Interference by 3-Methylcholanthrene and Phenobarbital on the Development of Liver Damage Caused by Carbon Tetrachloride and White Phosphorus Poisoning

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The toxicity of CCl₄ towards the liver is enhanced by pretreatment with various drugs that induce the proliferation of the hepatic smooth endoplasmic reticulum. Garner & McLean (1969) observed an increase in CCl₄-induced liver damage after prior administration of phenobarbital. This barbiturate stimulates a large number of metabolic pathways: it increases NADPH-cytochrome c reductase activity and cytochrome P-450 content (Conney, 1967). 3-Methylcholanthrene and other polycyclic hydrocarbons are also inducers of drug-metabolizing enzymes, but by contrast this induction affects the metabolism of relatively few compounds. Moreover 3-methylcholanthrene causes the formation of a different type of microsomal cytochrome P-450, namely cytochrome P-448 (Mannering, 1971; Imai & Siekevitz, 1971; Sladek & Mannering, 1966). In addition, Fouts & Rogers (1965) suggested, on the basis of electron-microscopical findings, that 3-methylcholanthrene induces less proliferation of hepatocellular smooth membranes than does the barbiturate phenobarbital.

The difference in behaviour of phenobarbital and 3-methylcholanthrene in the induction of drug-metabolizing enzymes stimulated us to investigate the action of these two drugs on CCl₄ hepatotoxicity. White phosphorus, another hepatotoxic agent, was thought to have some similarities, in mechanism of action, with CCl₄ (Ghoshal et al., 1969, 1971). However, contrasting results were obtained by Pani et al. (1972), who found that CCl₄-treated and white-phosphorus-treated rats were both protected by some antioxidants, but that they differed in that the hepatic microsomal lipids of white-phosphorus-poisoned animals were not affected by lipid peroxidation, as evaluated by diene conjugation absorption. In addition, phenobarbital does not seem to enhance the toxicity of white phosphorus (Pani et al., 1972; Hurwitz, 1972).

The present experiments were designed in an attempt to elucidate the relationship...
between CCl₄-induced and white-phosphorus-induced liver damage and effects on the microsomal fraction.

Male Wistar rats (200–250 g), fed on a semisynthetic diet (Fratelli Piccioni, Brescia, Italy), were starved for 16–18 h before poisoning was commenced. CCl₄ and white phosphorus were administered orally in mineral oil and in olive oil respectively. Control groups received the solvent alone. Phenobarbital (0.8% solution in 0.9% NaCl) was injected intraperitoneally at a dose of 80 mg/kg body wt. at 72 and 48 h, 50 mg/kg body wt. at 24 h and 30 mg/kg body wt. at 12 h before poisoning was commenced. 3-Methylcholanthrene (0.4% solution in olive oil) was given intraperitoneally at 72, 60 and 48 h before poisoning was commenced. Triton WR-1339 (Rohm and Haas, Philadelphia, Pa., U.S.A.) (20%, w/v, solution in 0.9% NaCl) was injected intravenously under light ether anaesthesia at a dose of 500 mg/kg body wt. Serum alanine aminotransferase (glutamate-pyruvate transaminase) and aspartate aminotransferase (glutamate-oxaloacetate transaminase) activities were determined with a Biochemica test kit (Boehringer, Mannheim, Germany). Plasma total lipids were extracted by the procedure of Folch et al. (1957). Triglycerides were measured colorimetrically by the method of Van Handel & Zilversmit (1957). For the study of polyribosomal profiles, all the operations were performed at 4°C after rapid removal of the liver; the organs were homogenized in 3 vol. (w/v) of 0.25 M-sucrose in buffer A (0.1 M-NH₄Cl–5 mM-magnesium acetate–20 mM-Tris–HCl buffer, pH 7.5) in a Potter–Elvehjem apparatus. The postmitochondrial supernatants were obtained by centrifuging the homogenates at 15000 g for 10 min; they were then treated with 1.2% (w/v) sodium deoxycholate. The deoxycholate-treated supernatants were layered on 16.7 ml convex sucrose density gradients (7.8 ml of 0.5 M-sucrose plus 8.9 ml of 1.5 M-sucrose, in an ISCO model 570 gradient-former). The

![Fig. 1. Effects of 3-methylcholanthrene and phenobarbital on lethality in the rat induced by CCl₄](image)

The rats were treated as follows: CCl₄; 3-methylcholanthrene + CCl₄; phenobarbital + CCl₄. Experimental details are indicated in the text.

Vol. 1
The rats were treated as follows: ○, CCl₄; □, 3-methylcholanthrene + CCl₄; ◊, phenobarbital + CCl₄. The CCl₄ was administered orally at a single dose of 1.5 ml/kg, body wt. Other experimental details are indicated in the text. The values refer to the means ± s.e.m. of results obtained with not less than five animals.

sucrose solutions were in buffer A. After the gradients had been centrifuged at 95000g for 5 h in a Beckman–Spinco ultracentrifuge with rotor SW 27.1, the absorbance profiles of the gradients were recorded continuously, with an ISCO–UA2 model D/184 apparatus provided with a flow cell having a 5 mm light-path.

The lethality induced by CCl₄, administered at different doses, is clearly increased in the phenobarbital-pretreated rats (Fig. 1). Our results are in agreement with the observations made by Hurwitz (1972). Garner & MacLean (1969) showed also an increased metabolism of CCl₄ to CO₂ in vivo and in vitro after oral administration of phenobarbitone; in addition, Rao et al. (1970) demonstrated that phenobarbital enhanced the response of the rats to CCl₄, namely increasing the lipid peroxidation phenomena as measured by diene conjugation. In contrast, the prior administration of 3-methylcholanthrene exerts a protective effect against the lethality of CCl₄ (Fig. 1). Further, the CCl₄-induced hepatic necrosis, as evaluated by the rise in serum alanine aminotransferase and aspartate aminotransferase activities, is worsened by the barbiturate, whereas 3-methylcholanthrene partially blocks the release of the aminotransferases into the serum (Fig. 2).

Reid et al. (1971) showed that 3-methylcholanthrene was able to protect hepatic necrosis induced by bromobenzene or CCl₄. They demonstrated the enhancement of bromo[¹⁴C]benzene metabolites, and they suggested that the protective action was due to a block of metabolic pathways possibly leading to the formation of toxic compounds.

At present there is no information on the enhancement of CCl₄ metabolism by 3-methylcholanthrene induction, but possibly, as in bromobenzene hepatotoxicity, the polycyclic hydrocarbon protects the liver by interfering with the metabolism of toxic compounds and consequently with their release from the hepatic tissue. There was no effect
of 3-methylcholanthrene on liver steatosis and triglyceride secretion, suggesting that the initial steps of CCl₄ hepatotoxicity are not associated with the interference by the polycyclic hydrocarbon; 3-methylcholanthrene would act on only those metabolites responsible for hepatic necrosis. To obtain a better understanding of the lack of effect by 3-methylcholanthrene on fatty liver and triglyceride secretion, we studied the polyribosomal disassociation induced by CCl₄ in rats pretreated with 3-methylcholanthrene. Even in this case no protection was found.

To evaluate the role of drug-metabolizing enzymes on hepatotoxicity, we studied the effects of phenobarbital and 3-methylcholanthrene on the liver damage produced by white phosphorus. As already shown by Pani et al. (1972), phenobarbital does not alter phosphorus hepatotoxicity, and as far as the lethality is concerned neither does 3-methylcholanthrene. As this hepatotoxic agent is probably not metabolized by the drug-metabolizing enzyme system, it is quite likely that the role of the drug-metabolizing enzyme system in drug toxicity concerns just those drugs that are primarily metabolized within its system. An extension of these studies to other hepatotoxins may clarify this point. Moreover, we may then better understand the role played by smooth membranes in drug metabolism and detoxification related with hepatic necrosis. In fact, the phenobarbital and 3-methylcholanthrene models are suitable ones, as the first enhances the toxicity of many drugs, whereas the latter seems to act by means of some protective mechanism.

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Lipid Alterations of Liver Microsomal Fraction and Plasma Lipoproteins after Carbon Tetrachloride Poisoning

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Evidence has been provided (Reynolds, 1967; Rao & Recknagel, 1968) that free radicals derived from the hepatic CCl₄ metabolism (probably CCl₃· free radicals) are incorporated into the fatty acid carbon chains of the lipids of the cell membranes, mainly those of the endoplasmic reticulum.

The original aim of the present research was to identify the fatty acids of microsomal lipids that undergo the free-radical addition reaction after CCl₄ poisoning, in order to get information on the pathological significance of the occurrence of chloromethylated fatty acids in the cell membranes.

Fatty acid methyl esters of liver microsomal lipids were examined by g.l.c. with an electron-capture detector. The column was split at the outlet, one branch going to the