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Conjugation of Pentachlorophenol to Palmitic Acid by Liver Microsomes

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Pentachlorophenol (PCP) has been used extensively as a pesticide in industry and agriculture (BEVENUE & BECKMAN 1967). Because of its diversified usage and chemical structure, PCP has a high potential as a hazardous and toxic environmental contaminant. It has been shown in in vitro studies to be a potent uncoupler of mitochondrial oxidative phosphorylation (WEINBACH 1954) and clinical symptoms indicate that this may also take place in vivo (JACOBSSON & YLLNER 1971). PCP has also been shown to inhibit microsomal detoxication enzymes (ARRHENIUS et al. 1977b). This inhibition is a serious toxic manifestation since an intact detoxification enzyme system is important in the protection of the body against many toxic substances.

The mechanism for the toxic action of PCP is not clear. However, the effect of uncoupling agents on mitochondrial oxidative phosphorylation has been shown to be an alteration in the properties of the lipid membrane which carries the enzymes of the electron transport chain (VAN DAM & MEYER 1971). PCP has also been shown to accumulate in microsomes (ARRHENIUS et al. 1977a), and to affect the electron transfer function between a flavin and a cytochrome (ARRHENIUS 1974).

A possible mechanism by which PCP may be retained in the lipid membranes of mitochondria and microsomes and alter their properties was recently discovered in our laboratories. In the initial studies long-retained metabolites of both Δ^8-and Δ^9-tetrahydrocannabinol (Δ^8-and Δ^9-THC) psychoactive components in marihuana, were identified in certain tissues of the rat as conjugates of fatty acids (LEIGHTY et al. 1976). These in vivo fatty acid conjugates could also be produced in vitro in a rat liver coenzyme A fortified microsomal system using the primary hydroxylated metabolite of Δ^8-THC or Δ^9-THC as the substrate (LEIGHTY 1979). DDOH [2,2-bis(p-chlorophenyl)ethanol], a known hydroxylated metabolite of the classical toxic environmental contaminant DDT (METCALF 1973), was also tested as a substrate in the microsomal system and found to conjugate to palmitic, stearic, oleic and linoleic fatty acids. Subsequent in vivo studies identified these fatty acid conjugates of DDT in livers and spleens of male and female rats given chronic intraperitoneal injections of DDT (LEIGHTY et al. 1980).

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The present in vitro study was performed to determine if PCP, which contains a hydroxyl group in its unmetabolized form, was conjugated to fatty acids in the rat liver coenzyme A fortified microsomal system.

METHODS AND MATERIALS

Palmitoylpentachlorophenol (palm-PCP), to be used as a reference standard, was synthesized from PCP using palmitoyl chloride in pyridine according to the procedure of SHRINER et al. (1956).

In the microsomal assay 1 mmole of PCP was incubated in a 37° C metabolic shaker for 2 hours in 25 ml of the rat liver coenzyme A fortified microsomal incubation mixture previously described (LEIGHTY 1979). After incubation the mixture was lyophilized, extracted exhaustively with chloroform, the solvent removed on a flash evaporator and the residue resuspended in a small volume of chloroform. The chloroform suspension was then partially purified using thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC).

For TLC, aliquots of the chloroform suspension were initially spotted on silica gel plates (K1, Whatman) and developed in 100 percent chloroform. Reference standards of PCP and palm-PCP were spotted on the same plates. The reference standards were detected on the plates by spraying with a 0.2 percent ethanolic solution of 2,7-dichlorofluorescein and examining under 254 UV. In this TLC system PCP has an Rf of 0.67 and palm-PCP moves with the solvent front. Sections of the TLC plates corresponding to the palm-PCP standard were scraped, eluted with methanol, dried on a flash evaporator and resuspended in chloroform. Aliquots of this suspension, and the standards, were then spotted on new plates, developed in 100 percent heptane and the plates treated and eluted as previously described. In this TLC system PCP does not move from the origin and palm-PCP has an Rf of 0.46.

The final TLC eluates were further purified by HPLC (DuPont 830 Liquid Chromatograph) using the following conditions: Whatman Partisil 10 standard PXS 10/50 column (50 cm x 4.6 mm x 6.4 mm); 50°C; 1.0 ml/min flow; chloroform/heptane (80:20) isocratic solvent; 254 UV detector. In this system palm-PCP has a Rt of 6.8. The peak area corresponding to palm-PCP was repeatedly collected, combined, dried under N_2 and then analyzed by gas chromatography-mass spectrometry (GC-MS).

All mass spectra were collected on a Finnigan 4000 GC-MS system combined with a INCOS data system. Gas chromatography was carried out on a 12 M SE-52 glass capillary column with H_2 carrier gas and a temperature program of 180°C (initial) for 2.0 minutes to 320°C (final) at 10°C/minute. The mass spectrometer was operated in the chemical ionization mode, using methane:ammonia as the reagent gas.

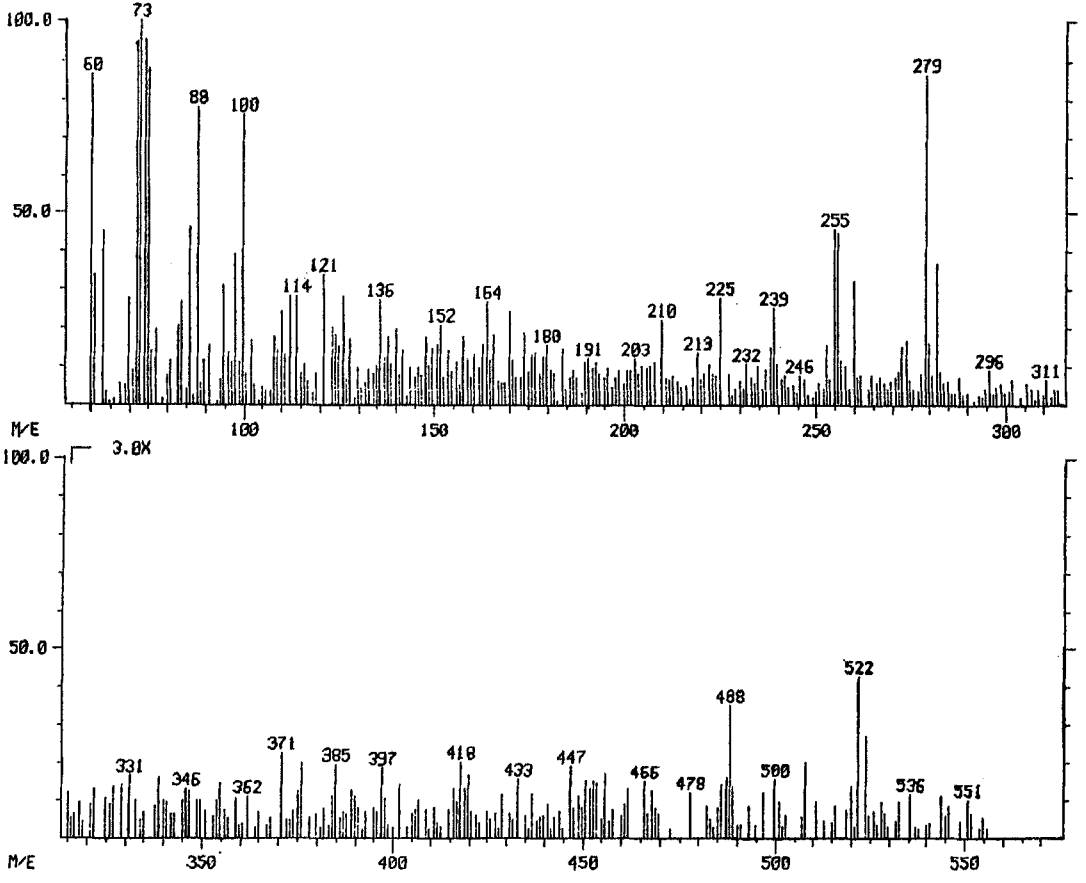

FIGURE 1. MASS SPECTRUM OF METABOLITE PRODUCED IN MICROSOMAL SYSTEM CONTAINING PCP

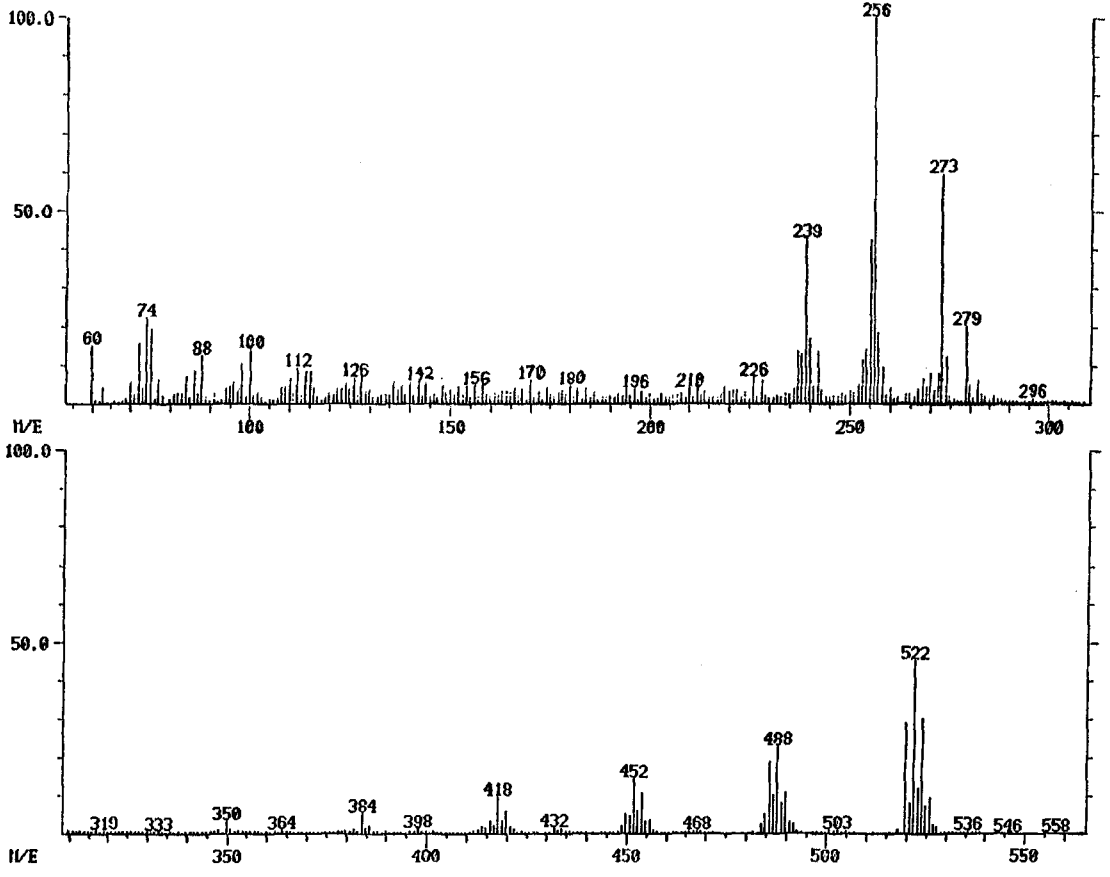

FIGURE 2. MASS SPECTRUM OF PALMITOYLPENTACHLOROPHENOL STANDARD

RESULTS

TLC analyses of the chloroform extract of the microsomal mixture containing PCP showed a spot corresponding to the Rfs of palm-PCP in both solvent systems. Subsequent HPLC analyses of the eluates of the TLC spot with Rf of 0.46 in the heptane solvent system showed a peak at the Rt (6.8) of palm-PCP.

Mass spectrometric analyses (Figure 1) of this HPLC peak identified it as palm-PCP, the PCP conjugate of palmitic acid. Figure 2 shows the CI_{NH3} mass spectrum of the palm-PCP standard. The molecular ions at 522 in both spectra correspond to $M(NH_4)^+$ of conjugated pentachlorophenol. Ions at m/e 488, 452, 418 correspond to loss of chlorine. Other representative ions in the two spectra are at 279, 273, 255-256 and 239.

DISCUSSION

This study shows that PCP can be conjugated to palmitic acid in a rat liver *in vitro* coenzyme A fortified microsomal system. The phenomenon of fatty acid conjugation to foreign compounds such as PCP may be a mechanism by which these compounds are retained *in vivo* in lipid-containing tissues of the body and ultimately exert a toxic effect.

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