sawyer (19). Aniline p-hydroxylase activity was measured at 37 C according to the method of Ikeda (20). The demethylation of aminopyrine was assayed at 37 C by the method of Ariyoshi and Takabatake (21), and formaldehyde formed was determined by the method of Nash (22). The concentrations of P-450 and cytochrome b₅ (b₅) were determined at room temperature in a Shimadzu
MPS-50L spectrophotometer as described by Omura and Sato (18).

The lipolytic enzyme activity was assayed by the method described in a previous paper (23), except that 5% triolein emulsion was used instead of tributyrin substrate.

**Difference spectrophotometry:** Difference spectra were measured in a Shimadzu MPS-50L spectrophotometer, using two sets of matched quartz cuvettes of 1.0 and 0.5 cm-optical path. Microsomal preparations (2 mg of protein per ml of 0.1 M phosphate buffer, pH 7.0) containing $10^{-5}$ or $10^{-6}$% PP or PC and 0.1 M phosphate buffer were placed in the cuvettes, separately.

**Protein determination:** Protein was estimated by the procedure described by Lowry et al. (24).

**Determination of phospholipids in microsomal fractions:** Extraction of microsomal membrane lipids and separation of the phospholipids were done by the method of Colbeau et al. (25). The phospholipid phosphorous content was estimated by the method of Ames (26).

**Quantitative estimation of lipid peroxidation:** The lipid peroxidation in vivo and in vitro were determined by measuring diene conjugate ultraviolet absorption of lipid extracted from the microsomal fraction according to the method of Klassen and Plaa (27) and by the thiobarbituric acid (TBA) reaction with the modification of Zalkin and Tappel to avoid interference by sucrose (28), respectively.

**Measurements of effect of Fe$^{2+}$, EDTA and drugs on lipid peroxidation:** To 3 ml of microsomal fraction (8 mg of protein/ml) 1 ml each of Fe$^{2+}$ (ferrous sulfate) solution and the drug solution were added at their final concentrations of 2-5 $\mu$M and $10^{-5}$-$10^{-4}$%, respectively. In the experiment using disodium ethylenediamine tetraacetate (EDTA), the final concentration was 10 and 50 $\mu$M. The mixture was incubated at 37°C for 30 min and the reaction was stopped by adding 1 ml of 30% trichloroacetic acid solution. After centrifugation, the peroxide content in the supernatant was assayed by the TBA reaction (28).

**RESULTS**

**Effect of PP and PC on microsomal drug-metabolizing enzyme activities and the components in CCl$_4$-poisoned liver**

As shown in Table 1, a single daily dose of CCl$_4$ for 2 days significantly decreased the aminopyrine N-demethylase and aniline p-hydroxylase activities by 68 and 78%, compared with the control group, respectively, and the contents of P-450 and b$_5$ by 48 and 68%, respectively, although at 8 days the decreased activities partially recovered. The decreases in aminopyrine N-demethylase and aniline p-hydroxylase activities were more remarkable than those of P-450 in CCl$_4$-poisoned rats. In PP-rats, aminopyrine N-demethylase activity was enhanced about 17% more than that in CCl$_4$-rats at 6 days and returned to control level at 8 days, while the activity in CCl$_4$-rats was depressed to 87% of control value. Aniline p-hydroxylase activity was also enhanced in PP-rats as compared with CCl$_4$-rats. Administration of PP was found to greatly increase the decreased P-450 and b$_5$ contents,
Table 1. Effect of treatment with CCl₄ or PP and the solvent on microsomal drug-metabolizing enzyme activities and the components in rat liver

| Enzymes                     | Groups       | 2 days        | 6 days        | 8 days        |
|-----------------------------|--------------|---------------|---------------|---------------|
| NADPH-cytochrome c reductase | Control-rats | 68.6±5.6      | 68.7±5.2      | 71.5±4.8      |
|                            | PP-rats      | 71.1±5.2      | 72.5±4.0      | 68.5±6.3      |
|                            | CCl₄-rats    | 71.3±3.2      | 74.2±6.5      | 71.8±3.6      |
| Aminopyrine                 | Control-rats | 3.02±0.44     | 2.80±0.35     | 3.00±0.20     |
|                            | PP-rats      | 1.13±0.08     | 2.04±0.20     | 2.95±0.36     |
|                            | CCl₄-rats    | 0.97±0.06*    | 1.56±0.20†    | 2.62±0.21     |
| Aniline                     | Control-rats | 0.55±0.02     | —             | 0.57±0.02     |
|                            | PP-rats      | 0.19±0.01     | —             | 0.53±0.03     |
|                            | CCl₄-rats    | 0.12±0.01*†   | —             | 0.48±0.04     |
| Cytochrome P-450            | Control-rats | 0.54±0.06     | 0.50±0.05     | 0.50±0.02     |
|                            | PP-rats      | 0.39±0.05     | 0.42±0.05     | 0.50±0.07     |
|                            | CCl₄-rats    | 0.28±0.03*†   | 0.36±0.04*    | 0.48±0.05     |
| Cytochrome b₂               | Control-rats | 0.38±0.03     | 0.40±0.04     | 0.39±0.06     |
|                            | PP-rats      | 0.19±0.01     | 0.29±0.06     | 0.33±0.06     |
|                            | CCl₄-rats    | 0.12±0.03*†   | 0.24±0.05*    | 0.30±0.05     |

Each value represents the mean of four rats ± standard error.

1): The activities of enzymes are expressed as nmoles of product per min per mg of protein.
2): The contents of cytochrome P-450 and b₂ are expressed as nmoles per mg of protein.

*p < 0.01 in Control-rats vs. CCl₄-rats  †p < 0.05 in PP-rats vs. CCl₄-rats

Table 2. Effect of treatment with CCl₄ or PC and the solvent on microsomal drug-metabolizing enzyme activities and the components in rat liver

| Enzymes                     | Groups       | 2 days        | 6 days        | 8 days        |
|-----------------------------|--------------|---------------|---------------|---------------|
| NADPH-cytochrome c reductase | Control-rats | 66.9±6.5      | 64.4±3.9      | 68.3±3.4      |
|                            | PC-rats      | 73.0±4.9      | 74.3±6.6      | 63.9±5.2      |
|                            | CCl₄-rats    | 76.1±5.1      | 76.4±2.7      | 68.5±2.8      |
| Aminopyrine                 | Control-rats | 3.03±0.30     | 2.81±0.31     | 2.83±0.31     |
|                            | PC-rats      | 1.58±0.18     | 2.20±0.12     | 2.54±0.30     |
|                            | CCl₄-rats    | 1.16±0.21*†   | 1.73±0.20†    | 2.47±0.24     |
| Aniline                     | Control-rats | 0.53±0.02     | —             | 0.52±0.05     |
|                            | PC-rats      | 0.15±0.02     | —             | 0.47±0.04     |
|                            | CCl₄-rats    | 0.15±0.02*    | —             | 0.44±0.05     |
| Cytochrome P-450            | Control-rats | 0.54±0.05     | 0.52±0.04     | 0.54±0.01     |
|                            | PC-rats      | 0.30±0.02     | 0.41±0.04     | 0.51±0.03     |
|                            | CCl₄-rats    | 0.30±0.03*    | 0.39±0.03*    | 0.52±0.04     |
| Cytochrome b₂               | Control-rats | 0.36±0.03     | 0.37±0.04     | 0.34±0.03     |
|                            | PC-rats      | 0.14±0.02     | 0.24±0.04     | 0.29±0.01     |
|                            | CCl₄-rats    | 0.14±0.02*    | 0.25±0.03*    | 0.31±0.04     |

Each value represents the mean of four rats ± standard error. The activities of enzymes and the contents of P-450 and b₂ are the same as expressed in Table 1.

*p < 0.01 in Control-rats vs. CCl₄-rats  ††p < 0.05 in PC-rats vs. CCl₄-rats
about 20\% at 2 days and 12\% at 6 days higher than those of CCl_4-rats. NADPH-cytochrome c reductase activity was not significantly changed by treatment with CCl_4 or PP.

Table 2 shows that administration of PC hardly affected the recovery of the microsomal components decreased by CCl_4 administered, although aminopyrine N-demethylase activity was slightly enhanced as compared with that of CCl_4-rats. Therefore, PC administered to CCl_4-poisoned rats does not appear to contribute directly to recovery of the components of hepatic microsomes.

Difference spectra of P-450 and b_6 in the microsomal fractions from each rat are shown in Fig. 1. This indicates that the decrease in P-450 content caused by administration of CCl_4 is attributed to the conversion of P-450 into P-420, and that administration of PP causes the increase in P-450 level, which is probably decreased by a direct attack of the free radicals on the pigment (29), and followed by the decrease in P-420.

FIG. 1. Difference spectra of liver microsomes from CCl_4-rats and PP-rats. Microsomal fractions, prepared from rats at 2 days, contained about 2 mg protein per ml. ---: Control-rats -----: PP-rats : CCl_4-rats

a) Difference spectra of P-450 (sample cuvette: dithionite reduced - CO, reference cuvette: dithionite reduced)

b) Difference spectra of b_6 (sample cuvette: air + NADH 0.2 mg, reference cuvette: air)

**Effects of PP and PC on microsomal lipolytic enzyme activity in CCl_4-poisoned liver**

Since it has been found that microsomes of rat liver have a high activity of triglyceride lipase (30), the activity of the microsomes from each group was measured on different days. As shown in Fig. 2, the activity was decreased by 50\% at 2 days and by 30\% at 5 days by CCl_4 administration as compared with that (102-110 \(\mu\)mole fatty acid/min/mg protein) of Control-rats. However, administration of PP enhanced the levels by 10-15\% in comparison with that of CCl_4-rats, while the levels of PC-rats did not exhibit any significant alteration.

**Effects of PP and PC on amount of phospholipid phosphorus in liver microsomes of CCl_4-poisoned rats**

It has been shown that phospholipids are required for the reduction of P-450 by NADPH-cytochrome c reductase (7) and for the stabilization of P-450 (31). As shown in Fig. 3, a single daily dose of CCl_4 for 2 days was found to decrease phospholipid phos-
Fig. 2. Effect of treatment with CCl₄ or PP and the solvent on activity of lipolytic enzyme in rat liver microsomes. Activity is expressed as percentage of the value (fatty acid μmoles/min/mg protein) in the fraction of Control-rats. Points are means of values in four rats and standard error is indicated by the bars.

○: PP-rats △: PC-rats ▲: CCl₄-rats

Fig. 3. Effect of treatment with CCl₄ or drugs and the solvent on concentration of phospholipid phosphorus in rat liver microsomes. The content of phospholipid phosphorus is expressed as percentage of the value in the fraction of Control-rats. Points are means of values in four rats and standard error is indicated by the bars.

○: PP-rats △: PC-rats ▲: CCl₄-rats

phosphorous content in the microsomes, a decrease of 25% at 2 days and 18% at 5 days as compared with that (13.8-16.5 μg P/mg protein) of control group. The amount was increased by administration of PC by 10-15% at 2 and 5 days, and at 8 days it returned to the control value. No enhancement was detected by administration of PP.

Effects of a dose of PP and PC on microsomal drug-metabolizing enzyme activities and the components in normal rat liver

Increased enzyme levels by administration of PP and PC to CCl₄-poisoned rats may be due to induction of the enzymes, thus, the microsomal drug-metabolizing enzyme ac-

Table 3. Response of microsomal drug-metabolizing enzyme activities and the components in normal rat liver to PP administration

| Enzymes                  | Groups   | 2 days     | 6 days     | 8 days     |
|--------------------------|----------|------------|------------|------------|
| NADPH-cytochrome c reductase | Control  | 69.2±0.9   | 73.2±1.4   | 67.0±3.4   |
|                          | PP       | 72.0±3.1   | 67.0±4.3   | 69.5±3.4   |
| Aminopyrine              | Control  | 3.05±0.32  | 3.43±0.10  | 3.38±0.06  |
|                          | PP       | 3.76±0.10* | 3.63±0.09  | 3.40±0.24  |
| N-demethylase            | Control  | 0.53±0.04  | 0.48±0.04  | 0.52±0.05  |
|                          | PP       | 0.58±0.05  | 0.64±0.04  | 0.51±0.03  |
| Aniline                  | Control  | 0.54±0.06  | 0.59±0.02  | 0.59±0.04  |
|                          | PP       | 0.66±0.04  | 0.54±0.04  | 0.66±0.06  |
| p-hydroxykase            | Control  | 0.39±0.06  | 0.38±0.02  | 0.40±0.05  |
|                          | PP       | 0.44±0.04  | 0.41±0.03  | 0.46±0.03  |

Each value represents the mean of four rats ± standard error. The activities of enzymes and the contents of P-450 and b₅ are the same as expressed in Table 1.

*p<0.05 in Control vs PP
tivities and the components of rats given the drugs were determined in comparison with those of control group. As can be seen in Table 3, microsomal aminopyrine N-demethylase and aniline p-hydroxylase activities and the contents of P-450 and b5 increased, to an only slight extent (10-33% increase), upon administration of PP, although NADPH-cytochrome c reductase activity was not affected. Alterations in the enzyme and the component levels were insignificant with a single daily dose of PC.

**Effects of PP and PC on microsomal drug-metabolizing enzyme activities and the components in vitro**

To determine whether or not the microsomal mixed function oxidase system can be activated by PP and PC, the drugs were added to microsomes in vitro at concentrations varying from $10^{-3}$ to $10^{-9}$%. But no activation of aminopyrine N-demethylase and aniline p-hydroxylase was observed. In addition, spectral changes were not induced upon addition of PP or PC to microsomes, indicating that microsomal P-450 and b5 did not directly interact with the drugs.

**Effects of PP and PC on lipid peroxidation**

*In vivo lipid peroxidation:* As demonstrated in Fig. 4, microsomal lipid peroxidation activity, as measured by the TBA reaction in vitro, increased upon CCl₄ administration, and the increased activity returned to the control value (11.7-13.0 OD × 10³/mg protein) within 8 days. Administration of PP prevented the CCl₄-induced increase in lipid peroxidation by 15% each at 2 and 5 days, while no significant effect was detected on PC administration.

Fig. 5 shows the ultraviolet absorption spectra of microsomal lipid extracts at 2 and
5 days. This also indicates that the increased amount of lipid peroxides was decreased by 50% in rats given PP for 2 days, as compared with that of CCl₄-rats.

**In vitro lipid peroxidation**: Table 4 demonstrates the ability of Fe²⁺ to enhance peroxidation in vitro, as shown by Schneider et al. (32). The amount of the products of lipid peroxidation was enhanced about 3.5 times higher than the original value at a concentration of 2 μM Fe²⁺. The addition of 10⁻⁵ % PP into the medium was found to decrease the amount, to a small extent (about 13% decrease in the presence of 2 μM Fe²⁺), whereas no effect of PC was found. It should be noted that even at the higher concentration of 10⁻³ %, the protecting action of PP against lipid peroxidation was equal to that at a concentration of 10⁻⁵ %, suggesting that its action may be due to an indirect protecting effect on lipid peroxidation rather than to direct action and its chelating effect. Since the lipid peroxidation is known to be protected by addition of EDTA (33), PP at a final concentration of 10⁻⁴ % and 10⁻³ % was added to the medium containing microsomal fraction and EDTA (at 10 and 50 μM), and the amount of lipid peroxides was measured. It is indicated from the results that PP added did not enhance the action of EDTA.

### DISCUSSION

PP and PC have indeed proven to be clinically effective for liver diseases. The mechanism of their action, however, is only partially clarified; PP is known to enhance the oxidative catalytic action (34), to prevent decreasing catalase levels (35), and to be used for the oxidation of fatty acids (36). PC is shown to be used in the biosynthesis of methyl groups (37) and cytidine diphosphate choline (38) in animal organisms and in oxidation of fatty acid in liver (39). The overall mechanism of the action of drugs for liver disease, however, has not yet been made clear. The effect of PP on lysosomal enzymes and some components of injured liver has already been reported by the present authors (23). To clarify the action of these drugs on microsomal drug-metabolizing enzyme activities and the components, during liver injury, some experiments were carried out in vivo and in vitro.

**Table 4. Effect of Fe²⁺ and drugs on lipid peroxidation**

| Drugs (%) | None | 2 μM* | 5 μM* |
|-----------|------|-------|-------|
|           | OD₃₅₀ | %     | OD₃₅₀ | %     | OD₃₅₀ | %     |
| None      | 0.063 | 100   | 0.223 | 355   | 0.464 | 737   |
| PP        | 10⁻⁵  | 0.069 | 110   | 0.212 | 337   | 0.445 | 706   |
|           | 10⁻⁴  | 0.060 | 95    | 0.195 | 310   | 0.440 | 698   |
| PC        | 10⁻⁵  | 0.065 | 103   | 0.235 | 373   | 0.460 | 730   |
|           | 10⁻⁴  | 0.069 | 110   | 0.235 | 373   | 0.456 | 724   |

*: concentration of Fe²⁺ in the assay medium
elements of the endoplasmic reticulum and mitochondria (11, 17), thus initiating the auto
catalytic, peroxidative breakdown of these membranes. Consequently, the liver cells are
unable to secrete triglycerides into the plasma and the fatty liver develops. The decrease
in P-450 (29, 40) and b5 levels (41) has also been reported in the liver microsomal fractions
of rats treated with CCl4.

In the present study, a dose of CCl4 for 2 days to rats was found to decrease the drug-
metabolizing enzyme and lipolytic enzyme activities in rat liver microsomal fractions (Tables
1, 2 and Fig. 1). This indicates that a structure bounded by a single lipoprotein membrane,
endoplasmic reticulum, would be far more sensitive to damage induced by free radical attack.
The decreased P-450 content probably produces, at least partially, damage of the electron
transport pathway of microsomal mixed function oxidase system. Administration of PP
to CCl4-poisoned rats was found to increase the microsomal drug-metabolizing enzyme
activities (Table 1). A possible explanation for the stimulating effect may be: (1) PP
increases the synthesis or protects against the decomposition of P-450 after the incorpora-
tion of PP into liver microsomal fractions (42), as suggested by the data obtained with a
single daily dose of PP to normal rats (Table 3); and (2) a protecting effect of PP against
the inhibition of the enzymes by lipid peroxides owing to an increase in the protecting ef-
fect of endogenous antioxidant materials, as described in our previous paper (23). The
decreased metabolic rate of the other xenobiotics (and some endogenous compounds) may
therefore return to normal levels earlier with administration of PP. PP administered
to CCl4-poisoned rats was also shown to reduce the inhibition of microsomal lipolytic en-
zyme activity, probably due to the protecting effect of PP against lipid peroxides. The
view is supported by such data that PP depressed the microsomal peroxide contents in
injured liver (Fig. 4). This also suggests that the protecting effect plays an important role
in depressing the fat accumulated in CCl4 poisoning, as we reported (23). On the other
hand, administration of PC to CCl4-poisoned rats hardly increased the microsomal drug-
metabolizing enzyme activities. Aminopyrine N-demethylase activity was, however, en-
hanced to a slight extent (Table 2). The fact that no significant effect was found on
NADPH-cytochrome c reductase in CCl4 intoxication (Tables 1 and 2) suggests that electron
transfer from NADPH to NADPH-cytochrome c reductase was not inhibited by the free
radicals and the lipid peroxides. PC administered to the injured rats enhanced a decreased
hepatic microsomal phospholipid phosphorous content in CCl4-rats. This phenomenon
may be interpreted as the incorporation of PC into the phospholipids in the membranes.
The increased phospholipids appear to take part in the reconstitution of the microsomal phospholipids, i.e. phosphatidylethanolamine, which is an essential component of microsomal membrane and is required for the hydroxylation of fatty acids, hydrocarbons and drugs in liver microsomal enzyme system (7). The fact that PC increased the microsomal amino-
pyrine N-demethylase activity in CCl4-poisoned rats (Table 2) may be attributed to its re-
quirement for phospholipids, as Chaplin and Manning (43) stated that a phospholipase
inhibited ethylmorphine demethylation in liver microsomal suspension.

These experiments on microsomal fractions lead to the conclusion that administra-
tion of PP increases the decreased microsomal drug-metabolizing enzyme and the lipolytic enzyme activities in CCl₄-poisoned liver. On the other hand, administration of PC appears to enhance the reconstitution of phospholipids in the injured membrane in order to restore the function of microsomes.

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