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Induction of Hepatic Cytochrome P-450 Activity in Wild Cotton Rats (*Sigmodon hispidus*) by Phenobarbital and 3-Methylcholanthrene

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Cytochrome P-450 enzymes are a family of hemoproteins which are important in the metabolism of drugs, carcinogens, steroid hormones, fatty acids, and endogenous and exogenous toxins (Guengerich and Liebler 1985). The bioactivation (enzyme) index of cytochrome P-450 has been used in fish as a criterion for monitoring environmental pollution (Payne et al. 1984).

For toxicological evaluation, small rodents are superior to fish and other aquatic species because they are phylogenetically closer to man and live in close proximity to man. Wild cotton rats (Sigmodon hispidus) are ubiquitous throughout the Southeast quadrant of the United States, easy to capture, have a generation interval of less than one year and a limited range of movement (less than one hectare). Adult cotton rats range in size from 110 to 225 g and 100 to 200 g for males and females respectively (Chipman 1965). This enables the collection of sufficient body fluids and tissues for pathological and toxicological assays on individual animals rather than having to pool fluids and tissues from several animals. Furthermore, this species may prove to be an excellent model for monitoring environmental contamination.

Traditionally, cytochrome P-450 inducing agents are grouped into two classes. One, represented by phenobarbital, induces P-450b and P-450e; the other, represented by 3-methylcholanthrene, induces P-450c and P-450d isoenzymes. The types and amounts of cytochrome P-450 vary among species, organs, health status, sex, and stress of the animal (Sipes and Gandolfi 1986, Thomford and Dziuk 1986). If the levels of cytochrome P-450 of wild cotton rats are to be used in monitoring environmental pollution, it is necessary to characterize the inducibility and concentration of cytochrome P-450 in this species.

This study was designed to determine the concentration and inducibility of cytochrome P-450 in the livers of cotton rats after intraperitoneal (ip) administration of phenobarbital and 3-methylcholanthrene.

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MATERIALS AND METHODS

Pregnant female cotton rats were collected from noncontaminated areas with Sherman live traps. They were housed in individual polycarbonate cages with wire tops containing wood shavings as bedding. They had a 12-hour photoperiod, were fed rat chow (Rodent laboratory chow 5001, Purina Mills, Inc.), and received water free choice. Their young were weaned at 3 weeks and housed 2 animals of the same sex to a cage until they weighed a minimum of 85 grams. Before initiating experiments, each rat was weighed and placed in an individual cage. Induction studies were conducted using phenobarbital, 70 mg/kg in 0.09 % saline ip for 4 days, and 3-methylcholanthrene, 25 mg/kg in corn oil ip for 3 days. Four males and four females received each agent. Dosage and route were based on previous studies in laboratory rats (Knight and Walker 1982, Krahn et al. 1986). Two groups of four males and four females each, one group receiving saline and one receiving corn oil by ip injection were served as controls. On the day after the last injection the animals were fasted overnight then killed by cervical dislocation. Animals were exsanguinated by severing the aorta. Livers from both control and treated rats were weighed and total cytochrome P-450 levels were assayed (Omura and Sato 1964). Microsomal protein was determined on solubilized microsome samples (Smith et al. 1985). Representative liver samples were fixed in 10% buffered formalin and processed for histopathologic examination. Results were analyzed using a Student's t-test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The liver weight to body weight ratio was increased 139% and 165% of the control value ($P < 0.05$) in rats receiving phenobarbital and 3-methylcholanthrene respectively (Table 1). Microscopically, the hepatic lobules of treated livers were enlarged due to hypertrophy of hepatocytes particularly in centrilobular areas (Fig. 1,2). The total hepatic cytochrome P-450 concentrations were higher in animals receiving phenobarbital and 3-methylcholanthrene than in control animals (Table 1). The concentration of cytochrome P-450 was similar between male and female animals. Furthermore, the concentration of hepatic cytochrome P-450 in treated rats was more than that for laboratory rat (Sumner and Lodola 1987): 1.3 ± 0.1 nmoles/mg protein and 1.0 ± 0.14 nmoles/mg protein for phenobarbital and 3-methylcholanthrene respectively. The cytochrome P-450 inducibility in laboratory rats is approximately 50 to 100% of the control value (Kahl et al. 1980). The value was 139% to 209% of the control value in wild cotton rats, indicating greater cytochrome P-450 response. Previous induction studies on cotton rats dealt only with 3-methylcholanthrene, an inducer of P-450c and P-450d. This response of higher P-450 concentration may be related to species differences in regulatory genes. It has been reported that P-450 induction is regulated by a cluster of genes (Kahl et al. 1980). Consequently, genetic differences in capacity for the induction of cytochrome P-450 can cause large differences in xenobiotic metabolism, thereby leading to significant differences in concentration and induction of cytochrome P-450

among different species.

Table 1. Liver weights and levels of hepatic cytochrome P-450 from control and treated animals

Treatment and sex	Control males (n=4)	Treated males (n=4)	Control females (n=4)	Treated females (n=4)
a. Phenobarbital:				
Liver weight (g)	2.61 ±0.13	4.36 ±0.35	2.84 ±0.42	3.57 ±0.18
Liver weight / Body weight x 100	2.75 ±0.11	3.82* ±0.10 (139%)	2.41 ±0.08	3.97* ±0.13 (165%)
P-450 nmoles/mg of protein	2.11 ±0.13	4.36* ±0.42 (206%)	1.74 ±0.31	3.64* ±0.14 (209%)
b. 3-Methylcholanthrene:				
Liver weight (g)	2.90 ±0.17	4.63 ±0.18	2.71 ±0.25	3.40 ±0.17
Liver weight / Body weight x 100	2.86 ±0.15	4.47* ±0.13 (156%)	2.50 ±0.09	3.95* ±0.07 (158%)
P-450 nmoles/mg of protein	2.10 ±0.14	3.07* ±0.21 (146%)	1.84 ±0.10	3.51* ±0.26 (191%)

(n) = Number of rats
Data expressed as $\bar{x} \pm SE$ * = Statistically different from control rats ($P < 0.05$)
Number in parenthesis indicates the percentage of the control value

Biotransformation enzyme activity in fish has been documented as criterion for determining water pollution (Payne et al. 1984). However, the use of cytochrome P-450 levels in fish has not been consistently successful due to low inherent levels of cytochrome P-450, and marked variation in inducibility (Lindstrom-Seppa et al. 1985). Cotton rats are a likely model due to their typically high cytochrome P-450, their marked inducibility and ubiquitous distribution in Southeast United States. Our initial Studies at a contaminated toxic waste disposal site suggest that hepatic cytochrome P-450 in the cotton rats may be a useful indicator of environmental contamination (Elangbam et al. 1987).

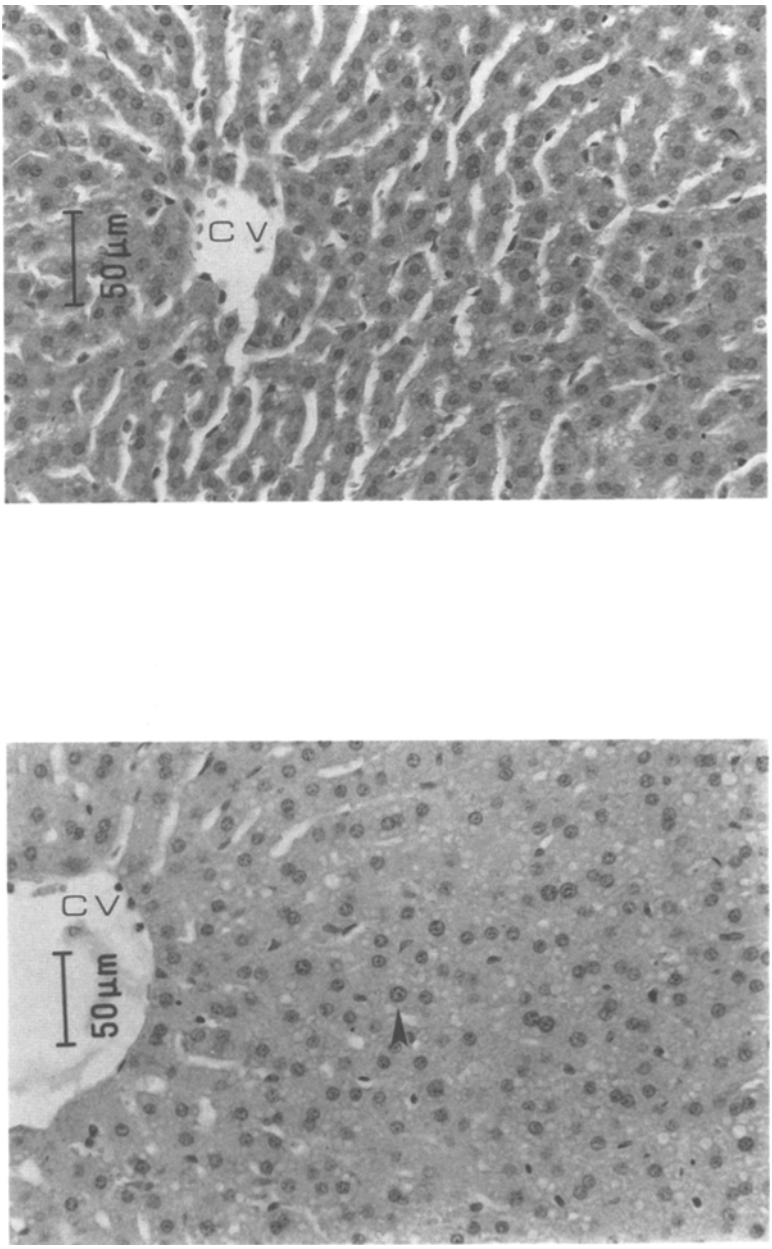

Fig. 2 Liver from animal treated with phenobarbital illustrating the enlargement of centrilobular hepatocytes and nuclei (arrow). Note increased cytoplasmic vacuoles. Central vein (CV).

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