NEPHROTOXICITY AND HEPATOTOXICITY OF CHLOROFORM IN MICE

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1982

ABSTRACT

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Administration of deuterium labelled chloroform and chloroform to male ICR and C57 and male and female DBA mice produced dose-dependent damage in the kidney and liver. Deuterium labelled chloroform produced less kidney and liver damage than chloroform. Chloroform caused the same degree of liver damage in male C57 and male and female DBA mice. Nephrotoxicity of chloroform was greater in male DBA than in C57 mice; female DBA mice failed to develop renal injury following treatment with chloroform. Phenobarbital increased hepatotoxicity but not nephrotoxicity of chloroform. β-Naphthoflavone enhanced chloroform hepatotoxicity in male C57 mice but had no effect on nephrotoxicity. Polybrominated biphenyls enhanced hepatotoxicity of chloroform in both strains but increased renal injury only in male C57 mice. The lack of correlation between chloroform hepatotoxicity and nephrotoxicity strongly suggests that the kidney is the site of formation of nephrotoxic chloroform metabolite(s).

ACKNOWLEDGEMENTS

I would like to express appreciation to Dr. Jerry Hook for this opportunity of studying toxicology and his encouragement. I would also like to express sincere gratitude to my other committee members. I thank Martha Thomas for her kind support and academic counseling and Dr. Charles Whitehair for his helpful advice. A special thank you is extended to Dr. Robert Echt for his guidance, encouragement and constructive criticism throughout the morphologic and cytochemical aspects of this investigation, as well as for his moral support during times of frustration.

I offer my thanks to Dr. Esther Roege for her technical assistance and to my dear friend Katherine Washko for her literary advice.

Finally, I am deeply grateful to my parents, brothers and sisters. Though they are geographically distant from me, they have always been with me in spirit and their moral and financial support has indeed made this entire academic experience possible for me.

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INTRODUCTION

Chloroform ($CHCl_3$) was initially used as a medical anesthetic. Later, it was found to cause heart and liver damage (Dipalma, 1971; Goodman and Gilman, 1970). With the discovery of safer agents, $CHCl_3$ lost importance as a medical anesthetic. However, $CHCl_3$ is still used in industry primarily as a solvent and chemical intermediate in the manufacture of artificial silk and plastic, as a cleaning agent, and as a chemical intermediate for the manufacture of fluorocarbon compounds. Until recently it was also employed as a preservative and/or flavor enhancer in pharmaceutical products such as cough medicine, mouth washes and for extraction and purification of penicillin and other antibiotics.

Recently it has been found that chronic oral administration of large doses of $CHCl_3$ increased the incidence of carcinoma in mice and rats (Melvin, 1979; Reuber, 1979). A low concentration of $CHCl_3$ has been reported in the surface water of industrialized areas of the United States. In addition, $CHCl_3$ may arise from the chlorination of organic materials already in the water during chemical sterilization of water supplies (Wade, 1977). These findings have generated widespread concern and caused renewed interest in the study of $CHCl_3$ toxicity.

Physical and chemical properties: Chloroform is a colorless, volatile liquid with a sweetish odor and taste and has a molecular weight of 119.38 daltons. It is insoluble in water but readily soluble in organic solvents such as ether, benzene and alcohols. Chloroform burns with a green flame at high temperatures (Hoover, 1970).

Chloroform can be absorbed through the lungs, from the gastrointestinal tract and, to a lesser degree, through the intact skin. In addition, it can penetrate the placental barrier and has been found in fetal liver (Von Oettingen, 1964). After CHC13 is absorbed, it is rapidly distributed to all organs of the body, with the concentration varying according to exposure level and duration. Chloroform is excreted unchanged primarily through the lung. This pulmonary excretion is slower than the absorption process, and CHC13 may persist for several hours or even days after exposure ends. Barret et al. (1939) showed that a small fraction of the absorbed CHC13 cannot be accounted for by excretory processes, indicating that some degree of decomposition must occur within the body after absorption.

Neither the precise reaction responsible for CHC13 breakdown in the body nor the metabolic products have been clearly established. Paul and Rubinstein (1963) injected carbon [14] labeled CHC13 into rats and were able to show that 74% of the radioactivity appeared as CHC13 in the exhaled breath; some appeared in the form of carbon dioxide (CO2). Reynolds and Yee (1967) hypothesized that the hepatotoxicity of CHC13 is related to the binding of

reduction products to the endoplasmic reticulum and to the formation of chloromethylated lipids and proteins in the liver.

Most reports of acute CHCl3 poisoning in man describe the results of accidental overdose during anesthesia or the characteristics of delayed poisoning following anesthesia. Chloroform also produces a local toxic response when in contact with intact skin because of its strong vesicant action. The symptomatology of CHCl3 poisoning varies according to the route of administration and the severity of exposure. Toxicity from ingestion of CHCl3 is marked by gastrointestinal disturbances as well as narcosis. Signs of CHCl3 toxicity include a characteristic sweetish odor on the breath, cold and clammy skin, and dilated pupils (Kaye, 1970). Ketosis develops as a result of incomplete oxidation of fats as well as increased blood sugar. In addition, ingested CHCl3 acts as a strong irritant to the gastrointestinal mucosa. Most CHCl3 poisoning results from inhalation of CHCl3; it produces strong central nervous system depression and can result in complete narcosis. These poisonings are characterized by loss of sensation and abolition of motor functions. Death may result from respiratory failure attributable to paralysis of the respiratory center or may result from the reduced blood supply to the brain caused by circulatory failure.

The pathological findings associated with CHCl3 toxicity are extensive. The outstanding pathological feature is centrolobular necrosis of the liver. The liver is the most

notably affected organ, but fatty degeneration of the kidneys and, to a lesser extent, the heart is also characteristic. Transient irritation of the kidneys has been observed during CHC13 narcosis; acute exposure may entail damage to the renal tubules. The degenerative changes are most commonly observed in adult or nephropathic animals and are manifested by the accumulation of stainable lipoid material (Von Oettingen, 1964; Casarett, 1975).

Although CHC13 has been known to cause renal damage (Von Oettingen, 1964), the precise biochemical mechanisms responsible for nephrotoxicity remain unclear. Increasing evidence indicates that CHC13-induced nephrotoxicity is independent of hepatic metabolism of CHC13; rather, it is the consequence of intrarenal bioactivation (Ilett et al., 1978; Kluwe et al., 1978; Kluwe and Hook, 1980). Susceptibility of CHC13-induced renal damage is sex-dependent. Female mice of any strain are resistant to nephrotoxicity, while male mice are sensitive to CHC13-induced renal damage (Deringer et al., 1953; Shubik and Ritchie, 1953; Hewitt, 1956; Jacobsen, 1964; Bennet and Whigman, 1964; Zaleska-Rutczynska and Krus, 1972; Clemens et al., 1979). However, the susceptibility of male mice to CHC13-induced kidney injury varies markedly among strains. The lack of significant difference in CHC13 hepatotoxicity in any strain and the difference in observed nephrotoxicity between male and female mice suggest that the mouse is a suitable species for the study and interpretation of renal damage produced by CHC13.

Strain-Related Differences in Nephrotoxicity of CHC13

Chloroform has been known to cause liver and kidney damage in humans and experimental animals (Von Oettingen, 1964). In mice, CHC13 produced liver damage in both sexes while causing renal lesions only in males (Eschenbrenner, 1944). Interestingly, the susceptibility of male mice to CHC13-induced renal necrosis varied among different strains. Shunbic and Ritchie (1953) reported a high death rate from kidney necrosis in the DBA strain of male mice following accidental exposure to a low concentration of CHC13 vapor. Deringer et al. (1953) noted a high death rate in C3H male mice; a smaller portion of the males of strains A and HR also died after exposure to CHC13. In contrast, the kidneys of male C57BL, C57BR/ed, C57L and ST mice were found to be free of any damage following an accidental exposure to CHC13. Hewitt (1956) observed a high death rate in adult male CBA mice; of surviving adult male CBA mice, 72% were found to have severe kidney damage. The renal lesions consisted of long areas of complete necrosis confined to the convoluted tubules. No evidence of renal damage was found in any other strain of male mice, although the mice were exposed under the same conditions as the affected mice. Bennet and Whigman (1964) noted that the CBA-P strain of male mice developed renal necrosis. No lesions were seen in albino ASW male mice following exposure to CHC13. Deringer et al. (1953) reported that the susceptibility to CHC13 increased with the age of mice; male mice which were immature at the time of the accidental exposure to CHC13

were not affected. Hewitt (1956) found that only adult males were susceptible and to varying degrees; he noted no renal damage in females or young males after exposure to CHC13. Such variation with age may reflect possible hormonal influence on CHC13-induced renal necrosis. Hill et al. (1975) reported that the C57BL/6J strain was about 4 times more resistant to the lethal effects of CHC13 than the male DBA/2J mice.

Histopathological studies of kidney lesions in male DBA mice have shown calcification in the kidney involving the entire cortex. No signs of damage were observed in other strains of male mice. Damage was localized in particular segments of the tubule; no changes were observed in Bowman's capsule or the first portion of the proximal convoluted tubule (Eschenbrenner and Miller, 1945; Eschenbrenner, 1944).

Several investigators have suggested that strain differences in CHC13-induced nephrotoxicity are genetically controlled, being transmitted in either a single or multifactorial manner (Hill et al., 1975; Zaleska-Rutczynska and Krus, 1973). The breeding of resistant C57 mice to sensitive DBA mice produced a generation of males with susceptibility to CHC13 nephrotoxicity intermediate to those of the parental strains.

Renal Regeneration

Following experiments on strain differences in CHC13-induced lethality, Zaleska-Rutczynska and Krus (1972).

reported that all males of the C3H/He strain died following administration of CHC13, while all of the C57/6J strain survived. Hybrid animals also survived, as did their resistant parents. This finding suggested that resistance to lethality is a dominant trait (Zaleska-Rutczynska and Krus, 1973). These investigators stated that all treated males developed renal tubular necrosis after administration of CHC13. Resistant animals, however, were able to regenerate new renal tubular epithelia and survive, whereas the C3H/He animals did not. Treated C3H/He males developed renal cortical calcification, which interfered with tubular regeneration, and died.

Hill et al. (1975) observed that male C3H/He mice were more susceptible to death because they were unable to repair renal injury. They noted that male DBA mice regenerated renal tubular epithelium, as did the male C57BL mice. Therefore, the sensitivity of male DBA mice to CHC13-induced lethality cannot be attributed to a total failure of repair, as observed in the male C3H/He mice. Clemens et al. (1979) reported that CHC13 induced renal damage in C57BL as well as DBA/2J male mice. However, the male DBA mice underwent regeneration of tubular epithelium, as did the male C57 strain. Regeneration was characterized by epithelial hyperplasia and increased cellular basophilia. It was concluded that strains differ in the capacity for regeneration of renal convoluted tubules.

Sex-Related Differences in Nephrotoxicity of CHCl3

In contrast to the strain differences observed in male mice, females of all strains of mice are resistant to CHCl3 nephrotoxicity. Castrated males, regardless of strain, are also resistant. It appears that testosterone plays a significant role in sex-related differences in renal toxicity and death produced by CHCl3. It may be important in the strain-related differences. In castrated mice, renal toxicity following exposure to CHCl3 increases with administration of increasing doses of testosterone. Analysis of various physiological and behavioral characteristics and determination of plasma testosterone levels suggests that male C57BL/10J mice are relatively testosterone deficient in comparison to male DBA mice (Shire and Bartke, 1972; Bartke and Shire, 1972).

If testosterone plays an important role in sensitizing the renal proximal convoluted tubular cells to CHCl3, it is important to understand the mechanism of this action. It has been suggested that the hormone enters the cells and binds to a specific cytoplasmic receptor. This is followed by movement of the hormone-receptor complex to the nucleus, where stimulation of nucleic acid and protein synthesis occur (Wilson, 1972). Other mechanisms have been suggested for the initial step in androgen action (Kochakian and Harrison, 1961; Koth et al., 1971). These include the metabolism of testosterone to another androgen or an estrogen before complexing with a specific receptor and possible testosterone action without mediation of a receptor (Wilson, 1972; Brown et al., 1976).

Flutamide is a nonsteroidal antiandrogen that inhibits testosterone action by competing with the hormone for its cytoplasmic receptor (Wilson, 1972; Brown et al., 1976). Kidneys of all C57BL female mice were sensitized by testosterone to the toxic effects of a subsequent dose of CHC13; kidneys of all animals treated with both testosterone and flutamide failed to show damage from CHC13 (Clemens et al., 1979). These experiments suggested that testosterone sensitization in the mouse kidneys acts through the testosterone receptor mechanism. The protective role of castration in mice and the production of kidney lesions in testosterone-treated female mice appears to indicate that that or a metabolite of testosterone in the kidney may be responsible for development of CHC13-induced renal damage in the mouse.

The lack of sensitivity of female mice to CHC13-induced nephrotoxicity could be due to the possible protective effect of female hormones. However, oophorectomized females are also insensitive to CHC13-induced renal injury. Therefore, it is unlikely that these hormones are involved as necrosis-preventing agents (Krus et al., 1974).

Morphology of the Kidney in Nephrotoxicity of CHC13

Sex-related differences in kidney morphology have been reported by Crabtree (1941), who found that the parietal epithelium of Bowman's capsule in male mice was composed of cuboidal cells similar to those lining the proximal convoluted tubules. Later, she observed brush borders lining the cuboidal type cells in Bowman's capsule (Crabtree, 1941). Eschenbrenner and Miller (1945) confirmed this finding. In

addition, they noted renal lesions following exposure to CHCl3 in male mice. In contrast, female mice were not affected. These investigators also reported that castrated mice failed to develop renal necrosis following exposure to CHCl3. However, female mice treated with testosterone developed renal tubular necrosis following CHCl3 administration. Selye (1939) stated that testosterone-treated female mice showed increased kidney weight due to hypertrophy of the epithelium of the proximal and distal convoluted tubules and of the epithelium lining Bowman's capsule. Pfeiffer et al. (1940) reported that in the kidney of mice receiving testosterone propionate, a portion of the normal squamous epithelium of Bowman's capsule was replaced in many instances by cuboidal cells, cytologically indistinguishable from those of the proximal tubules. However, the source of the thickened cells of Bowman's capsule was not clear. Pfeiffer et al. (1940) suggested that either the epithelial cells of the proximal tubule entered the capsule by migration and gradually replaced the squamous cells, which degenerated and dropped out, or the parietal cells themselves underwent metaplasia and converted to a type indistinguishable from those of the tubules. Crabtree (1940) noted that castration produces in the kidney of male mice a return to the immature or female type cell morphology but does not result in a complete layer of cuboidal cells in the capsule. However, castration affects the mouse kidney by causing a slight decrease in the kidney ratio of kidney weight to body weight

and a fall in the percentage of cuboidal cells in the capsule to approximately the female level for mice of the same age.

The administration of testosterone propionate to castrated mice results in marked hypertrophy of all parts of the kidney and an increase in the percentage of cuboidal cells in Bowman's capsule to nearly the normal value for males of the same age (Selye, 1939; Pfeiffer, 1940). These observations indicate that testosterone appears to play a significant role in sex-related differences in kidney morphology as well as renal toxicity of CHCl3. However, these authors did not indicate a cause and effect relationship between the two phenomena. Since hepatotoxicity of CHCl3 is not related to changes in hormone activities (Culliford and Hewitt, 1957), the data suggest that either the nephrotoxicity and hepatotoxicity of CHCl3 occur by different mechanisms or that the formation of a CHCl3 metabolite is stimulated by testosterone in the kidney of the mouse.

Metabolites of CHCl3

The toxicity of many drugs is due to the formation of toxic metabolites. This also appears to be true for CHCl3; reactive metabolite(s) of CHCl3 are responsible for toxicity. Several investigators have suggested that the initial step leading to CHCl3-induced tissue injury is biotransformation of CHCl3 to the reactive intermediate phosgene (COCL2) by enzymes of the mixed-function oxygenase system (MFO) (Pohl et al., 1977; Mansuy et al., 1977; Sipes et al., 1977). Formation of COCL2 was postulated to proceed through an oxidative dechlorination mechanism which involved oxidation

of the C-H bond of CHCl3 to produce the trichlomethanol (CCl3-OH) derivative. This unstable derivative would then spontaneously dehydrochlorinate to COCl2. The electrophilic COCl2 could react with water to form carbon dioxide (CO2), a known metabolite of CHCl3 *in vitro* (Paul and Rubinstein, 1963) and *in vivo* (Pohl and Krishna, 1978), or with microsomal enzyme to yield a covalently-bound product (Ilett et al., 1973; Brown et al., 1974; Uehleke and Werner, 1975; Gillette and Pohl, 1977; Sipes et al., 1977; Uehleke et al., 1977).

To support the hypothesis that intermediate product of CHCl3 is toxic and causes cell injury, the relative hepatotoxicity of CHCl3 and deuterium-labeled chloroform (CDCl3) was compared. The approach was based upon the fact that an isotope effect was observed in the *in vitro* formation of COCl2 by liver microsomes at approximately half the rate of CHCl3. *In vivo* studies also indicated that CDCl3 was less hepatotoxic than CHCl3 (Pohl and Krishna, 1978). These observations suggested that the cleavage of the C-H bond is the rate-limiting step in the activation of CHCl3.

The observation was made that, when rats or mice are treated with [^{14}C] CHCl3, the extent of hepatic necrosis parallels the amount of [^{14}C] label bound irreversibly to liver protein (Ilett et al., 1973). This supported the concept that a reactive metabolite of CHCl3 is responsible for its toxicity. Although CHCl3 has been known to cause renal damage (Von Oettingen, 1964), the precise biochemical mechanism responsible for nephrotoxicity remains unclear.

The strain and sex differences in susceptibility to CHCl-induced renal necrosis raise the possibility that the nephrotoxicity is caused by a metabolite of CHC13. However, increasing evidence indicates that CHC13-induced nephrotoxicity is independent of hepatic metabolism of CHC13; rather, it is the consequence of intrarenal bioactivation (Ilett et al., 1973; Kluwe et al., 1978; Kluwe and Hook, 1980).

In summary, drug metabolizing enzyme systems in the liver and kidney convert CHC13 to one or more chemically reactive metabolites. Cellular necrosis caused by this toxic metabolite(s) appears to be related to the alkylation of macromolecules in the target cells. However, the biological process by which alkylation of macromolecules may lead to cell necrosis is not completely understood.

Covalent Binding of CHC13

The covalent binding of CHC13 metabolite(s) to liver and kidney protein was observed by Ilett et al. (1973), who showed that the livers of male and female C57BL/6J mice not only developed similar degrees of hepatic necrosis but also covalently bound a similar amount of radioactivity to hepatic protein following administration of [^{14}C]CHC13. In contrast, more radioactivity was bound to renal protein by male than female mice. In addition, they noted that female mice were relatively resistant to CHCL3-induced renal injury. Taylor et al. (1974) found that in the kidneys of male mice the concentration of [^{14}C] was much higher than in female CBA mice. Testosterone treatment of female mice resulted in a

greater concentration of radioactivity in the cortex and castration reduced the amount of renal cortical radioactivity in males (Taylor et al., 1974) and decreased the renal damage produced by CHCl3. Clemens et al. (1979) reported that radioactivity from [^{14}C]CHCl3 bound to renal protein appeared to be greater in male DBA/2J mice which are sensitive to CHCl3-induced nephrotoxicity than in relatively resistant male C57/6J mice. Ilett et al. (1973) suggested that a cause and effect relationship may exist between the covalent binding of a CHCl3 metabolite and acute renal and hepatic necrosis. Pretreatment of mice with phenobarbital (PB) increased both the degree of liver damage and the level of radioactivity bound to protein in this tissue. In contrast, pretreatment of mice with an inhibitor of MFO activities such as piperonyl butoxide decreased the hepatotoxicity and covalent binding of [^{14}C]CHCl3. Thus, considerable evidence indicates that the extent of renal and hepatic necrosis in mice parallels the amount of CHCl3 covalently bound to protein in these tissues (Ilett et al., 1973).

The metabolic activation of CHCl3 by liver microsomes appears to be catalyzed by cytochrome P-450, because the binding to microsomal protein is NADPH-dependent and inhibited by carbon monoxide, SKF 525-A and piperonyl butoxide (Sipes et al., 1977). In contrast, omission of NADPH from mouse kidney microscomal preparation caused only a slight decrease in the amount of covalent binding (Sipes et al., 1977). In addition, carbon monoxide and SKF 525-A failed to inhibit the irreversible binding of CHCl3 to renal microsomes (Pohl

and Krishna, 1978). Thus, the failure of SKF 525-A and carbon monoxide to protect the kidney from CHCl3 toxicity indicates that CHCl3 may be metabolized to a nephrotoxic product by an enzyme system sensitive to inhibition by piperonyl butoxide but not blocked by SKF 525-A or carbon monoxide.

Depletion of Glutathione

Glutathione (GSH), a sulfhydryl tripeptide, found in high concentration in the liver, may play a key role in protecting the liver from the toxic effects of CHCl3 (Brown et al., 1974). It has been found that one of the physiological functions of GSH is to protect cellular components from attack by electrophilic chemicals or metabolites which may cause tissue damage by reaction with macromolecules (Jakoby, 1977). Results of several observations suggested that treatment of rats with CHCl3 depelets hepatic GSH (Brown et al., 1974; Johnson, 1965), but acute liver damage occurs only with doses of CHCl3 sufficient to reduce the concentration of hepatic GSH below a critical level. It has been suggested that toxic, electrophilic metabolites of CHCl3 are detoxified by conjugation with GSH. However, when hepatic GSH has been depleted below a critical level, the electrophilic metabolites can attack essential cellular components.

Pretreatment of mice with a liver microsomal inducer such as phenobarbital markedly increased the depletion of liver GSH by CHCl3; no corresponding depletion of GSH was found in the kidney (Dock, 1976). In addition, although

massive liver necrosis was observed in mice treated with phenobarbital, no kidney damage was noted in these mice. This finding further supports the concept that GSH protects the kidney from the injurious effects of CHC13. A protective role of GSH is supported by the observation that the hepatotoxicity of CHC13 is potentiated when liver GSH is depleted by pretreatment of animals with diethyl maleate (Brown et al., 1974).

Since CDC13 is not nearly as potent as CHC13 in depleting GSH, it would indicate that the hydrogen in CHC13 may be involved in the formation of the reactive intermediate that might deplete GSH conjugate (Docks and Krishna, 1976). The finding that CDC13 was also less hepatotoxic than CHC13 (Docks and Krishna, 1976; Pohl and Krishna, 1978) suggests that CHC13 is presumably metabolized in the liver to an electrophilic molecule, possibly phosgen, which depeletes GSH by formation of GSH conjugates (Docks et al., 1974).

Classes of Microsomal Enzyme Inducers and Effects of These Agents on CHC13-Induced Toxicity

The mechanism by which CHC13-induced nephrotoxicity and hepatotoxicity develops is not completely understood. However, the initial step in the pathway leading to CHC13 toxicity is generally believed to be biotransformation of CHC13 to a reactive intermediate, possibly phosgen (COC12) by a mixed-function oxygenase (MFO) present in the endoplasmic reticulum of susceptible tissue (Ilett et al., 1973; Pohl et al., 1977; Manusy et al., 1977; Sipes et al., 1977; Brown et al., 1974). The presence of MFO systems in both

the kidney and liver of various animal species, including mice, has been reported by many investigators (Kuntzman, 1969; Gillette, 1963; Conney, 1967; Parke, 1968; Brodie, 1958; Goldstein et al., 1968).

A number of compounds have been reported to induce microsomal enzymes. Based on differences in the profile of biological effects, most of these agents can be placed into one of three general groups.

1. Phenobarbital type: Phenobarbital (PB) administration enhances the metabolism of drugs by microsomal enzymes by increasing total liver mass, microsomal protein and enzyme-specific activity. It also increases the amount of both NADPH and cytochrome P-450.

2. Polycyclic hydrocarbon types: Two of the most common examples of this group are 3-methylcholanthrene (3MC) and 3,4-benzo(a)pyrene (BP). Although many compounds in this group are carcinogens, there is no relation between their carcinogenic capacity and enzyme induction. There are 3 general differences between the induction effect of PB and 3MC:

   a. 3-Methylcholanthrene produces more selective stimulation of hepatic enzymes; i.e., it increases the metabolism of fewer substrates.

   b. 3-Methylcholanthrene increases the amount of cytochrome P-450 but does not alter the amount of NADPH cytochrome c reductase.

   c. The delay between exposure and maximum enzyme induction is in the order of hours for 3MC and days for PB.

3. Anabolic steroids: Comparatively little is known about the mechanism of induction of microsomal drug metabolism by anabolic steroids. Administration of testosterone or methyltestosterone to female or castrated male rats increases drug metabolism by hepatic microsomes. However, co-administration of anabolic steroids and phenobarbital results in a summation of their inductive effects, suggesting that they act through different mechanisms. The time required for maximal enzyme induction by the anabolic steroids differs from both other groups. Whereas 3MC produces maximum effects within 12 to 24 hours and PB within 2 to 3 days, the anabolic steroids require 2 to 4 weeks. Their administration is not accompanied by significant effect on liver weight or microsomal protein content. Moreover, the steroids do not greatly increase the amount of cytochrome P-450 in liver microsomes.

Stimulation of hepatic MFO activity by PB not only increased hepatic MFO activities but also increased hepatic damage following $CHCl_3$ administration in mice (Burger and Herdson, 1966; Kluwe et al., 1978). In contrast, PB did not affect renal MFO activity or alter the sensitivity of mice to $CHCl_3$-induced nephrotoxicity. Pretreatment of mice with 3MC enhanced MFO activities in both liver and kidney. This agent did not alter the liver damage produced by $CHCl_3$ but markedly decreased the nephrotoxic effect of $CHCl_3$ (Kluwe et al., 1978). Polybrominated biphenyls (PBB) have been found to enhance enzyme sensitive to both PB and 3MC (Dent et al., 1976). Pretreatment of mice with PBB both increased

MFO activities in liver and kidney and enhanced the nephrotoxicity and hepatotoxicity of $CHCl_3$ (McCormack et al., 1978; Kluwe et al., 1978).

PURPOSE

The overall purpose of these studies was to test the hypothesis that chloroform-induced nephrotoxicity is due to biotransformation of chloroform into one or more toxic metabolites intrarenally and not by translocation of the toxic agent from the liver.

These investigations were divided into 3 segments:

1. Determination of nephrotoxicity and hepatotoxicity of chloroform in male ICR mice and determination of the effect of deuterium substitution on toxicity.

2. Study of induction of drug metabolizing enzymes in kidney and liver and their effects on renal and hepatic toxicity of chloroform in inducible and noninducible strains of male mice.

3. Comparison of renal and hepatic injury produced by chloroform in male and female mice.

MATERIALS AND METHODS

Materials

Chloroform (CHCl3) and phenobarbital (PB) were obtained from Mallinckrodt (St. Louis, MO); deuterium-labelled chloroform (CDCl3) and β-naphthoflavone (BNF) were purchased from Sigma Chemical Company (St. Louis, MO). Polybrominated biphenyls (PBB) were received from the Michigan Chemical Company (St. Louis, MI).

Animals

Male ICR mice (25-30 g) were purchased from Spartan Farms (Haslett, MI); male C57BL/BJ (C57) and male and female DBA/2J (DBA) strain mice (25-30 g) were obtained from Jackson Laboratory (Bar Harbor, MO) and housed in groups of 5 in plastic shoebox cages in temperature and humidity controlled rooms with a light cycle of 12 hours light and 12 hours dark.

Treatments

Chloroform (CHCl3) and deuterium-labelled chloroform (CDCl3) were dissolved in corn oil and administered intraperitoneally (ip) at doses of 0.1, 0.25, 0.5, 0.75, and 1.0 ml/kg into male ICR mice. Control mice were given

corn oil only. β-Naphthoflavone (BNF) was dissolved in acetone and mixed slowly and evenly into the ground rodent diet (Wayne Lab Blox, Chicago, IL) to produce a final concentration of 1 g/kg BNF diet. Male C57 and male DBA mice were maintained on a 1 g/kg BNF diet for 5 days before exposure to CHC13. Control mice were fed a regular rodent diet.

Polybrominated biphenyl (PBB) was dissolved in acetone and mixed slowly and evenly into ground diet (Wayne Lab Blox, Chicago, IL) to produce a final concentration of 100 parts per million (ppm). Male C57 and male DBA mice treated with PBB were placed on 0 or 100 ppm of PBB for 28 days before exposure to CHC13. In another series of experiments, phenobarbital (PB) was dissolved in saline (13.3 mg/ml saline) and administered ip into mice (both male C57 and male DBA mice) at a dose of 80 mg/kg once daily for 3 consecutive days. Twenty-four hours after the last injection, animals were exposed to CHC13. Following pretreatment with BNF, PBB or PB, mice were administered ip 0.025, 0.05, 0.1 or 0.25 ml/kg of CHC13 (in peanut oil). Control mice received peanut oil alone. In another series of experiments, male C57 and male and female DBA mice received CHC13 (in peanut oil) ip at doses of 0.05, 0.1, 0.25 or 0.5 ml/kg. All animals were killed 24 hours after administration of CHC13.

Microsomal Enzymes

Some control and inducer-treated animals were killed prior to exposure to CHCl3 and livers and kidneys were quickly removed, weighed, minced and homogenized in 3 volumes of ice-cold 1.15 KCl (livers) or 66 mM Tris-HCl buffer, pH 7.4 (kidneys). The homogenates were centrifuged at 10,000 x g for 20 minutes. All assays were performed on the day of supernatant preparation; protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard. Arylhydrocarbon hydroxylase (AHH) activity was determined by the method of Nebert and Gelboin (1968).

Toxicity Tests

Male ICR, male C57 and male and female DBA mice and male C57 and male DBA mice pretreated with BNF, PB or PBB with controls (each set had separate controls) were challenged with a single injection of CDCl3 (male ICR mice) or CHCl3 and killed 24 hours later. Blood was collected and livers and kidneys were quickly removed and weighed. The blood sample was allowed to clot for 1 hour at room temperature and serum was prepared.

Hepatotoxicity Tests

Serum glutamic pyruvic transaminase (SGPT) activity, which has been suggested to be a sensitive index for hepatotoxicity (Balazs et al., 1962), was determined by the method of Reitman and Frankel (1957). A Sigma Chemical

Company reagent kit was used. The results were expressed as SF units/ml serum.

Nephrotoxicity Tests

Blood urea nitrogen (BUN) was determined as described in the Sigma Technical Bulletin No. 640 using Sigma Chemical Company reagents. Renal cortical slice transport capacity for organic anions (p-aminohippurate, PAH) and organic cations (tetraethylammonium, TEA) was determined. For estimation of PAH and TEA uptake, renal cortical slices were prepared (30-100 mg) and placed in 4.0 ml of phosphate buffered (pH 7.4) incubation medium (Cross and Targart, 1950) containing 7.4×10^{-5} M PAH and 1.0×10^{-5} M [^{14}C] TEA. Renal cortical slices were incubated for 90 minutes at 25 C under an atmosphere of 100% oxygen in a Dubnoff metabolic shaker. Following incubation, slices were removed, blotted, weighed and homogenized with 3% trichloroacetic acid (TCA) in a final volume of 10 ml. Two milliliters of medium was treated in a similar manner. After centrifugation, the supernatant was assayed for PAH and TEA. p-Aminohippuric acid (PAH) was determined spectrophotometrically by the method of Smith et al. (1945). Radioactivity of TEA in tissue and medium supernatant was determined by liquid scintillation spectrometry using 1.0 ml aliquots of tissue or medium following addition of 10 ml aqueous counting scintillant (ACS). Accumulation of PAH or TEA was expressed as the slice-to-medium (S/M) ratio, where S represents mg of PAH or TEA per g of tissue and M represents mg of PAH or TEA per ml of medium.

Light Microscopy

In another series of experiments, untreated male C57 and male and female DBA mice were killed by decapitation. Kidneys were quickly removed. For light microscopy, the tissue was sectioned and several samples were immersed in 10% buffered formalin fixative, conventionally processed, and embedded in paraffin. Sections were cut at 5 μm and stained by either hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) stain. Observations were recorded for at least 5 animals in each group.

All glomeruli in 1 section of each kidney were counted and the epithelial cells of the parietal layer of Bowman's capsule were classified as either squamous or cuboidal as described by Crabtree (1941). Ten to fifteen microscopic fields for each kidney were observed, and the percentage of squamous and cuboidal cells was determined.

Transmission Electron Microscopy

For ultrastructural studies, kidneys from each animal were excised. The cortex was cut into pieces of approximately 1 mm^3 and immersed in 4% glutaraldehyde in 0.1 M phosphate buffer. Following a 24-hour fixation period, they were washed in Zetterqvist's wash solution and then postfixed for 2 hours in Zetterqvist's 1% osmium fixative. The tissue was dehydrated in ascending grades of alcohol and cleared in propylene oxide for 2 hours. Tissues were embedded in a mixture of Epon-Araldite. One micron sections were cut; selected areas for tissue orientation by light

microscopy were stained with toluidine blue. Selected areas for further examination were sectioned (approximately 900 Å). Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 9 electron microscope.

Cytochemistry

For study of cytochrome P-450 in microsomal reactions, fresh cryostat sections of unfixed liver and kidney tissue of male C57 and male DBA mice were prepared and incubated in the reaction medium for 10 minutes at 37 C. Following incubation, slides were washed with distilled water and mounted in Farran's medium (Chayen et al., 1972). To determine the effect of the inhibition of microsomal enzyme activities, piperonyl butoxide or SKF 525-A was added to the reaction medium.

The reaction medium included the following: Tris buffer (pH 7.8) was prepared; 0.3 g of nitroblue tetrazolium (NBT) was added to 100 ml of the buffer. Twenty grams of polyvinyl alcohol (PVC) was also added to the Tris buffer to inhibit diffusion of reaction products. NADPH2 (5 mg/ml) was added to the PVC-buffered solution. To test the effect of piperonyl butoxide or SKF 525-A on hepatic and renal microsomal activities, these compounds were added into the incubation medium (pH 7.8).

Statistical Analyses

Data were expressed as mean ± standard error. The results were analyzed by analysis of variance in a completely randomized design. Treatment differences were identified by the method of least significant difference (Steel and Torrie, 1965). The 0.05 level of probability was used as the criterion of significance.

RESULTS

Effect of CHC13 or CDC13 on Body Weight, Liver Weight to Body Weight, and Kidney Weight to Body Weight in Male ICR, C57, Male and Female DBA Mice: Effect of BNF, PB or PBB on These Parameters

Body weight (BW) was not affected by treatment of animals with CHC13 or CDC13 (male ICR mice). Similarly, BW did not change in male C57 or male DBA mice treated with BNF, PB or PBB alone or following administration of CHC13. Liver weight to body weight ratio (LW/BW) was not significantly affected by treatment of male ICR mice with CHC13 or CDC13 (Table 1). Treatment of male C57 and male and female DBA mice with CHC13 did not alter LW/BW ratio (Tables 2 and 4). However, these ratios were significantly increased in PB- and PBB-treated animals. Liver weight to body weight ratio increased in male C57 but not in male DBA mice following BNF treatment (Table 4). Administration of CHC13 following these inducer treatments did not further increase LW/BW ratios (Table 4).

The kidney weight to body weight ratio (KW/BW) increased in male ICR mice after administration of CHC13 or CDC13 (Table 1). The ratio of kidney weight to body weight was not affected in male C57 and female DBA mice following exposure to CHC13 (Table 3). In contrast, these ratios

Table 1. Effect of a single dose of CHC13 or CDC13 on liver weight to body weight and kidney weight to body weight ratio[a]

Solvent	Dosage (ml/kg)	$\frac{\text{Liver Weight}}{\text{Body Weight}} \times 100$	$\frac{\text{Kidney Weight}}{\text{Body Weight}} \times 100$
---	0(corn oil)	6.14 ± 0.18	1.44 ± 0.01
CDC13	0.10	5.37 ± 0.29	1.53 ± 0.04[b]
CHC13	0.10	6.23 ± 0.13	1.61 ± 0.04[b]
CDC13	0.25	6.25 ± 0.16	1.48 ± 0.03[b]
CHC13	0.25	5.88 ± 0.11	1.62 ± 0.04[b]
CDC13	0.50	5.88 ± 0.14	1.90 ± 0.08[b,c]
CHC13	0.50	5.72 ± 0.19	2.32 ± 0.14[b]
CDC13	0.75	5.99 ± 0.30	1.80 ± 0.08[b]
CHC13	0.75	6.74 ± 0.22	1.87 ± 0.14[b]
CDC13	1.00	6.21 ± 0.42	2.26 ± 0.13[b]
CHC13	1.00	6.33 ± 0.30	2.34 ± 0.12[b]

[a]Mice received a single injection of various doses of CHC13 or CDC13 dissolved in corn oil and were killed 24 hours later. Control animals received corn oil alone. Each number represents the mean ± S.E. of five or more animals.

[b]Significantly different from mice receiving corn oil only ($P<0.05$).

[c]Significantly different from mice receiving the same dose of CHC13 ($P<0.05$).

Table 2. Effect of a single dose of CHC13 on ratio of liver weight to body weight (LW/BW)

Sex	Strain	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW X100	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100
M	C57	0	4.91 ±0.21	0.05	5.38 ±0.03	0.1	5.14 ±0.21	0.25	4.69 ±0.10	0.5	5.26 ±0.29
M	DBA	0	5.01 ±0.28	0.05	4.83 ±0.27	0.1	4.84 ±0.21	0.25	4.68 ±0.10	0.5	4.80 ±0.24
F	DBA	0	5.35 ±0.37	0.05	5.38 ±0.17	0.1	5.91 ±0.19	0.25	5.33 ±0.45	0.5	5.29 ±0.05

Male C57 and male and female DBA mice received a single injection of various doses of CHC13 dissolved in peanut oil and were killed 24 hours later. Control animals received peanut oil alone. Each value represents the mean ± S.E. for five or more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

Table 3. Effect of a single dose of CHC13 on ratio of kidney weight to body weight (KW/BW)

Sex	Strain	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100
M	C57	0	1.03 ±0.02	0.05	1.10 ±0.01	0.1	1.12 ±0.24	0.25	1.15 ±0.02	0.5	1.35 ±0.03
M	DBA	0	1.32 ±0.06	0.05	1.47 ±0.05	0.1	1.80 ±0.14*	0.25	1.92 ±0.13*	0.5	2.02 ±0.17*
F	DBA	0	1.36 ±0.07	0.05	1.49 ±0.06	0.1	1.52 ±0.09	0.25	1.35 ±0.07	0.5	1.28 ±0.05

Male C57 and male and female DBA mice received a single injection of various doses of CHC13 dissolved in peanut oil and were killed 24 hours later. Control animals received peanut oil alone. Each value represents the mean ± S.E. for five or more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

Table 4. Effect of CHC13 and inducers of MFOs on liver weight to body weight (LW/BW) ratio

Strain	Treatment	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100	CHC13 (mg/kg)	LW/BW x100
C57	none	0	4.73 ±0.06	0.025	4.70 ±0.07	0.05	4.55 ±0.05	0.1	4.47 ±0.10	0.25	4.51 ±0.15
DBA	none	0	4.92 ±0.12	0.025	5.06 ±0.13	0.05	5.20 ±0.17	0.1	4.45 ±0.04	0.25	4.59 ±0.08
C57	BNF	0	5.61 ±0.14*	0.025	5.34 ±0.05	0.05	5.19 ±0.20	0.1	4.85 ±0.14	0.25	4.95 ±0.04
DBA	BNF	0	5.37 ±0.24	0.025	5.29 ±0.15	0.05	5.16 ±0.32	0.1	4.81 ±0.24	0.25	4.61 ±0.17
C57	PB	0	5.68 ±0.41*	0.025	---	---	5.35 ±0.13*	0.1	6.19 ±0.28*	0.25	6.15 ±0.18*
DBA	PB	0	5.41 ±0.14*	0.025	---	---	5.29 ±0.18	0.1	5.48 ±0.19*	0.25	5.88 ±0.42*
C57	PBB	0	11.07 ±0.73*	0.025	12.77 ±0.30*	0.05	11.75 ±0.54*	0.1	10.25 ±0.50*	0.25	10.43 ±0.29*
DBA	PBB	0	6.82 ±0.33*	0.025	6.67 ±0.03*	0.05	6.99 ±0.11*	0.1	6.07 ±0.40*	0.25	6.03 ±0.28*

Control male C57 and DBA or animals pretreated with BNF, PB or PBB were challenged with a single IP injection of CHC13. Mice were killed 24 hours later. Each value represents the mean ± S.E. of five or more animals.

*Significantly different from untreated mice receiving same dose of CHC13 ($P<0.05$).

significantly increased in male DBA mice following CHC13 administration (Table 5). Pretreatment with BNF, PB or PBB had no effect on KW/BW ratio following CHC13 administration (Table 5).

Hepatotoxicity of CHC13 or CDC13 in Male ICR, C57 Male and Female DBA Mice: Effect of BNF, PB or PBB on Hepatotoxicity of CHC13 in Male C57 and DBA Mice

A dose-related increase in SGPT activity was observed in male ICR mice following the exposure of animals to CHC13 or CDC13 (Figure 1). Chloroform elevated SGPT more than CDC13. For instance, at 0.75 and 1.0 ml/kg, CHC13 increased SGPT activity 28- and 84-fold above the control, respectively, while CDC13 increased SGPT only 14- and 35-fold (Figure 1). A dose-dependent relation in SGPT activity was also observed in male C57 and male and female DBA mice after treatment with CHC13 (Figures 5 and 9). Interestingly, CHC13 produced the same degree of liver damage in male C57 and male and female DBA mice (Figures 5 and 9). Treatment with BNF, PB or PBB alone did not affect SGPT activity in either strain of male mice. However, an elevation of SGPT was observed in BNF-treated male C57 mice following administration of CHC13 (Figure 9). In contrast, administration of CHC13 did not alter SGPT activity in BNF-treated male DBA mice (Figure 9). Administration of CHC13 in PB- or PBB-treated male C57 and male DBA mice markedly increased SGPT activity (Figure 9). However, SGPT activity was considerably higher in PBB-treated male C57 mice compared to SGPT activity in PBB-treated male DBA mice following exposure to CHC13 (Figure 9).

Table 5. Effect of CHC13 and inducers of MFOs on kidney weight to body weight (KW/BW) ratio

Sex	Strain	Treat-ment	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100	CHC13 (mg/kg)	KW/BW x100
M	C57	none	0	1.17 ±0.01	0.025	1.14 ±0.01	0.05	1.21 ±0.02	0.1	1.23 ±0.05	0.25	1.33 ±0.02
M	DBA	none	0	1.47 ±0.03	0.025	1.68 ±0.14	0.05	1.82 ±0.08	0.1	1.96 ±0.02	0.25	1.98 ±0.03
M	C57	BNF	0	1.21 ±0.02	0.025	1.23 ±0.04	0.05	1.26 ±0.04	0.1	1.35 ±0.02	0.25	1.43 ±0.13
M	DBA	BNF	0	1.52 ±0.08	0.025	1.49 ±0.09	0.05	1.86 ±0.09	0.1	1.80 ±0.11	0.25	2.09 ±0.15
M	C57	PB	0	1.14 ±0.03	0.025	1.10 ±0.03	0.05	---	0.1	1.12 ±0.04	0.25	1.15 ±0.02
M	DBA	PB	0	1.34 ±0.03	0.025	1.36 ±0.14	0.05	---	0.1	1.68 ±0.05	0.25	1.84 ±0.05
M	C57	PBB	0	1.20 ±0.02	0.025	1.25 ±0.04	0.05	1.26 ±0.04	0.1	1.35 ±0.02	0.25	1.43 ±0.13
M	DBA	PBB	0	1.58 ±0.05	0.025	1.73 ±0.13	0.05	1.66 ±0.15	0.1	1.88 ±0.07	0.25	1.98 ±0.07

Control male C57 and male DBA or pretreatment animals with BNF, PB or PBB for varying periods of time were challenged with single IP injections of various doses of CHC13. Mice were killed 24 hours later. Each value represents the mean ± S.E. of five or more animals. *Significantly different from the untreated mice receiving the same dose of CHC13 ($P<0.05$).

Figure 1. Effect of chloroform (CHC13) and deuterium labelled chloroform (CDC13) on serum glutamic pyruvic transaminase (SGPT) activity. Male ICR mice were challenged with a single IP injection of CHC13, CDC13 or corn oil. Animals were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving the same dose of CDC13 ($P<0.05$).

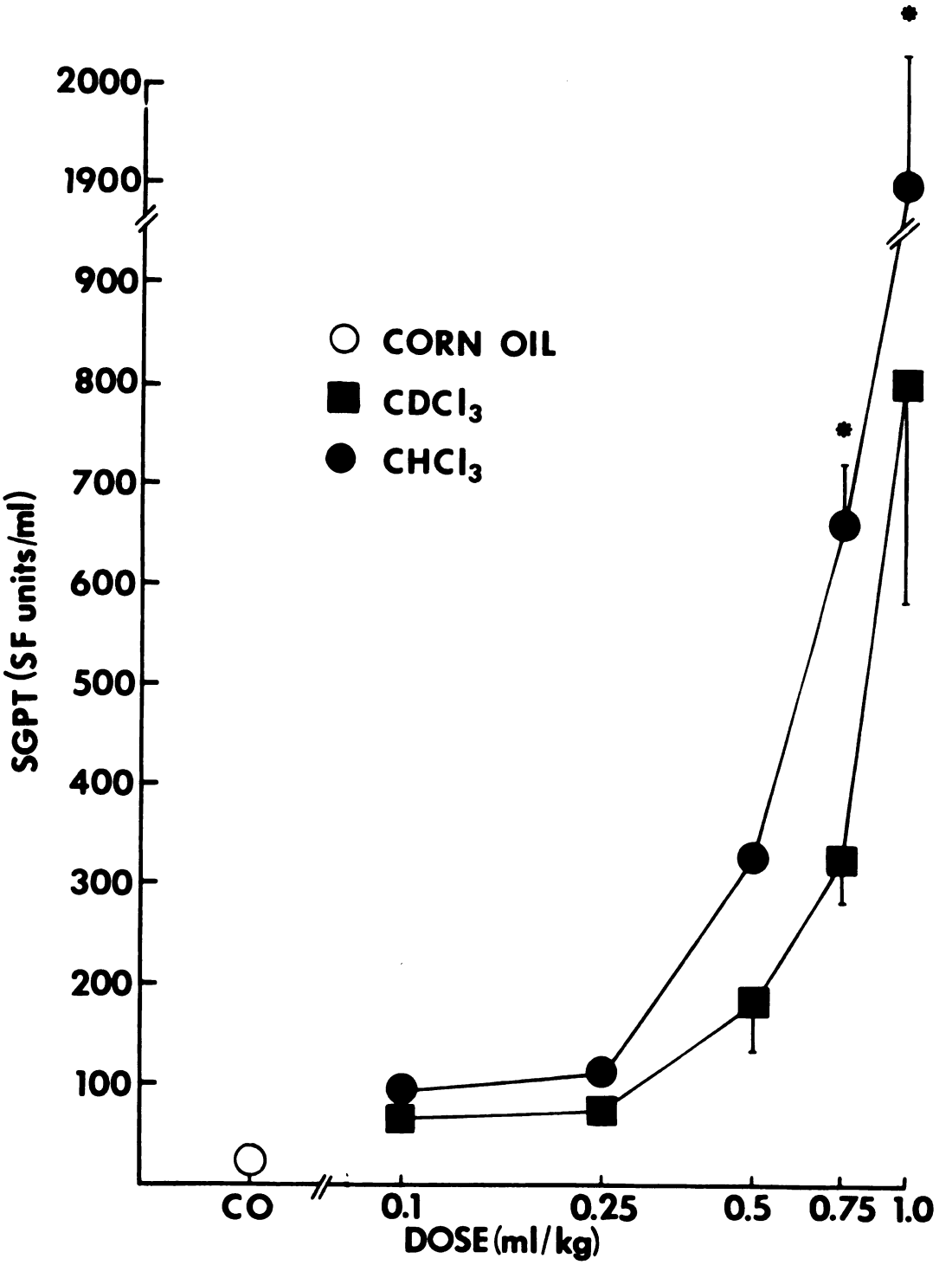
2000
1900
900
800
700
600
500
400
300
200
100
SGPT (SF units/ml)
CORN OIL
CDCl3
CHCl3
*
*
CO
0.1
0.25
0.5
0.75
1.0
DOSE (ml/kg)

Figure 1

Nephrotoxicity of CHC13 or CDC13 in Male ICR, C57 Male and Female DBA Mice: Effect of BNF, PB or PBB on Nephrotoxicity of CHC13 in Male C57 and DBA Mice

Twenty-four hours after a single administration of CHC13 or CDC13, accumulation of PAH and TEA into renal cortical slices *in vitro* was depressed in a dose-dependent manner (Figures 2, 3, 6, 7, 10 and 11). The effect was considerably greater in kidneys from male ICR mice treated with CHC13 than in those treated with CDC13 (Figures 2 and 3). Administration of an equivalent dose of CHC13 reduced PAH and TEA S/M ratios to a greater extent in male DBA mice than in male C57 mice (Figures 6, 7, 10 and 11). In contrast, administration of CHC13 in female DBA mice had no effect on PAH and TEA S/M ratios (Figures 6 and 7). Treatment of male C57 and male DBA mice with BNF, PB or PBB alone did not affect these ratios (Figures 10 and 11). Neither BNF nor PB affected PAH and TEA S/M ratios following CHC13 administration in male C57 or male DBA mice (Figures 10 and 11). However, dietary PBB caused considerable depression in PAH and TEA S/M ratios following CHC13 administration in male C57 mice. In contrast, male DBA mice receiving PBB failed to alter PAH and TEA S/M ratio following exposure to CHC13 (Figures 10 and 11).

A dose-dependent increase in BUN was found after exposure of male ICR, C57 and DBA mice to CHC13 or CDC13 (male ICR mice). In contrast, administration of CHC13 in female DBA mice had no effect on BUN content (Figures 4, 8 and 12). Blood urea nitrogen increased following treatment

Figure 2. Effect of chloroform (CHC13) and deuterium labelled chloroform (CDC13) on p-amminohippurate (PAH) accumulation (S/M ratio) by renal cortical slices. Male ICR mice were challenged with a single IP injection of CHC13, CDC13 or corn oil. Animals were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving the same dose of CDC13 ($P<0.05$).

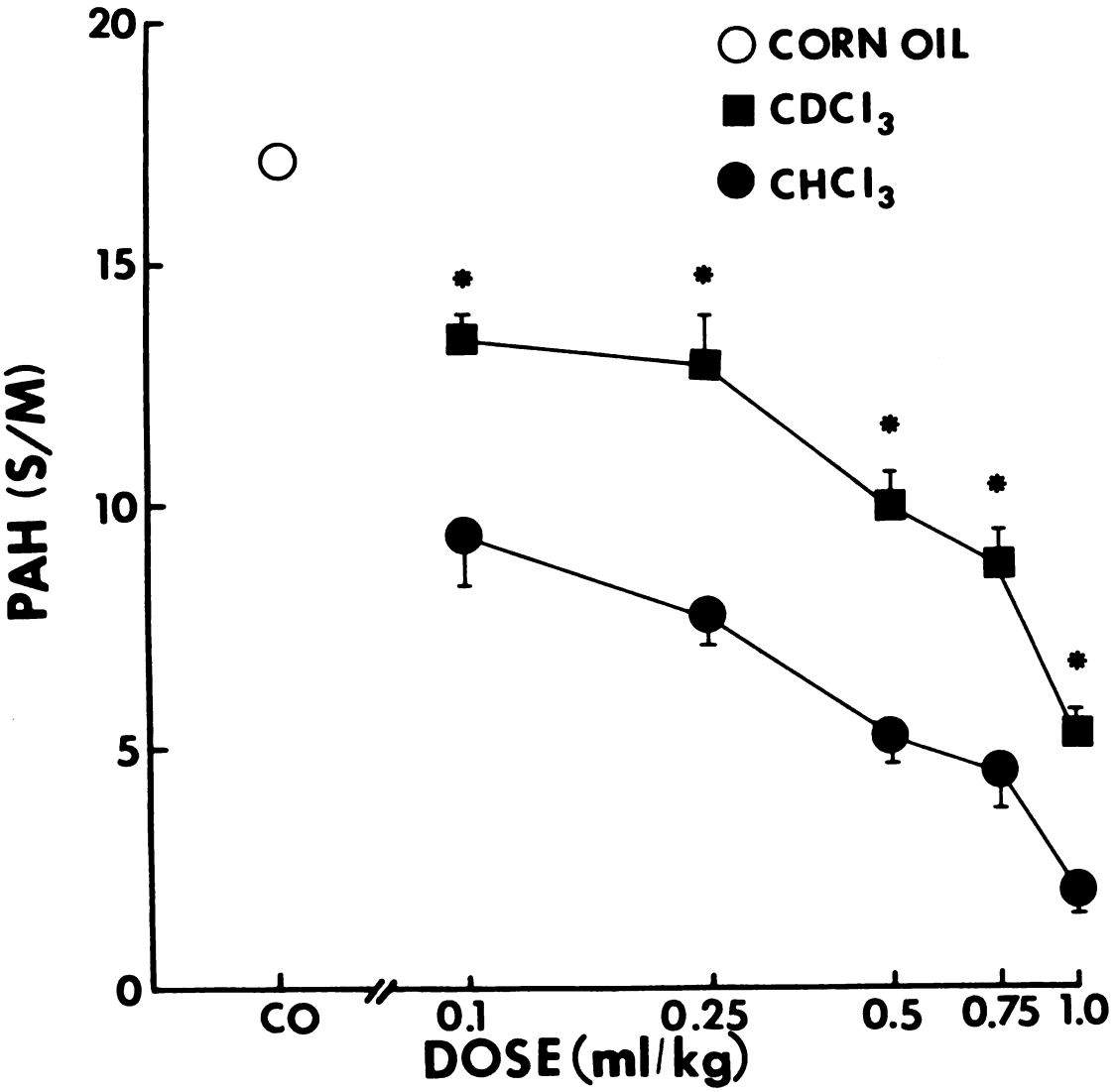
20
15
10
5
0
PAH (S/M)
CORN OIL
CDCl3
CHCl3
*
*
*
*
*
CO
0.1
0.25
0.5
0.75
1.0
DOSE (ml/kg)

Figure 2

Figure 3. Effect of chloroform (CHC13) and deuterium labelled chloroform (CDC13) on tetraethylammonium (TEA) accumulation (S/M) by renal cortical slices. Male ICR mice were challenged with a single IP injection of CHC13, CDC13 or corn oil. Animals were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving the same dose of CDC13 ($P<0.05$).

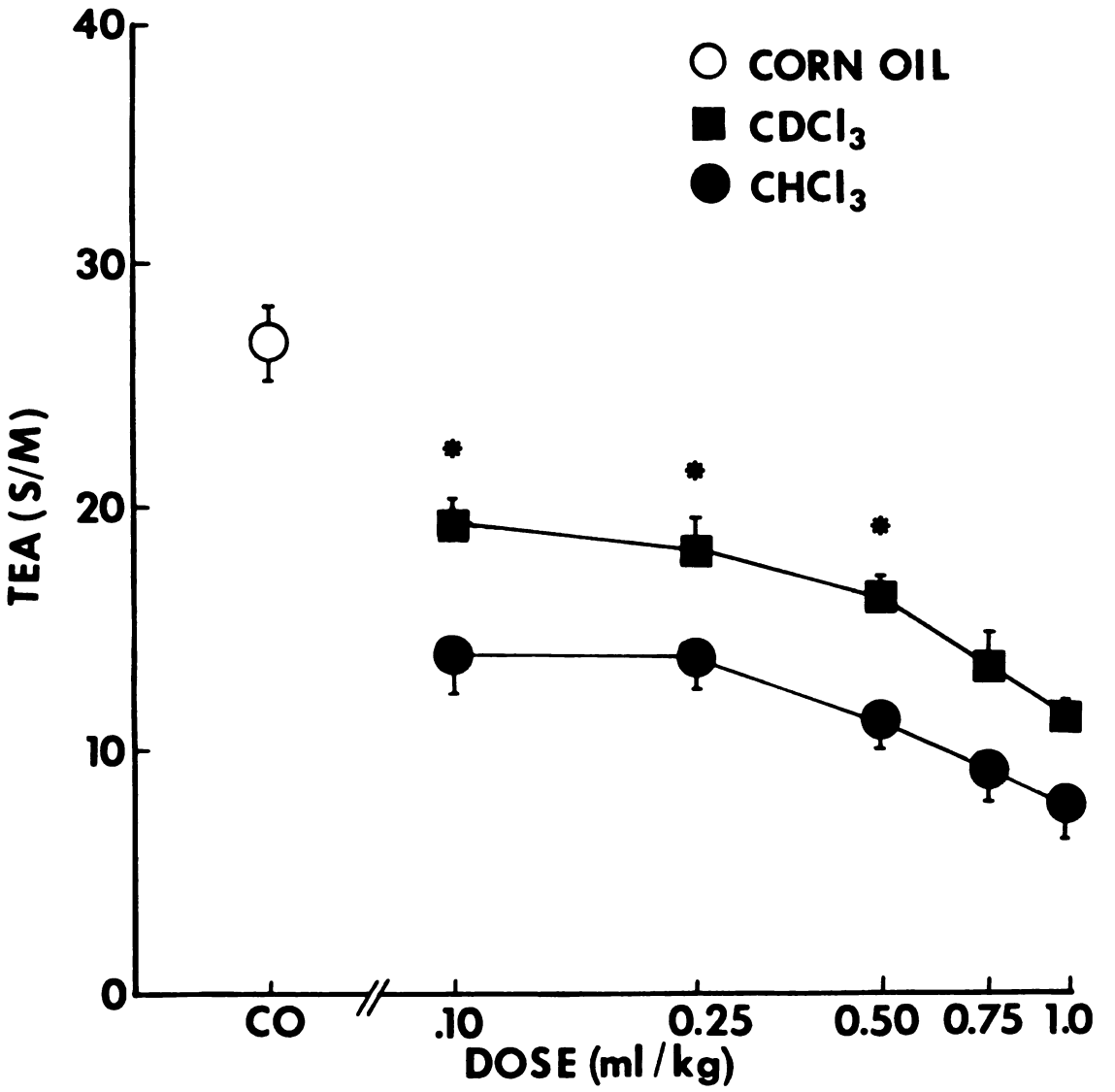
40
30
20
10
0
TEA (S/M)
CORN OIL
CDCl3
CHCl3
CO
.10
0.25
0.50
0.75
1.0
DOSE (ml / kg)

Figure 3

Figure 4. Effect of chloroform (CHC13) and deuterium labelled chloroform (CDC13) on blood urea nitrogen (BUN). Male ICR mice were challenged with a single IP injection of CHC13, CDC13 or corn oil. Animals were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving the same dose of CDC13 ($P<0.05$).

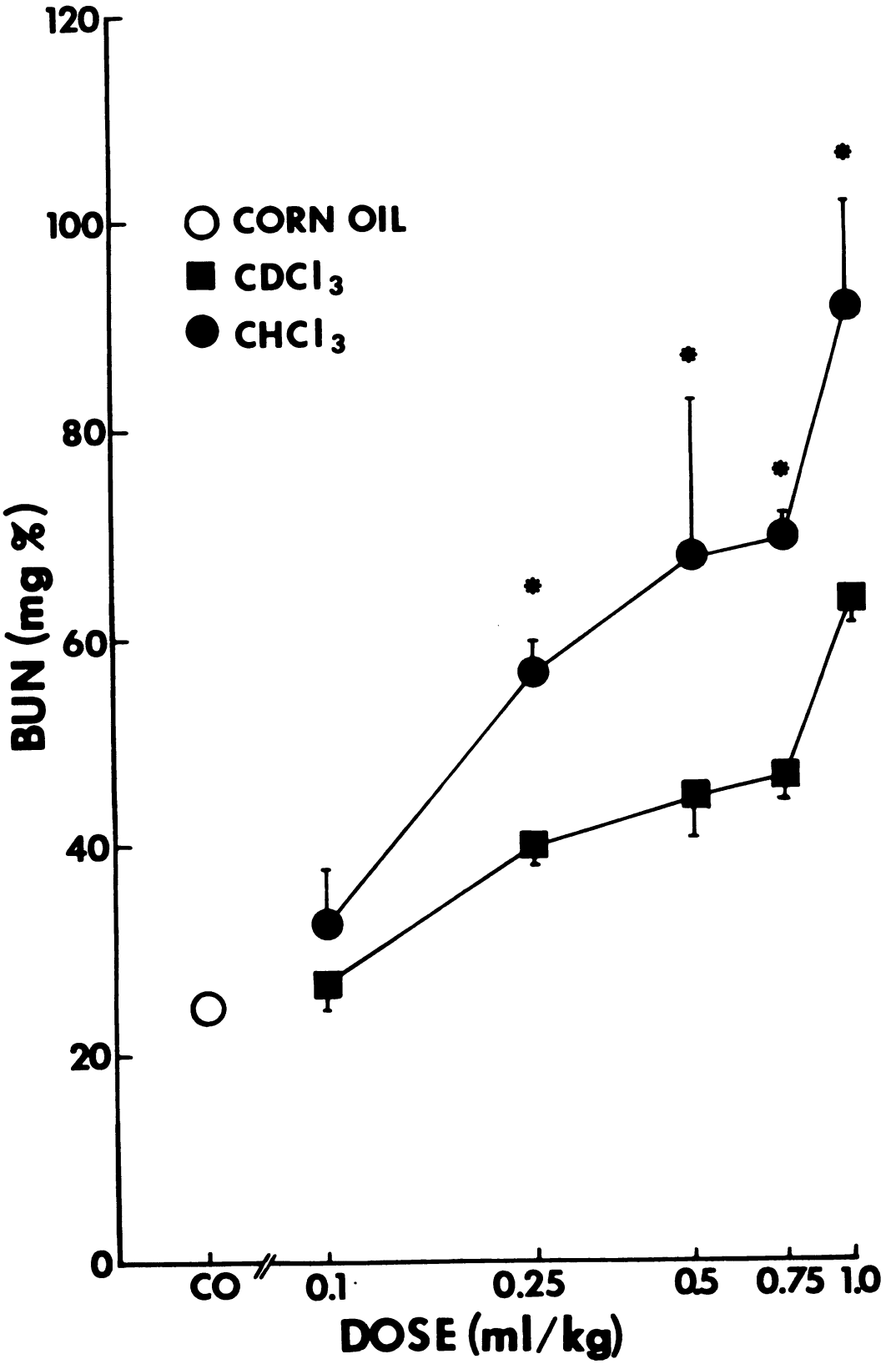
120
100
80
60
40
20
0
BUN (mg %)
CORN OIL
CDCl3
CHCl3
CO
0.1
0.25
0.5
0.75
1.0
DOSE (ml/kg)

Figure 4

Figure 5. Effect of chloroform (CHCl3) on serum glutamic pyruvate transaminase (SGPT) activity. Male C57 and male and female DBA mice were challenged with a single IP injection of various doses of CHCl3 dissolved in peanut oil and were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

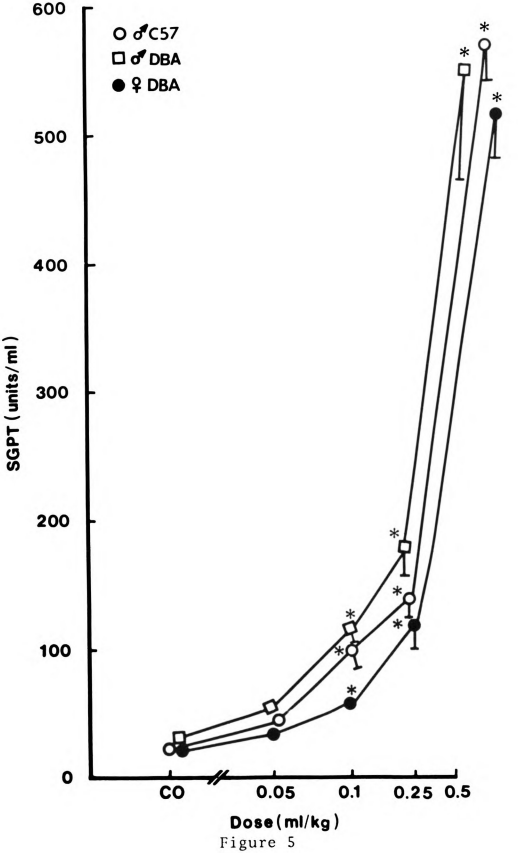
600
500
400
300
200
100
0
SGPT (units/ml)
○ ♂C57
□ ♂DBA
● ♀DBA
CO
0.05
0.1
0.25
0.5
Dose(ml/kg)

Figure 5

Figure 6. Effect of chloroform (CHCl3) on p-ammino-hippurate (PAH) accumulation (S/M ratio) by renal cortical slices. Male C57 and male and female DBA mice were challenged with a single IP injection of various doses of CHCl3 dissolved in peanut oil and were killed 24 hours later. Control animals received peanut oil alone. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

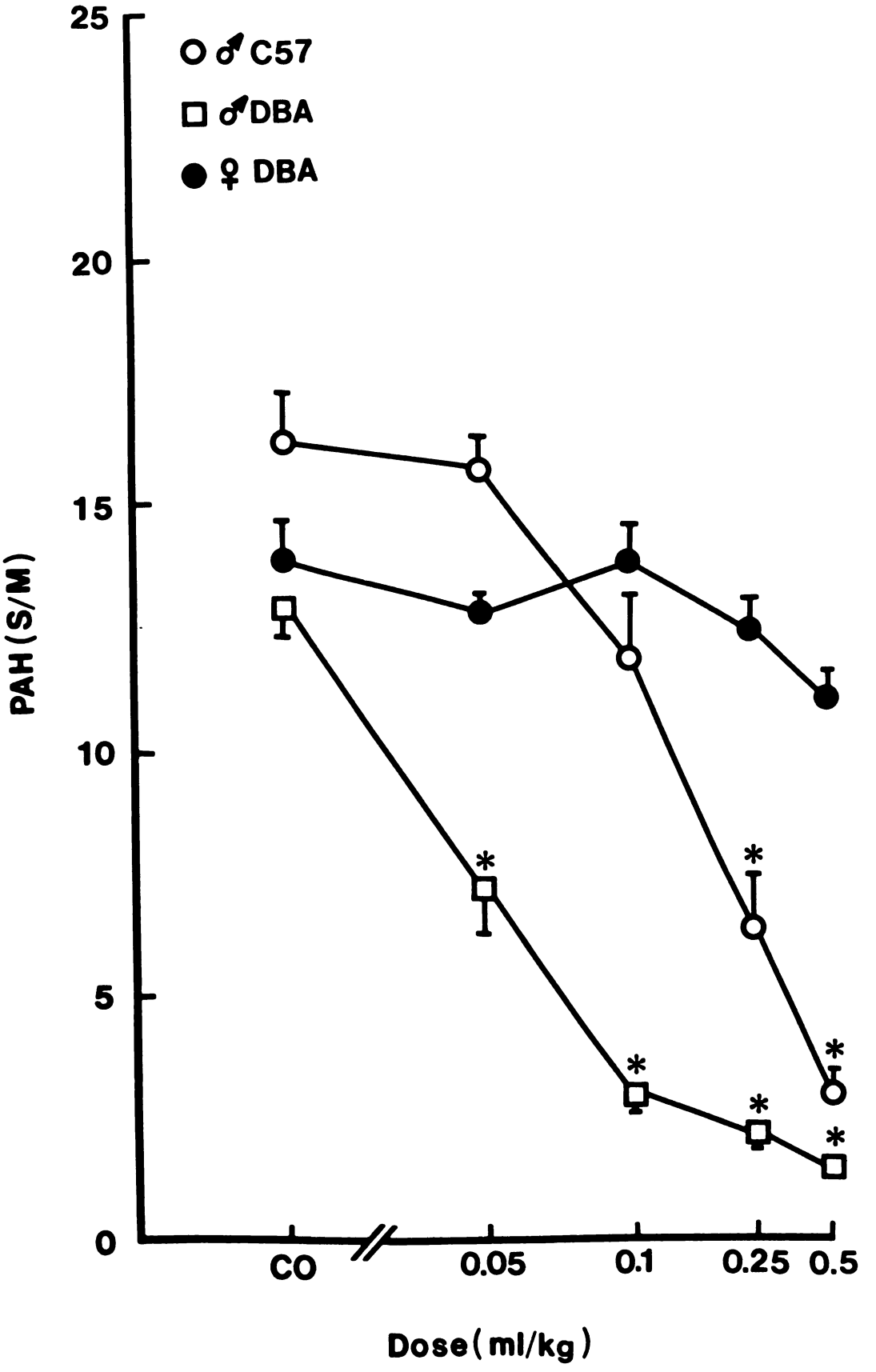

Figure 6

Figure 7. Effect of chloroform (CHCl3) on tetraethylammonium (TEA) accumulation (S/M ratio) by renal cortical slices. Male C57 and male and female DBA mice were challenged with a single IP injection of various doses of CHCl3 dissolved in peanut oil and were killed 24 hours later. Control animals received peanut oil alone. Each point and vertical bar represents the mean ± S.E. of five ore more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

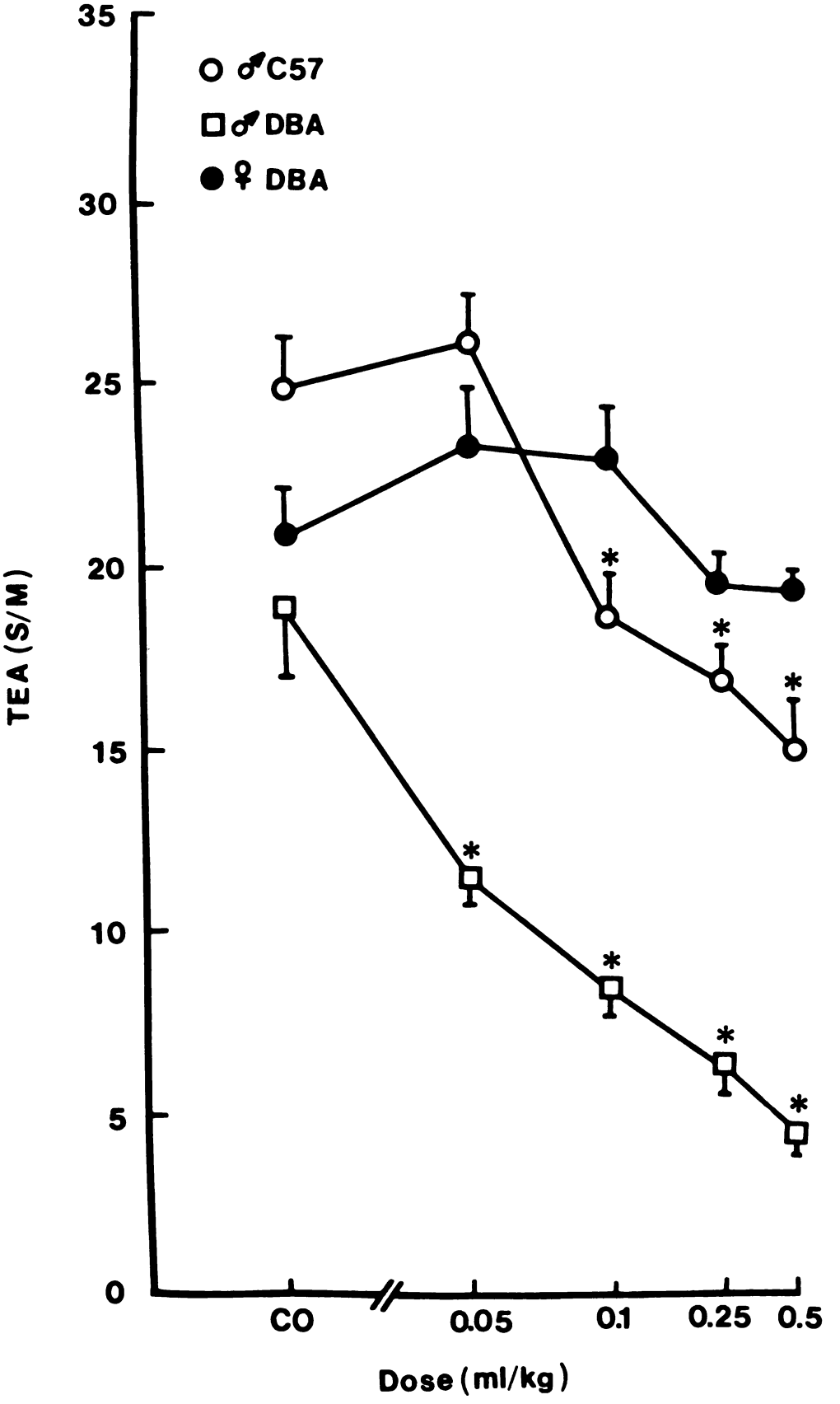

Figure 7

Figure 8. Effect of chloroform (CHC13) on blood urea nitrogen (BUN). Male C57 and male and female DBA mice were challenged with a single IP injection of various doses of CHC13 dissolved in peanut oil and were killed 24 hours later. Control animals received peanut oil alone. Each point and vertical bar represents the mean ± S.E. of five or more animals.

*Significantly different from mice receiving peanut oil only ($P<0.05$).

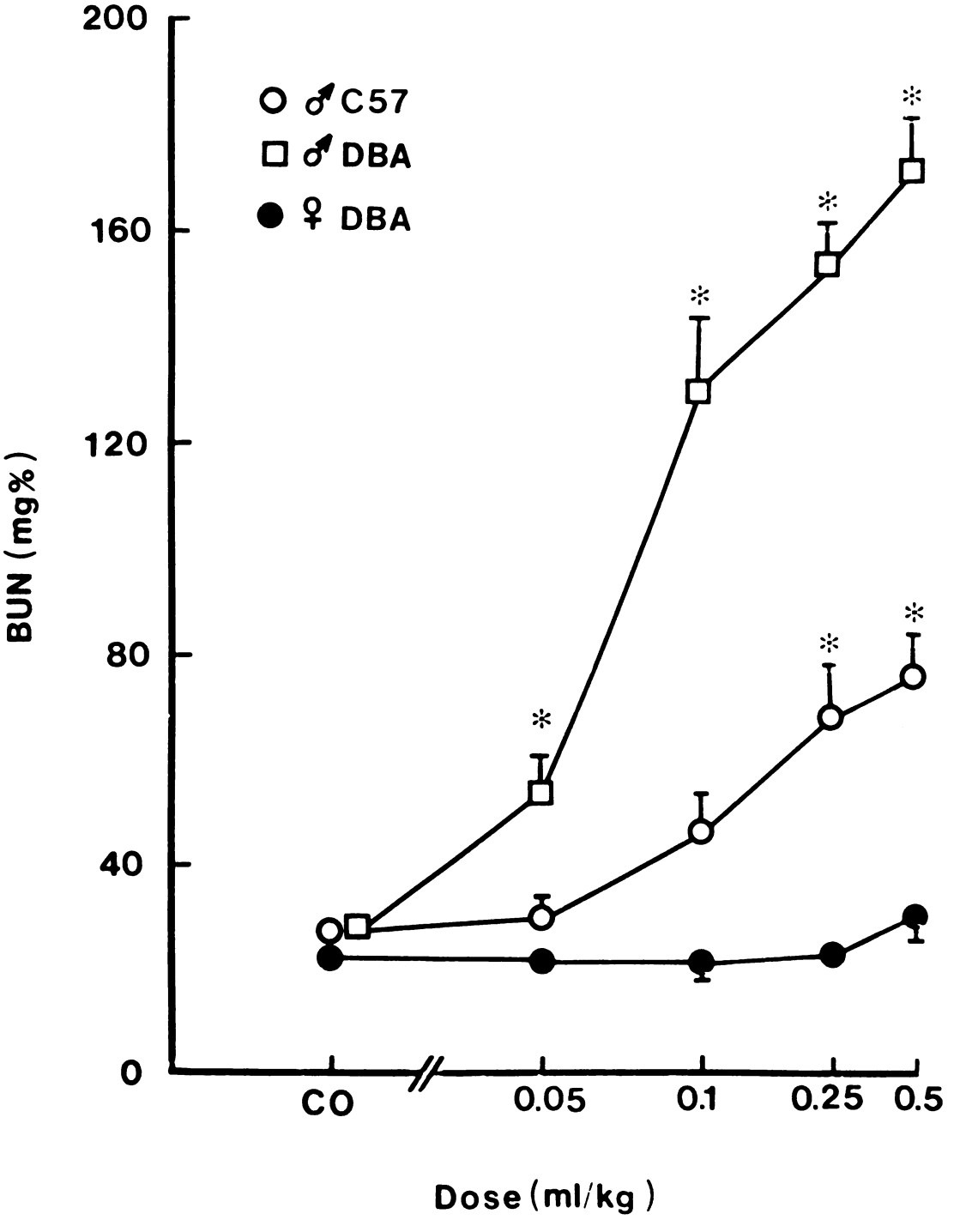

Figure 8

Figure 9. Effect of chloroform (CHC13) and inducer of MFOs on serum glutamic pyruvic transaminase (SGPT). Control male C57 and DBA mice or pretreated animals with BNF, PB or PBB for varying periods of time were challenged with a single IP injection of CHC13. Mice were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals; SGPT activity was expressed as percentage of control male C57 or DBA mice.

*Significantly different from mice receiving the same dose of CHC13 ($P<0.05$).

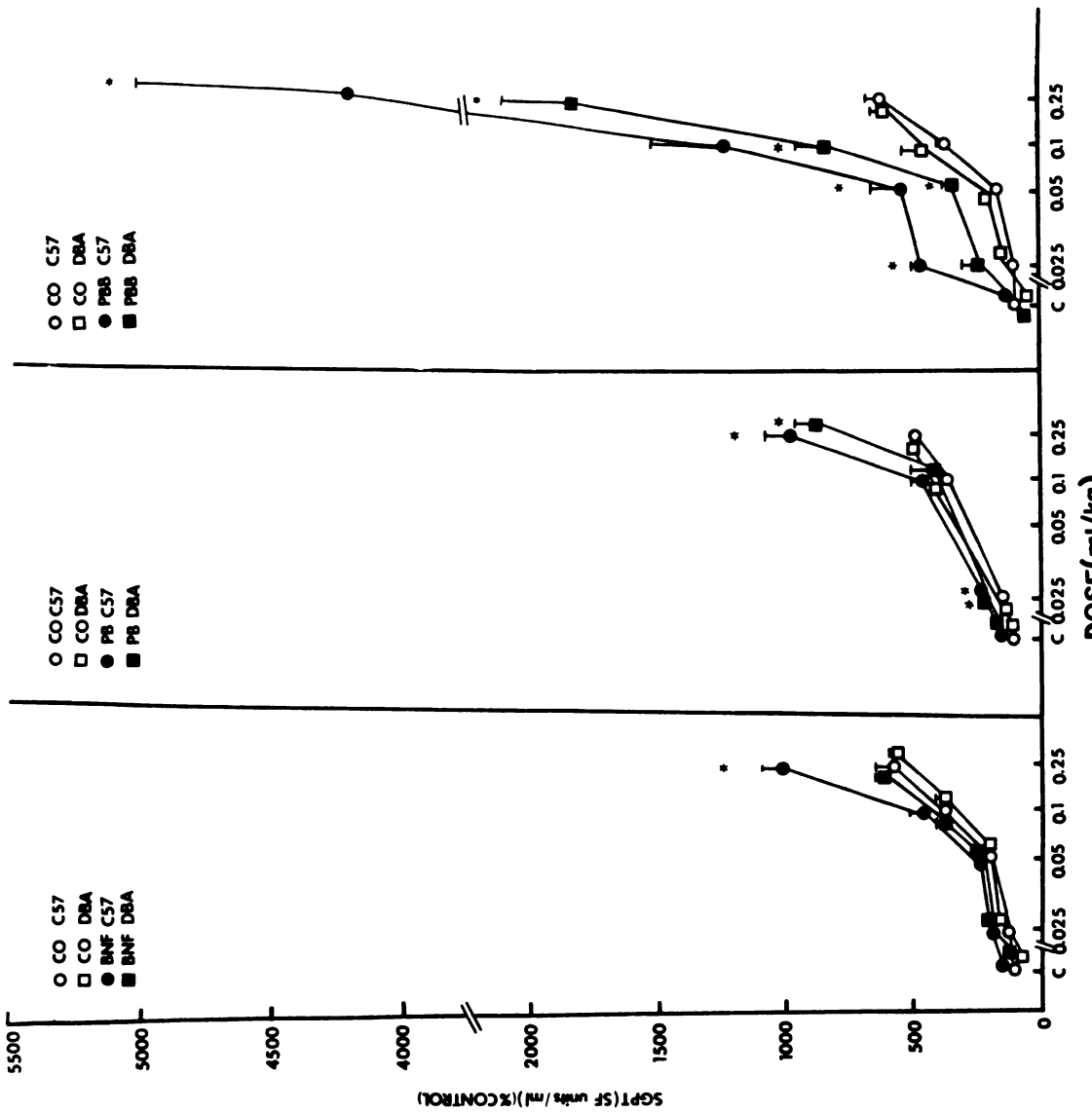
SGPT(SF units/ml)(%CONTROL)
5500
5000
4500
4000
2000
1500
1000
500
0
C 0.025 0.05 0.1 0.25
DOSE(ml/kg)
O CO C57
□ CO DBA
● BNF C57
■ BNF DBA
O CO C57
□ CO DBA
● PB C57
■ PB DBA
O CO C57
□ CO DBA
● PB C57
■ PB DBA

Figure 9

Figure 10. Effect of chloroform (CHCl3) and inducer of MFOs on p-amminohippurate (PAH) accumulation (S/M ratio) by renal cortical slices. Control male C57 and DBA mice and pretreated animals with BNF, PB or PBB for varying periods of time were challenged with a single IP injection of CHCl3. Mice were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals; PAH was expressed as percentage of control male C57 or DBA mice.

*Significantly different from mice receiving the same dose of CHCl3 ($P<0.05$).

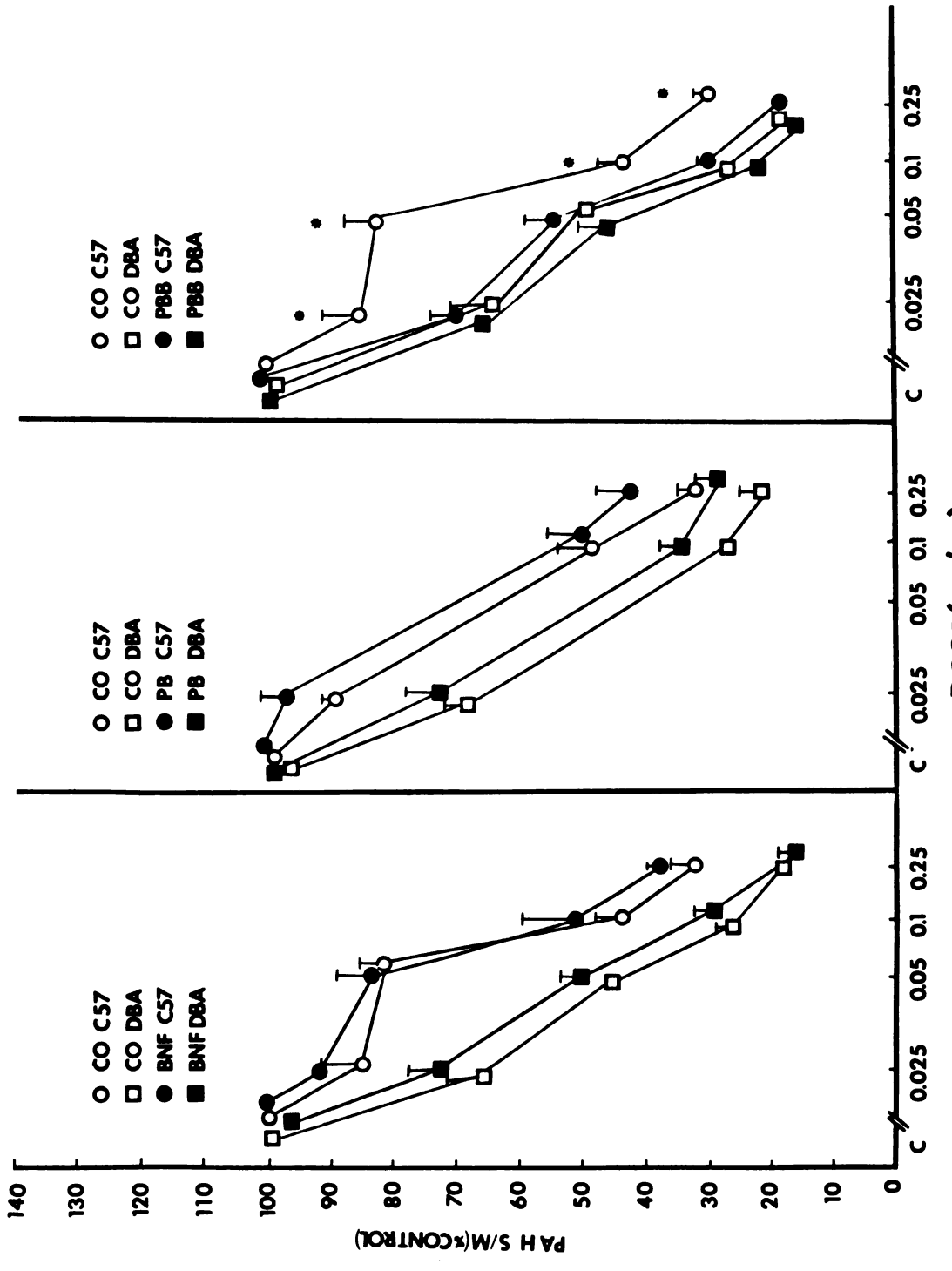
PAH S/M(%CONTROL)
DOSE(ml/kg)
CO C57
CO DBA
BNF C57
BNF DBA
PB C57
PB DBA
PBB C57
PBB DBA
C
0.025
0.05
0.1
0.25

Figure 10

Figure 11. Effect of chloroform (CHC13) and inducer of MFOs on tetraethylammonium (TEA) accumulation (S/M) by renal cortical slices. Control male C57 and DBA mice and pretreated animals with BNF, PB or PBB for varying periods of time were challenged with a single IP injection of CHC13. Mice were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals; TEA S/M ratio was expressed as percentage of control male C57 or DBA mice.

*Significantly different from mice receiving the same dose of CHC13 ($P<0.05$).

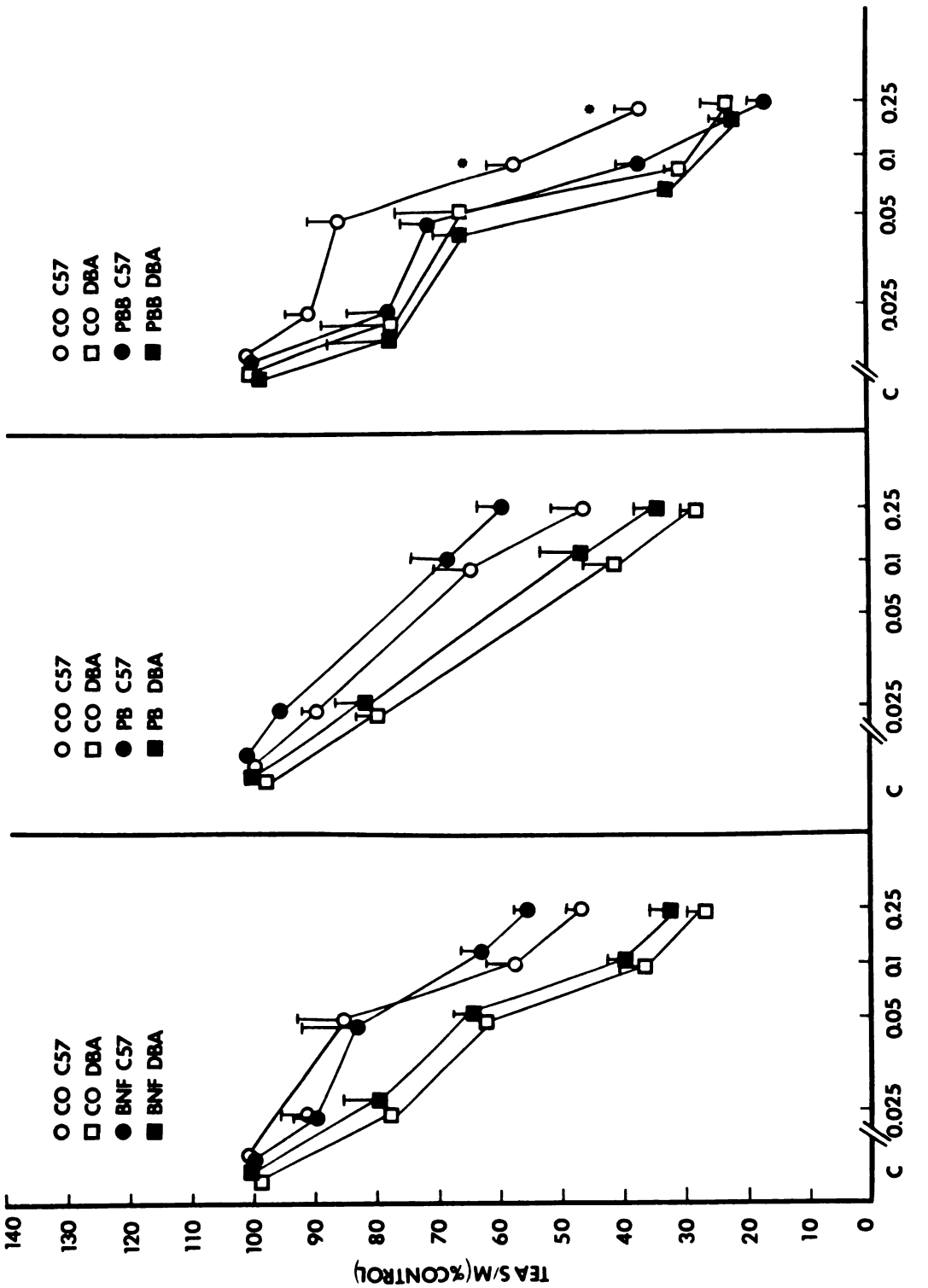
TEA S/M(%CONTROL)
DOSE(ml/kg)
O CO C57
□ CO DBA
● BNF C57
■ BNF DBA
O CO C57
□ CO DBA
● PB C57
■ PB DBA
O CO C57
□ CO DBA
● PBB C57
■ PBB DBA

Figure 11

Figure 12. Effect of chloroform (CHC13) and inducer of MFOs on blood urea nitrogen (BUN). Control male C57 or DBA mice and pretreated animals with BNF, PB or PBB for varying periods of time were challenged with a single IP injection of CHC13. Mice were killed 24 hours later. Each point and vertical bar represents the mean ± S.E. of five or more animals; BUN was expressed as percentage of control male C57 or DBA mice.

*Significantly different from mice receiving the same dose of CHC13 ($P<0.05$).

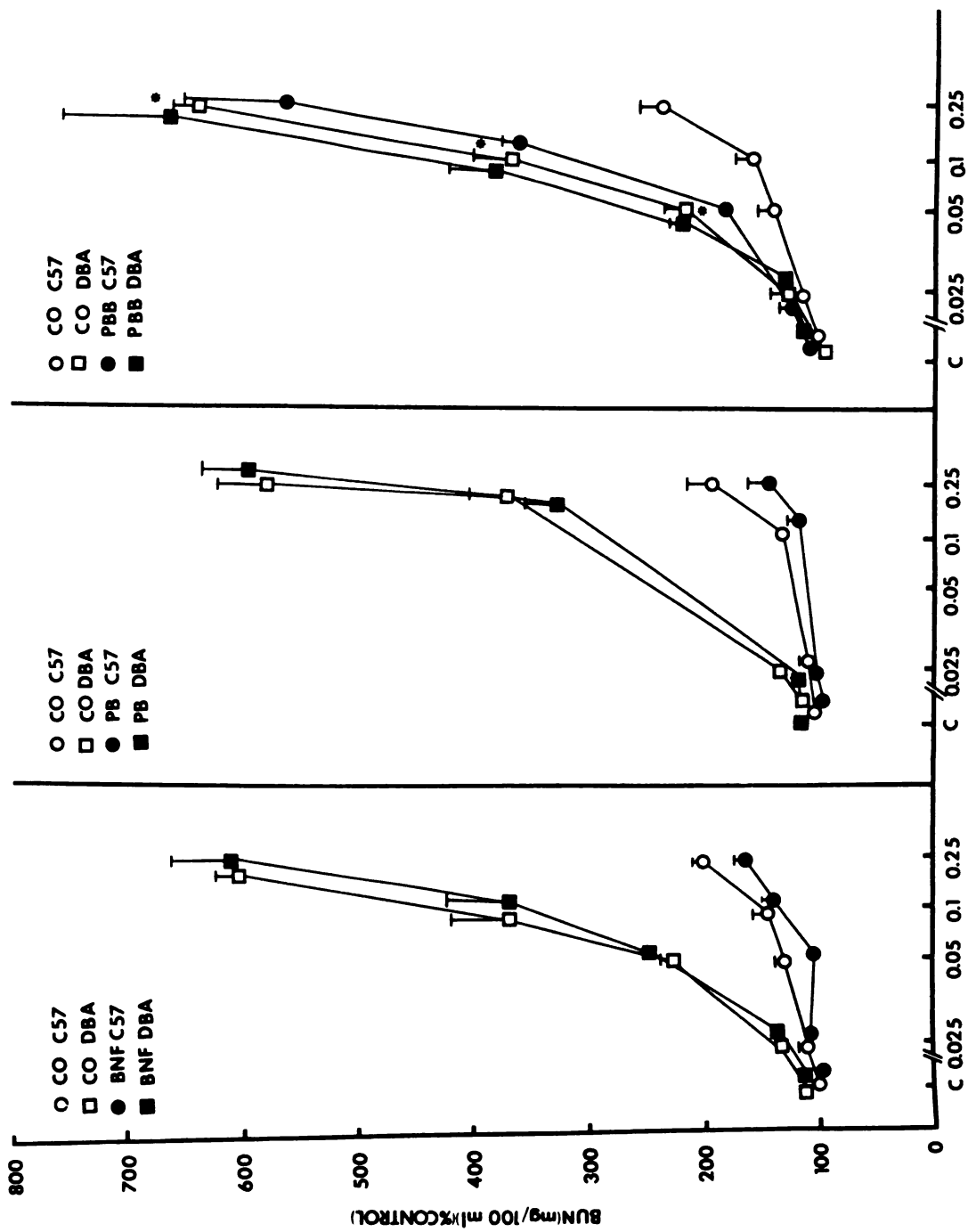
BUN(mg/100 ml)x%CONTROL)
800
700
600
500
400
300
200
100
0
C 0.025 0.05 0.1 0.25
DOSE (ml/kg)
CO C57
CO DBA
BNF C57
BNF DBA
PB C57
PB DBA
PBB C57
PBB DBA

Figure 12

of male ICR mice with CDC13 to a lesser extent than with CHC13 (Figure 4). However, BUN was considerably higher in male DBA mice when compared to that in male C57 mice following injection of CHC13. Pretreatment with BNF, PB or PBB alone did not alter BUN (Figure 12). Neither BNF nor PB altered BUN in male C57 or male DBA mice following administration of CHC13. However, pretreatment with PBB increased BUN in male C57 mice to a greater extent than nontreated animals following CHC13 exposure. In contrast, BUN was not affected by administration of CHC13 in male DBA mice pretreated with PBB (Figure 12).

Effect of BNF, PB or PBB on Hepatic Microsomal Activities in Male C57 and Male DBA Mice

Hepatic arylhydrocarbon hydroxylase (AHH) activity was significantly higher in male C57 mice compared to AHH activity in male DBA mice (Table 6). Hepatic AHH activity was not affected in male DBA mice treated with BNF (Figure 13). In contrast, BNF significantly increased hepatic AHH activity in male C57 mice (Figure 13). Stimulation of hepatic AHH was observed following pretreatment of both strains of male mice with PB or PBB (Figure 13). However, the degree of inducibility was considerably higher in male C57 than in male DBA mice following pretreatment of animals with dietary PBB (Figure 13).

Table 6. Hepatic and renal AHH activities in adult male C57 and DBA mice

Strain	Liver	Kidney
C57	715.56 ± 87.01	0.84 ± 0.06
DBA	412.61 ± 45.69*	1.74 ± 0.21*

Arylhydrocarbon hydroxylase (AHH) activity was measured in the 10,000 x g supernatant fraction of homogenates of livers and kidneys of adult male C57 or DBA strain mice. Values are means ± S.E. of 10 or more animals. AHH activity was expressed as relative fluorescence units per milligram protein per minute.

*Significantly different from male C57 mice ($P<0.05$).

Figure 13. Effect of inducers of MFOs on hepatic arylhydrocarbon hydroxylase (AHH) activity. Arylhydrocarbon hydroxylase activities were determined in the 10,000 x g supernatant fraction of homogenates of livers of control male C57 or DBA mice or treated animals with BNF, PB or PBB. Activities are presented as a percentage of controls. Each bar represents the mean ± S.E. of five or more animals.

*Significantly different from control mice ($P<0.05$).

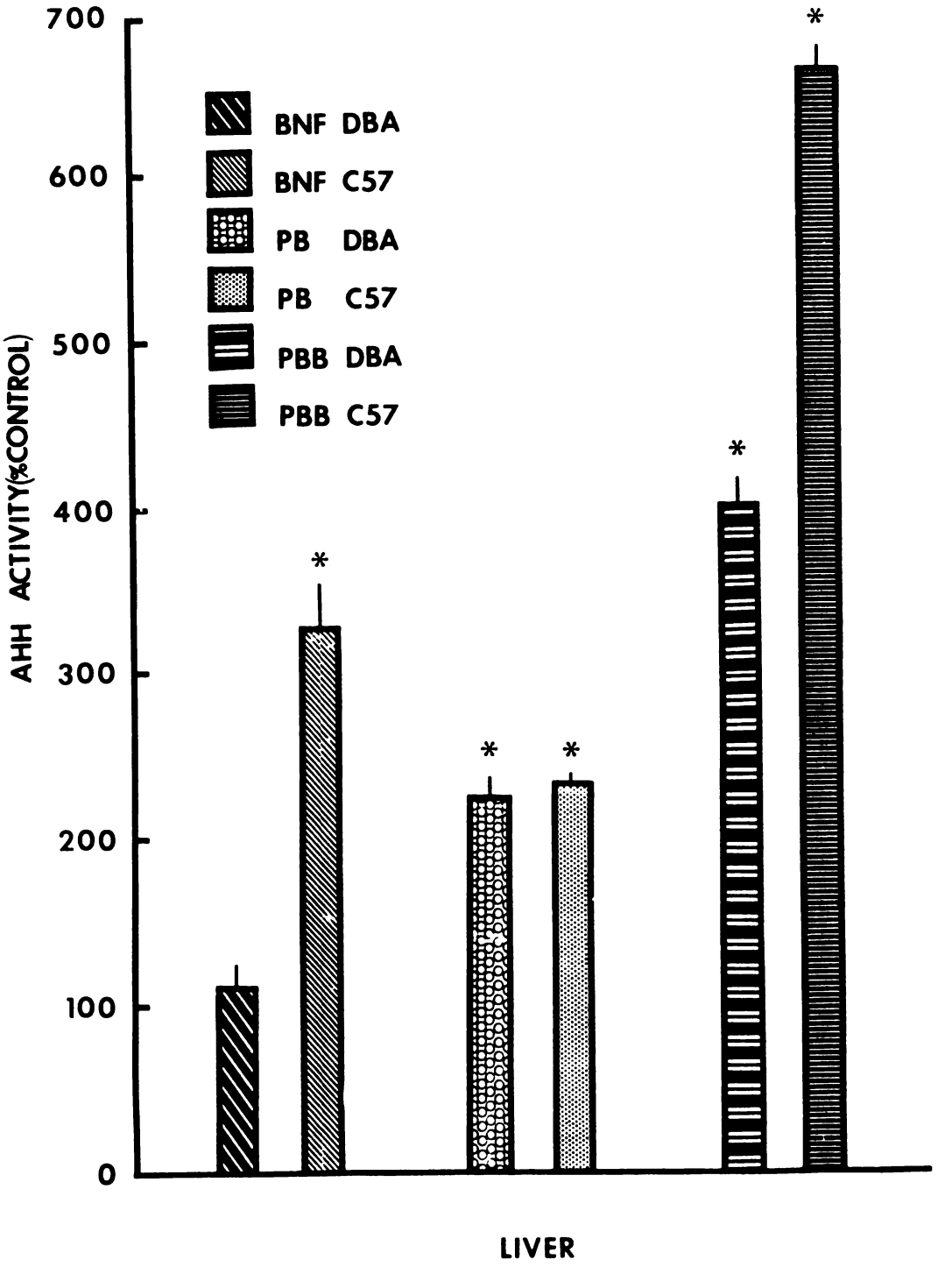
700
600
500
400
300
200
100
0
AHH ACTIVITY(%CONTROL)
BNF DBA
BNF C57
PB DBA
PB C57
PBB DBA
PBB C57
*
*
*
*
*
*
LIVER

Figure 13

Effect of BNF, PB or PBB on Renal Microsomal Activities in Male C57 and Male DBA Mice

In the kidney, the constitutuent level of AHH activity was very low in both strains. However, renal AHH activity was significantly higher in male DBA mice compared to renal AHH in male C57 mice (Table 6). Renal AHH was not affected by treatment of male DBA mice with BNF. In contrast, BNF enhanced kidney AHH activity in male C57 mice (Figure 14). Phenobarbital (PB) did not alter renal AHH in male C57 or DBA mice (Figure 14), but PBB markedly induced renal AHH in male C57 mice. Kidney AHH activity in male DBA mice was not affected following pretreatment with PBB (Figure 14).

Morphology of Bowman's Capsule in Male C57 and Male and Female DBA Mice

The parietal epithelial lining of Bowman's capsules in all strains of mice studied contained either a mixture of simple cuboidal and squamous cells or all cuboidal or squamous cells. In male C57 and female DBA mice, parietal epithelial cells were predominantly composed of squamous cells (Figures 15 and 16). Parietal cells in male DBA mice were predominantly composed of cuboidal cells (Figure 17). Male DBA mice had a higher percentage of cuboidal cells in the parietal layer (Table 7); male C57 mice had a higher percentage of squamous cells when compared to male DBA mice (Table 7). Female DBA mice had the least number of the cuboidal cells in the parietal epithelium of Bowman's capsule (Table 7).

Figure 14. Effect of inducers of MFOs on renal aryl-hydrocarbon hydroxylase (AHH) activities. Arylhydrocarbon hydroxylase activities were determined in the 10,000 x g supernatang fraction of homogenates of kidneys of control male C57 or DBA mice or treated animals with BNF, PB or PBB. Activities are presented as a percentage of controls. Each bar represents the mean ± S.E. of five or more animals.

*Significantly different from control mice ($P<0.05$).

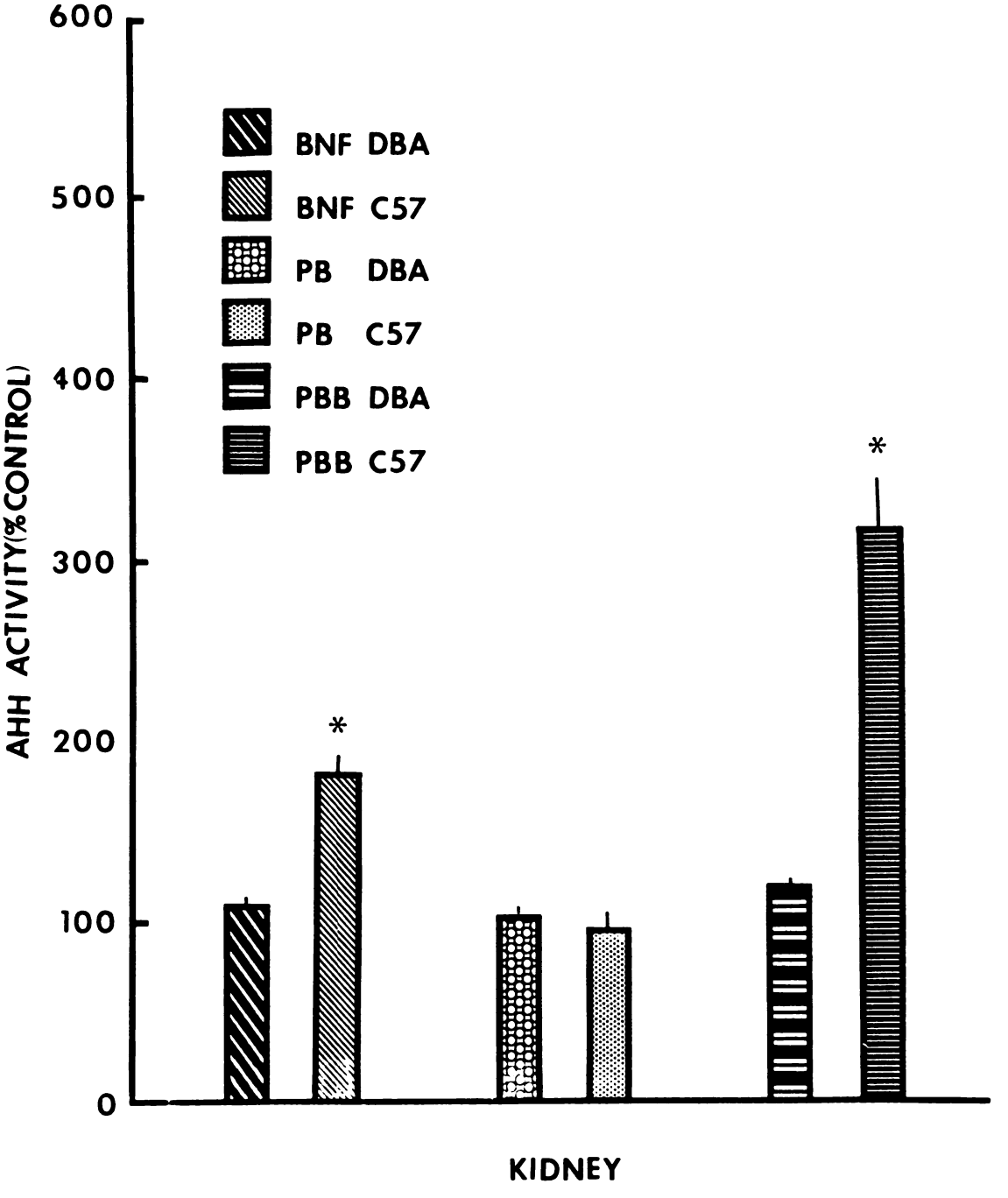
600
500
400
300
200
100
0
AHH ACTIVITY(%CONTROL)
BNF DBA
BNF C57
PB DBA
PB C57
PBB DBA
PBB C57
*
*
KIDNEY

Figure 14

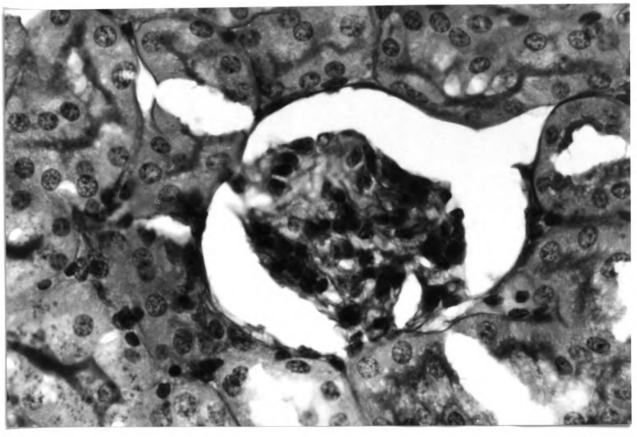

Figure 15. Structure of Bowman's capsule in male C57 mice. Male C57 mice were killed by decapitation. Kidneys were removed, fixed with 10% buffered formalin, stained with H&E or PAS. Bowman's capsules were analyzed with light microscopy. (X400)

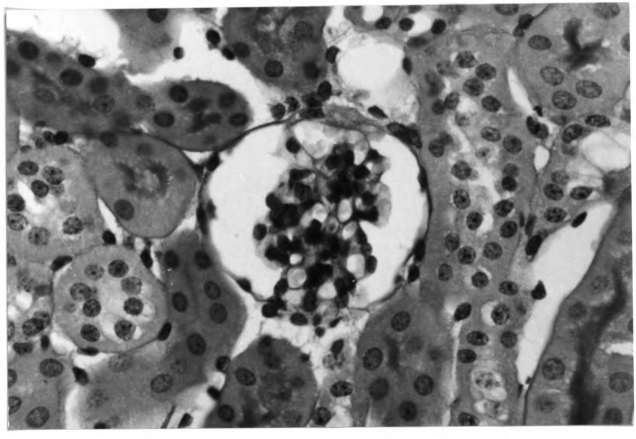

Figure 16. Structure of Bowman's capsule in female DBA mice. Female DBA mice were killed by decapitation. Kidneys were removed, fixed with 10% buffered formalin, stained with H&E or PAS. Bowman's capsules were analyzed with light microscopy. (X400)

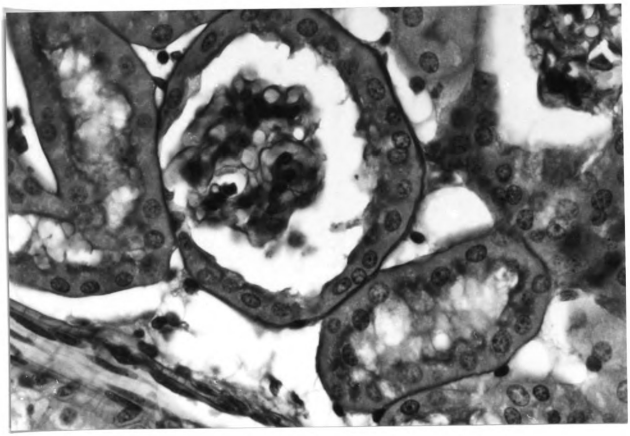

Figure 17. Structure of Bowman's capsule in male DBA mice. Male DBA mice were killed by decapitation. Kidneys were removed, fixed with 10% buffered formalin, stained with H&E or PAS. Bowman's capsules were analyzed with light microscopy. (X400)

Table 7. A comparison of parietal epithelium in Bowman's capsule (BC) of male C57 and male and female DBA mice

Sex	Species	No. of Squamous BC	No. of Cuboidal or Cuboidal & Squamous BC	Percent Squamous BC	Percent Cuboidal or Cuboidal & Squamous BC
M	C57	56	16	71.43	28.57
M	C57	64	25	71.80	28.10
M	C57	46	12	79.30	20.70
M	C57	50	12	80.65	19.35
M	C57	53	14	79.11	20.89
M	DBA	20	64	15.79	84.21
M	DBA	10	45	18.18	81.82
M	DBA	10	50	16.67	83.33
M	DBA	16	52	23.53	76.47
M	DBA	17	58	22.67	77.33
F	DBA	60	8	88.24	11.76
F	DBA	57	3	95.00	5.00
F	DBA	63	4	94.33	5.97
F	DBA	70	9	88.61	11.39
F	DBA	61	8	89.33	10.67

Male C57 and male and female DBA mice were killed by decapitation. Kidneys were removed, fixed with 10% buffered formalin, stained with H&E or PAS. Bowman's capsules were analyzed with light microscopy.

Ultrastructural features of the parietal cuboidal epithelial cells of Bowman's capsule in male C57 and male and female DBA mice were generally similar to those of the epithelium lining proximal convoluted tubules (PCT) (Figures 18 and 19). The cuboidal cells lining Bowman's capsule contain microvilli constituting a brush border at the apical cell surface. However, this brush border does not project from the entire cell surface and microvilli appear shorter and not as dense numerically when compared to PCT epithelium (Figures 18 and 19). The lateral and basal plasma membranes of the cuboidal capsule cells exhibit infolding, which appears to be more extensive in the cuboidal capsule than that observed in PCT epithelial cells. Although cuboidal cells of Bowman's capsule and PCT both contain numerous mitochondria as well as ribosomes, PCT epithelial cells appeared to have more mitochondria per unit of cytoplasm and fewer ribosomes associated with endoplasmic reticulum than Bowman's capsule cells. The morphology of the lysosomes and vacuoles associated with the endocytotic apparatus was similar in PCT and Bowman's capsule cuboidal epithelium. Bowman's capsule cells contain a round to oval nucleus with a prominent nucleolus and extensive peripheral heterochromatin. The Golgi apparatus was not observed in Bowman's capsule (Figures 18 and 19).

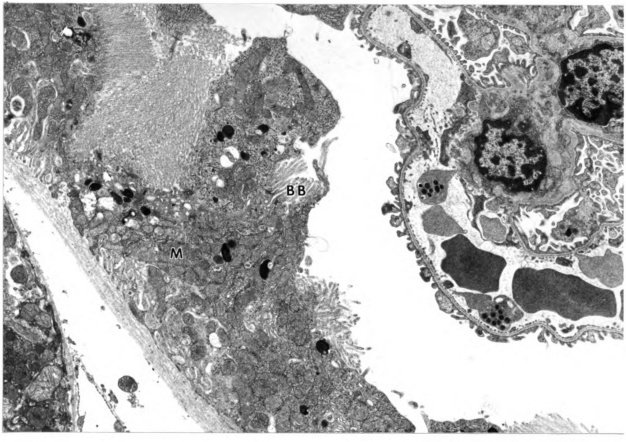

Figure 18. Ultrastructure of the cuboidal epithelial cell lining Bowman's capsule in male DBA mice, showing the microvilli which project from the apical surface. Note that the brush border (BB) does not project from the entire surface and that there are fewer mitochondria (M) per unit area of cytoplasm. (X6077.5)

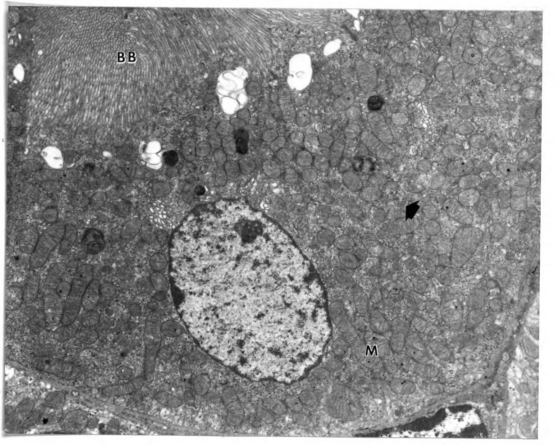

Figure 19. Ultrastructure of the cuboidal epithelial cell lining the proximal convoluted tubule (PCT) in male DBA mice, showing a brush border (BB) which projects from the entire apical surface. There appears to be more mitochondria (M) per unit area of cytoplasm but fewer ribosomes (arrow) associated with endoplasmic reticulum when compared to cells lining Bowman's capsule (Figure 18). (X7350)

Cytochemistry of the Hepatic and Renal Cytochrome P-450: Effect of Inhibitors of Microsomal Enzyme Activities on Kidney and Liver

The presence of cytochrome P-450 in kidney as well as in liver of male C57 and male DBA mice was noted (a deposit of the colored formazan) (Chayen et al., 1972) (Figures 20 and 21). However, the activity of renal and hepatic microsomal enzymes was markedly reduced when piperonyl butoxide was added into the reaction medium (Figures 22 and 23). Interestingly, SKF 525-A only inhibited hepatic microsomal activity; this agent failed to reduce renal microsomal activity in either male C57 or male DBA mice (Figures 24 and 25).

The presence of microsomal enzyme activity in the cuboidal epithelium of Bowman's capsule was observed. The activity of the microsomal enzyme in the cuboidal cells was reduced by piperonyl butoxide but not by SKF 525-A (Figures 22 and 24).

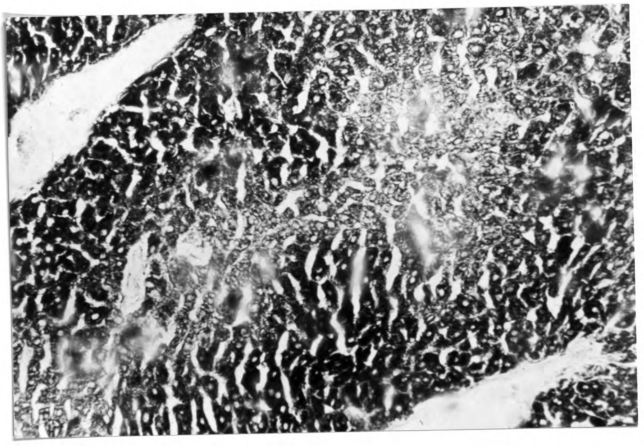

Figure 20. Cytochemistry of hepatic cytochrome P-450 in male C57 mice. The dark area indicates the presence of microsomal enzyme activities in the centrolobular region. (X400)

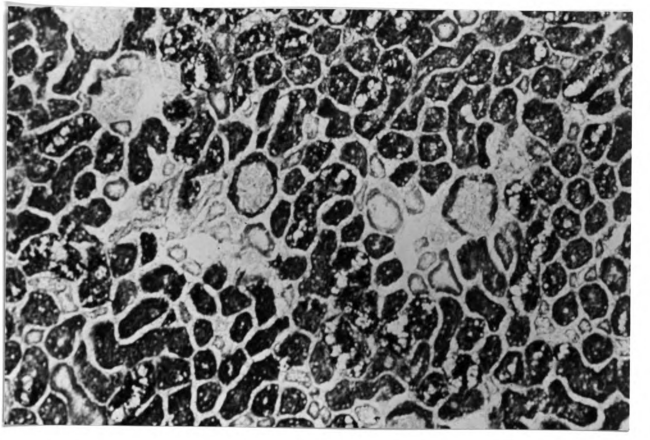

Figure 21. Cytochemistry of renal cytochrome P-450 in male DBA mice. The dark areas indicate the presence of microsomal enzyme activities in proximal convoluted tubular cells as well as the cuboidal epithelial cells of Bowman's capsule. (X250)

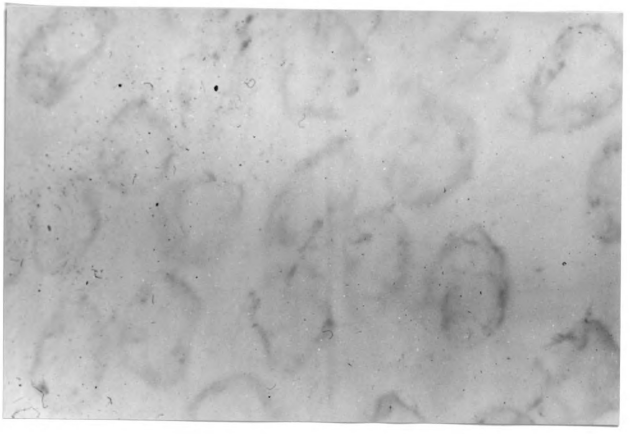

Figure 22. Effect of piperonyl butoxide on renal cytochrome P-450 in male DBA mice, showing inhibition of microsomal enzyme activities in both proximal convoluted tubular cells and cuboidal epithelial cells of Bowman's capsule (absence of dark areas). (X400)

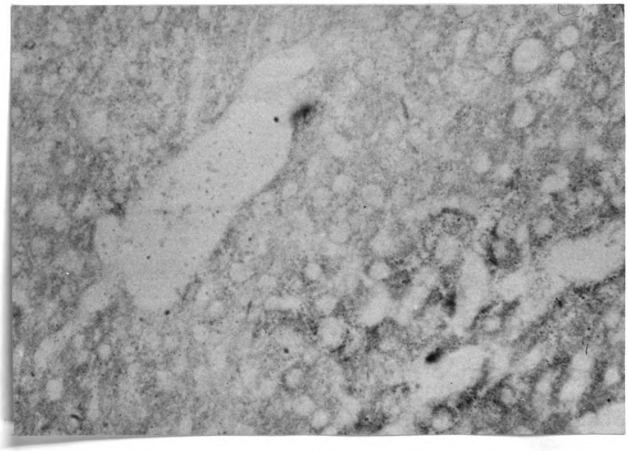

Figure 23. Effect of piperonyl butoxide on hepatic cytochrome P-450 in male C57 mice, showing inhibition of microsomal enzyme activities by this agent (absence of dark areas). (X400)

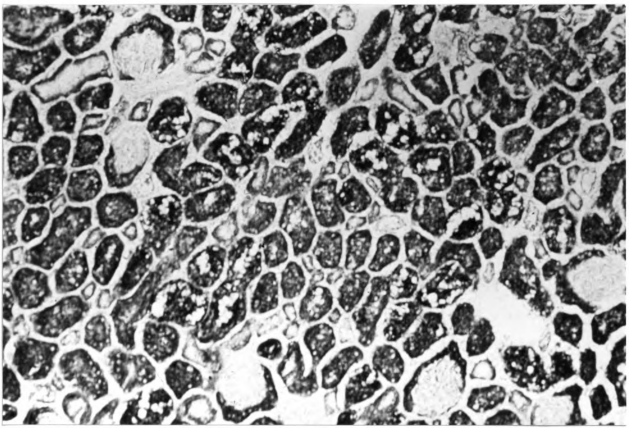

Figure 24. Effect of SKF 525-A on renal cytochrome P-450 in male DBA mice. Dark, cytochemically stained areas reflect the presence of microsomal enzyme activity in the cuboidal epithelial cells lining proximal convoluted tubules and Bowman's capsules. (X250)

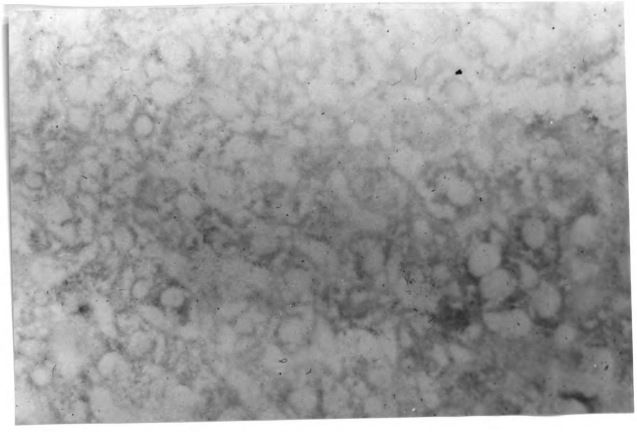

Figure 25. Effect of SKF 525-A on hepatic cytochrome P-450 in male C57 mice, showing inhibition of microsomal enzyme activities by this agent (absence of dark areas). (X400)

DISCUSSION

The mechanism by which CHC13 produces toxicity is not completely understood. The initial step in the pathway leading to CHC13 toxicity is generally assumed to be the biotransformation of CHC13 to a reactive intermediate, possibly phosgene (COC12) by mixed-function oxygenases (MFO) present in the endoplasmic reticulum of susceptible tissue (Ilett, 1973; Brown et al., 1974; Sipes et al., 1977; Pohl et al., 1977; Mansuy et al., 1977). The MFO system is a heterogeneous group of enzymes responsible for oxidative metabolism of polycyclic hydrocarbons and drugs and for the manifestation of the nephrotoxic and hepatotoxic effects of a variety of compounds including CHC13. The presence of the MFO system in kidney as well as liver microscomes in various animal species has been described by many investigators (Ichihara, 1969; Levis, 1970; Ichihara et al., 1971; Ellin et al., 1972; Jakobsson et al., 1970; Jakobsson and Cinti, 1973; Ichikawa, 1975). An important function of these enzymes is the metabolism of various exogenous and endogenous compounds. Broide et al. (1958) introduced the term "drug metabolizing enzyme." The reactions involved in the biotransformation include oxidation, reduction, demethylation and hydroxylation.

Biotransformation of xenobiotics does not necessarily result in substrate detoxification. It has been shown that the resulting metabolite can be as toxic as the foreign chemical itself, or more toxic. This mechanism is at least partly responsible for the hepatotoxic and nephrotoxic effect of compounds such as carbon tetrachloride and chloroform (McLean, 1970; Scholler, 1970; Hewitt et al., 1979).

Phosgen (COCl2) has been postulated to be a possible reactive intermediate(s) formed upon CHCl3 oxidation (Pohl et al., 1977; Mansuy et al., 1977; Sipes et al., 1977). Phosgen is a very reactive electrophilic compound and reacts with tissue macromolecules; formation of irreversible covalent binding occurs and causes tissue necrosis (Ilett et al., 1973; Pohl and Krishna, 1978).

Increased serum glutamic pyruvic transaminiase (SGPT) activity has been suggested as a sensitive index of hepatic injury (Balazs et al., 1962). By this criterion, CDCl3 produced less liver damage in mice than the same dose of CHCl3 (Figure 1), consistent with findings in rats (Pohl and Krishna, 1978). Pohl and Krishna (1978) reported that 2.49 mmol/kg CDCl3 produced only minor histological changes and no elevation of SGPT relative to a sesame oil control; at this dose CHCl3 produced considerable necrosis of the centrolobular region of the liver and elevation of SGPT. Thus, a deuterium isotope effect appears to influence hepatotoxicity of CDCl3 in rats and mice, suggesting that

similar pathways and mechanisms of CHCl3 toxicity exist in the liver in both species.

Accumulation of p-aminohippuric acid (PAH) or tetraethylammonium (TEA) into renal cortical slices *in vitro* is an energy-dependent active transport process. Altered uptake of these ions is a sensitive index of nephrotoxicity, especially injury of proximal tubular cells (Hirsch, 1976), and has been used in quantifying CHCl3-induced nephrotoxicity (Watrous and Plaa, 1972; Kluwe et al., 1978; Hewitt et al., 1979). Treatment with CHCl3 depressed accumulation of PAH and TEA by rat and mouse renal cortical slices *in vitro*. Deuterium-labelled chloroform appeared to be less nephrotoxic than CHCl3 (Figures 2 through 4).

Since kidneys usually have low drug-metabolizing enzyme activities (Litterst et al., 1975), chemically induced nephrotoxicity has been assumed to be produced by toxic intermediate(s) generated in the liver and transported to the kidney. If this were the case, phosgene could be produced from CHCl3 in the liver and transported to the kidneys to produce nephrotoxicity. Male C57 and male and female DBA mice which developed the same degree of liver damage following exposure to CHCl3 (Figures 5 and 9) should show the same degree of kidney damage as well. However, results of renal function tests indicated that male DBA mice were more sensitive to CHCl3-induced nephrotoxicity than male C57 mice. In contrast, female DBA mice failed to develop renal damage after administration of CHCl3 (Figures 6 through 8). This observation was consistent with Clemmens et al.

(1979) and Hill et al. (1975), who reported that covalent binding of [^{14}C]CHCl3 to renal microsomes was greater in male DBA mice than in male C57 mice. Female DBA mice failed both to develop tubular lesions and to accumulate [^{14}C]CHCl3 bound to renal microsomal protein.

Since renal injury does not parallel hepatotoxicity, it is unlikely that CHCl3 nephrotoxicity is due to intermediate(s) formed in the liver. Thus, kidney damage following administration of CHCl3 appears to be caused by toxic reactive intermediate(s) generated within the kidney.

Strain-dependent nephrotoxicity following CHCl3 administration to male mice, in the absence of significant differences in hepatic injury, strongly suggests that CHCl3 is metabolized intrarenally and that generation of toxic metabolites in the kidney is responsible for renal injury. Male C57 mice have lower renal MFO activity than male DBA mice (Table 6); they also are relatively resistant to CHCl3-induced nephrotoxicity. It seems reasonable to attribute reduced susceptibility to CHCl3-induced renal necrosis to lower renal microsomal drug metabolizing enzyme activity.

The activity of microsomal enzymes can be altered by administration of various agents such as phenobarbital (PB), 3-methylcholanthrene (3MC), tetrachlorodibenzo-p-dioxane (TCDD), polybrominated biphenyls (PBB), polychlorinated biphenyls (PCB) and β-naphthoflavone (BNF). Both liver enlargement and increased hepatic activity of drug metabolizing enzymes with PB-induced proliferation of

smooth endoplasmic reticulum in the hepatic cells in rats have been reported (Gopinath and Ford, 1975; Burger and Herdson, 1966). Induction of the MFO system in male C57 and DBA mice and CHC13-induced hepatotoxicity were observed following treatment of animals with PB (Figures 9 and 13). In contrast, PB did not alter renal MFO activity, nor did it affect CHC13-induced nephrotoxicity. Similar observations were made by Kluwe et al. (1978), who reported that treatment with PB increased the activity of drug metabolizing enzyme in the liver but not in the kidney of male ICR mice. Since PB only enhances hepatotoxicity and not nephrotoxicity of CHC13, it is unlikely that CHC13 nephrotoxicity is due to intermediate(s) formed in the liver. 3-Methylcholanthrene appears to be less effective than PB in promoting the hepatotoxicity of CHC13. β-Naphthoflavone (BNF), which is a 3MC type inducer (Boobis et al., 1976), significantly induced both renal and hepatic MFO activity in liver and kidney in male C57 mice (Figures 13 and 14). These figures are consistent with the observation of Nebert et al. (1972). Nephrotoxicity of CHC13 was not altered in either strain of male mice treated with BNF (Figures 10 through 12). Pretreatment of animals with BNF only enhanced liver injury by CHC13, further supporting the idea that liver is not the source of nephrotoxic CHC13 metabolites.

Polybrominated biphenyls (PBB) were used commercially as fire retardants. Recently these were accidentally mixed into animal feed, resulting in heavy exposure of domestic farm animals to dietary PBB. Increased MFO activities in

kidney as well as liver following PBB administration have been reported (McCormack et al., 1980). A similar class of compounds, the polychlorinated biphenyls (PCB), have been extensively studied. Polybrominated biphenyls (PBB) may share many of the biological and toxic properties of PCB. In laboratory animals, PCB and PBB cause an alteration of hepatic function (Johnstone et al., 1974) and induce both hepatic and renal microsomal enzyme activities (Alvores et al., 1973; Kluwe and Hook, 1978; McCormack et al., 1979; McCormack et al., 1980). Although PCB is similar to PBB in many respects, they differ in their toxic effects. Polychlorinated biphenyls have been reported to protect against CHCl3 administration (Kluwe and Hook, 1978). The differences between the effects of PBB and PCB on CHCl3 toxicity suggest that these agents may have different effects on the overall metabolism of CHCl3 (Kluwe et al., 1978).

The effect of PBB on hepatic microsomal activities was compared with the effect produced by PB and 3MC, agents which represent two distinct classes of inducing agents. The PBB are unique in that they stimulate hepatic enzymes sensitive to both PB and 3MC (Dent et al., 1976). Polybrominated biphenyls induce hepatotoxicity as well as nephrotoxicity of CHCl3 in mice (Kluwe et al., 1978). These results suggest that PBB ingestion, in subtoxic amounts, stimulates the metabolism of CHCl3 to hepatotoxic and nephrotoxic agents and enhances the sensitivity of the liver and kidney to CHCl3 injury (Kluwe and Hook, 1978). Hepatic microsomal enzyme activities, as well as

CHCl3-induced hepatotoxicity, have also been studied in male C57 and DBA mice (Figures 9 and 13). The degree of MFO inducibility and the toxic effect of CHCl3 are considerably higher in male C57 than in male DBA mice. In male DBA mice, PBB did not affect renal MFO activities or alter the CHCl3-induced renal injury. In contrast, pretreatment of male C57 mice with PBB resulted in markedly increased renal MFO activity as well as enhanced CHCl3 nephrotoxicity (Figures 14 and 10 through 12). These findings further support the idea that nephrotoxicity of CHCl3 is likely to be caused by CHCl3 metabolites formed intrarenally. If the liver were the site of production of nephrotoxic CHCl3 metabolites, pretreatment with PBB should induce renal damage in male DBA mice as well as hepatic injury. Since this was not the case, it is unlikely that the liver is responsible for the generation of nephrotoxic CHCl3 metabolites.

Arylhydrocarbon hydroxylase (AHH) is an example of a mixed-function oxidase (MFO) (Nebert et al., 1972). The *in vitro* enzyme assay was used as an index for enzymatic conversion of CHCl3 both in the liver and kidney. Following treatment of male C57 and DBA mice with PBB, hepatic AHH activity was significantly higher in male C57 than in male DBA mice (Figure 13). Similarly, SGPT activity, which is a sensitive index of hepatic injury (Balazs et al., 1962), was considerably higher in PBB treated male C57 mice than in male DBA mice after CHCl3 (Figure 9). Hepatic AHH activity and CHCl3 hepatotoxicity were also

enhanced in male C57 and male DBA mice following administration of PB (Figures 13 and 9). Stimulation of hepatic AHH activity and CHCl3 hepatotoxicity was noted in BNF-treated male C57 mice (Figures 13 and 9). Interestingly, BNF did not affect hepatic AHH activity or CHCl3-induced hepatic injury in male DBA mice (Figures 13 and 9). These findings may suggest that hepatic AHH activity may parallel the activity of enzyme(s) responsible for the metabolite of CHCl3.

Untreated male C57 mice had higher hepatic AHH activity in comparison to the hepatic AHH activity in male DBA mice (Table 6). However, when both strains of male mice were challenged with the same dose of CHCl3, hepatic injury produced by CHCl3 was the same (Figure 9). Similar observations on male ICR mice were carried out by Kluwe et al. (1978), who reported that hepatic AHH activity increased following treatment of male ICR mice with PCB, 3MC or TCDD, but hepatotoxicity of CHCl3 was not affected by these agents. These findings, which are contrary to the previous observations, indicate that determination of AHH activity alone is not a suitable index for interpretation of the MFO activity responsible for metabolism of CHCl3 in the liver.

In kidney, AHH activity was higher in untreated male DBA mice than in untrated male C57 mice (Table 6). Similarly, CHCl3-induced renal injury was greater in male DBA than in male C57 mice (Hill et al., 1975; Clemens et al., 1979) (Figures 14 and 10-12). In addition, when both male C57 and male DBA mice were treated with PBB, nephrotoxicity of

CHC13 was enhanced as well as renal AHH activity in male C57 mice (Figures 14 and 10-12). In contrast, PBB did not alter nephrotoxicity or renal AHH activity in male DBA mice (Figures 14 and 10-12). Renal AHH was not altered by treatment of male C57 and DBA mice with PB. Similarly, PB did not affect nephrotoxicity of CHC13 in either strain of male mice. However, treatment of male C57 mice with BNF induced renal AHH activity, but there were no significant effects by this agent on nephrotoxicity of CHC13 (Figures 10-12). In addition, Hook et al. (1978) and Kluwe et al. (1978) found that TCDD, 3MC or PCB induced renal AHH; in contrast, nephrotoxicity of CHC13 was reduced following treatment of male ICR mice with these agents. From these observations, it was also concluded that determination of renal AHH activity alone also is not a suitable index for the detection of renal MFO activity responsible for CHC13 metabolism.

In conclusion, failure to correlate the induction of renal and hepatic AHH activities and nephrotoxicity and hepatotoxicity of CHC13 suggests that CHC13-induced renal and hepatic toxicity may not parallel AHH activity. As a result, enzymes responsible for metabolism of CHC13 comprise a subpopulation of the MFO system, the activity of which does not correlate with the degree of inducibility of AHH activity.

Inhibition, as well as stimulation, of drug metabolizing enzyme systems can alter the nephrotoxicity and hepatotoxicity of CHC13. Piperonyl butoxide is an inhibitor of

the microsomal drug metabolizing enzyme system in both liver and kidney (Figures 22 and 23). Ilett et al. (1973) reported that pretreatment of male C57 mice with piperonyl butoxide reduced the hepatotoxicity and nephrotoxicity of CHCl3. Interestingly, SKF 525-A inhibited the activity of the MFO system in liver but not in kidney of male C57 or male DBA mice (Figures 24 and 25). Similarly, decreased CHCl3-induced hepatotoxicity is found when mice were pretreated with SKF 525-A. This compound, however, failed to protect the kidney from CHCl3 injury.

The precise effects of these agents on CHCl3 metabolism are not completely understood. However, these two inhibitors are believed to affect different subpopulations of the MFO enzyme systems in the kidney. Decreased CHCl3 nephrotoxicity in animals pretreated with piperonyl butoxide and the failure of SKF 525-A to block renal injury produced by CHCl3 suggest that CHCl3 is metabolized in the kidney by enzyme system(s) sensitive to inhibition by piperonyl butoxide but not by SKF 525-A.

The mechanism by which CHCl3 produces renal necrosis in male but not in female mice is not clear. Sex-related differences in kidney morphology of mice have been reported by Crabtree (1940). She observed that most cells in the parietal epithelium of Bowman's capsule in male albino mice was composed of cuboidal cells resembling those which line the proximal convoluted tubule cells. In female albino mice the parietal epithelium of Bowman's capsule is composed of squamous cells. The observation of Eshenbrenner and Miller

(1945) on strain A mice confirmed these findings. In addition, they noted renal lesions occurred in males following exposure to CHCl3 but not in similarly treated female mice. Therefore, they suggested that there is a correlation between the structure of Bowman's capsule in male and female mice and sex dependent nephrotoxicity of CHCl3.

Male DBA mice have a higher percentage of cuboidal epithelial cells in the parietal layer of the Bowman's capsule. Male C57 mice, which are relatively resistant to CHCl3 nephrotoxicity (Figures 6-8, 10-12), have a higher percentage of squamous epithelial cells (Table 7). However, female DBA mice which are resistant to renal damage following exposure to CHCl3 (Figures 6-8) have the lowest percentage of cuboidal cells in the pareital epithelium of Bowman's capsule (Table 7). These studies also demonstrated that the ultrastructural features of cuboidal cells in Bowman's capsule of male C57 or DBA mice are generally similar to the epithelial cells lining proximal convoluted tubules (Figures 18 and 19). The finding that the effect of CHCl3-induced renal damage parallels the percentage of the cuboidal cells in the pareital epithelium of Bowman's capsule (Table 7 and Figures 6-8) suggests a correlation between the structure of Bowman's capsule in male and female mice and susceptibility to nephrotoxicity of CHCl3 (Eshenbrenner and Miller, 1945). These correlations may exist among different strains of male mice. However, the data do not indicate a cause and effect relationship between the

morphology of Bowman's capsule and susceptibility of CHCl3-induced renal necrosis.

The role of testosterone in developing cuboidal epithelial cells in Bowman's capsule has been reported. Crabtree (1941) noted that castrated male mice failed to develop cuboidal cells. Setye (1939) found development of cuboidal cells in the parietal epithelium of castrated mice treated with testosterone and in testosterone-treated female mice. Furthermore, Crabtree (1941) observed that the cuboidal cells do not increase in number in male mice until sexual maturity.

Testosterone also plays a significant role in CHCl3-induced nephrotoxicity. Renal tubular necrosis in testosterone-treated female mice following exposure to CHCl3 has been noted (Krus et al., 1974). Renal injury with CHCl3 exposure has been found also in castrated mice treated with testosterone (Eshenbrenner and Miller, 1945). The role of testosterone may also be important in strain-related differences in renal injury produced by CHCl3 (Shire and Bartke, 1972). Interestingly, testosterone is not affected in hepatotoxicity of CHCl3 (Culliford et al., 1957).

Results clearly establish that kidney of mice is particularly sensitive to testosterone and under the influence of the hormone undergoes histological changes in Bowman's capsule, as well as testosterone-induced CHCl3 toxicity in the kidney but not in the liver. It should be emphasized that an explanation of these two phenomena is not clear. However, a lack of correlation between nephrotoxicity and

hepatotoxicity of CHCl3 strongly suggests that the kidney is the site of formation of CHCl3 metabolite(s).

There is considerable evidence to support a cause and effect relationship between [^{14}C]CHCl3 covalent binding and tissue necrosis. In kidney, the covalent binding of [^{14}C]-CHCl3 and CHCl3-induced renal necrosis was carried out with male and female C57 mice by Ilett et al. (1973). They observed that the amount of covalent binding and severity of necrosis were significantly higher in male C57 mice when compared to female C57 mice. Similarly, Clemens et al. (1979) observed that covelant binding of CHCl3 by renal microsomal protein was greater in male DBA than in male C57 mice, and male DBA mice were more sensitive to CHCl3-induced renal injury than male C57 mice. Taylor et al. (1974) reported that the amount of radioactivity of [^{14}C]-CHCl3 in the kidneys of male CF/LP, CBA and C57BL mice was significantly greater when compared to the female kidneys of each strain of mice. These observations support the idea that there is a cause and effect relationship between [^{14}C]CHCl3 covalent binding and kidney necrosis.

Pretreatment of male C57 and male DBA mice with PB enhanced covalent binding of [^{14}C]CHCl3 by renal microsomes in male DBA but not in male C57 mice (Clemens et al., 1979). In addition, Ilett et al. (1973) reported that pretreatment of male C57 mice with PB decreased covalent binding of [^{14}C]CHCl3 in the kidney. Interestingly, administration of CHCl3 to male C57 and male DBA mice pretreated with PB did not alter nephrotoxicity of CHCl3 in either strain of

male mice (Figures 10-12). This finding, which is in contrast to the previous suggestion of cause and effect relationship between covalent binding and kidney necrosis, suggests that covalent binding of radioactivity of CHCl3 by kidney microsomes may not parallel the nephrotoxicity of CHCl3.

SUMMARY

Administration of deuterium-labelled chloroform (CHC13) to male ICR mice and chloroform (CHC13) to male ICR, C57 and male and female DBA mice produced dose-dependent damage in the liver (elevated SGPT activity) and kidney (increased BUN, decreased accumulation of PAH and TEA). Deuterium-labelled chloroform produced less kidney and liver damage than CHC13 in male ICR mice, suggesting that the kidney may metabolize CHC13 in the same manner as the liver. Chloroform caused the same degree of liver damage in male C57 and male and female DBA mice. However, nephrotoxicity of CHC13 was greater in male DBA than in male C57 mice. Female DBA mice failed to develop renal injury following treatment with CHC13. Pretreatment of male C57 and DBA mice with phenobarbital (PB) markedly increased hepatotoxicity of CHC13 but did not affect nephrotoxicity of CHC13. β-Naphthoflavone enhanced CHC13 hepatotoxicity in male C57 mice but had little effect on nephrotoxicity. Polybrominated biphenyl (PBB) enhanced hepatotoxicity of CHC13 in both strains. In contrast, PBB increased renal injury in male C57 mice but not in male DBA mice. Therefore, the lack of correlation between hepatotoxicity and nephrotoxicity produced by CHC13 strongly suggests that the liver is not the site of formation of nephrotoxic CHC13 metabolite(s).

Arylhydrocarbon hydroxylase (AHH) is an example of a mixed-function oxygenase. Following treatment of male C57 and DBA mice with PBB, hepatic AHH activity was significantly higher in male C57 than in male DBA mice. Similarly, hepatotoxicity of CHCl3 was considerably higher in PBB treated male C57 than in male DBA mice pretreated with PBB. Hepatic AHH activity and CHCl3 hepatotoxicity were also enhanced in male C57 and DBA mice following administration of PB. Stimulation of hepatic AHH activity and hepatotoxicity of CHCl3 were noted in BNF-treated male C57 mice. Interestingly, BNF did not affect hepatic AHH or CHCl3-induced hepatic injury in male DBA mice. However, untreated male C57 mice had higher hepatic AHH activity in comparison to the hepatic AHH activity in male DBA mice. But when both strains of male mice were challenged with the same doses of CHCl3, hepatic injury produced by CHCl3 was the same.

In kidney, AHH activity was higher in untreated male DBA than in untreated male C57 mice. Similarly, CHCl3-induced renal injury was greater in male DBA than in male C57 mice. In addition, when both male C57 and male DBA mice were treated with PBB, nephrotoxicity of CHCl3 and renal AHH activity were increased in male C57 mice. In contrast, PBB did not alter nephrotoxicity or renal AHH activity in male DBA mice. Renal AHH was not altered by treatment of male C57 and DBA mice with PB. Similarly, PB did not affect nephrotoxicity of CHCl3 in either strain of male mice. However, treatment of male C57 mice with BNF induced renal AHH activity, but there were no significant

effects by this agent on nephrotoxicity of CHCl3 in male C57 mice.

In conclusion, renal and hepatic AHH activities may not parallel the CHCl3-induced renal and hepatic injury. Enzymes responsible for metabolism of CHCl3 comprise a subpopulation of the MFO system and their activity does not correlate with the degree of inducibility of AHH activity in the target organ.

Inhibition as well as stimulation can alter nephrotoxicity and hepatotoxicity of CHCl3. Piperonyl butoxide, an inhibitor of microsomal drug metabolizing enzymes, inhibits both liver and kidney microsomal enzyme activities. Similarly, it has been reported that nephrotoxicity and hepatotoxicity of CHCl3 are markedly reduced following pretreatment of mice with piperonyl butoxide. However, SKF 525-A only inhibits hepatic microsomal enzyme activity and has little effect on renal microsomal enzyme activity. In addition, it has been found that hepatotoxicity of CHCl3 decreased in mice pretreated with SKF 525-A. In contrast, this agent failed to protect the kidney from the toxicity of CHCl3, suggesting that the kidney may metabolize CHCl3 by a subpopulation of the MFO system which is sensitive to inhibition by piperonyl butoxide but not by SKF 525-A.

Male DBA mice had the highest percentage of cuboidal epithelial cells in Bowman's capsule in comparison to male C57 and female DBA mice. Female DBA mice had the least percentage of cuboidal cells in the parietal layer. Nephrotoxicity of CHCl3 in male DBA mice was considerably greater

than in male C57 mice, and female mice failed to develop renal injury following exposure to CHC13. This suggests that there is a correlation between the structure of Bowman's capsule and nephrotoxicity of CHC13 in mice.

BIBLIOGRAPHY

BIBLIOGRAPHY

Alvores, A. P., Bickers, D. R., and Kappas, A. (1973) Polychlorinated biphenyls: a new type of inducers of cytochrome P-448 in the liver. Proc. Nat. Acad. Sci. USA 70:1321-1325.

Balazs, T. Airth, J. M., and Grice, H. C. (1962) The use of the serum glutamic pyruvic transaminase test for the elevation of hepatic necrotropic compounds in rats. Can. J. Biochem. Physiol. 40:1-6.

Barrett, H. M., Cunningha , J. G., and Johnston, J. H. (1939) Study of fate in organic of some chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 21:479-490.

Bartke, A., and Shire, J. G. M. (1972) Differences between mouse strain in testicular cholesterol level and androgen target organs. J. Endocr. 55:173-184.

Bennet, R. A., and Whigman, A. (1964) Chloroform sensitivity of mice. Nature (London) 204:1328.

Boobis, A. R., Nebert, D. W., and Felton, J. S. (1976) Comparison of β-naphthoflavon and 3-methylcholanthrene as an inducer of hepatic cytochrome(s) P-448 and arylhydrocarbon hydroxylase (benzo[α]pyrene) hydroxylase activity. Mol. Pharmacol. 13:259-268.

Brodie, B. B., Gillett, J. R., and Ladu, B. N. (1958) Enzymatic metabolism of drug and other foreign compounds. Ann. Rev. Biochem. 27:427-454.

Brown, T. R., Greene, F. E., and Bardin, C. W. (1976) Androgen receptor dependent and independent activities of testosterone on hepatic microsomal drug metabolism. Endocrinology 99:1353-1362.

Brown, B. R., Sipes, I. G., and Sagalyn, A. M. (1974) Mechanism of acute hepatic toxicity: chloroform, halothane and glutathione. Anesthesiology 41:554-561.

Burger, P. C., and Herdson, P. B. (1966) Phenobarbital-induced fine structure changes in rat liver. Am. J. Pathol. 48:793-809.

Casarett, L. J., and Doull, J. (1975) Toxicology: the basic science of poisons. Macmillan Publishing Co., Inc., New York, pp. 153, 504, 505.

Chayen, J., Bitensky, J. L., and Butcher, R. G. (1972) Practical histochemistry. Wiley, New York.

Clemens, T. I., Hill, R. N., Bullock, L. P., Johnson, W. D., Sultatos, L. G., and Vesell, E. S. (1979) Chloroform toxicity in the mouse - role of genetic factors and steroids. Toxicol. Appl. Pharmacol. 48:117-130.

Conney, A. H. (1967) Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19:317-366.

Crabtree, C. E. (1940) Sex differences in the structure of Bowman's capsule in the mouse. Science 91:299.

Crabtree, C. (1941) The structure of Bowman's capsule as an index of age and sex variations in normal mice. Anat. Rec. 79:395-412.

Crabtree, C. (1941) The structure of Bowman's capsule in castrate and testosterone treated male mice as an index of hormonal effects on the renal cortex. Endocrinology 29:197-203.

Cross, R. J., and Taggart, J. V. (1950) Renal tubular transport: accumulation of p-amminohippuric acid by rabbit kidney slices. Am. J. Physiol. 161:181-190.

Dent, J. G., Netter, K. J., and Gibson, J. E. (1976) The induction of hepatic microsomal metabolism in rats following acute administration of mixture of polybrominated biphenyls. Toxicol. Appl. Pharmacol. 38:237-249.

Deringer, M. K., Dunn, T. B., and Heston, W. E. (1953) Results of exposure of strain C3H mice to chloroform. Proc. Soc. Exp. Biol. Med. 83:474-479.

Diplama, J. R. (1971) Drill's pharmacology in medicine, 4th ed. McGraw-Hill, New York, p. 162.

Docks, E. L., and Krishna, G. (1976) The role of glutathione in chloroform-induced hepatotoxicity. Exp. Mol. Path. 24:13-22.

Docks, E. L., Sipes, I. G., and Krishna, G. (1974) Mechanism of chloroform induced depletion of liver glutathione. Pharmacologist 16:239-244.

Ellin, A., Jakobsson, S. V., Schenkman, J. B., and Orrenius, S. (1972) Cytochrome P-450K of rat kidney cortex microsomes. Its involvement in fatty acid w- and (w-1) hydroxylation. Arch. Biochem. Biophys. 150: 64-71.

Eschenbrenner, A. B. (1944) Induction of hepatomas in mice by repated oral administration of chloroform with observation on sex differences. J. Natl. Cancer Inst. 5:251-255.

Eschenbrenner, A. B., and Miller, E. (1945) Sex differences in kidney morphology and chloroform necrosis. Science 102:302-303.

Gillette, J. R., and Pohl, L. R. (1977) A prospective on covalent binding and toxicity. J. Toxicol. Environ. Health 2:849-877.

Gillette, J. R. (1963) Metabolism of drug and other foreign compounds by enzymatic mechanism. Progress in Drug Research 6:13-23.

Goldstein, A., Aronow, L., and Kalman, S. M. (1968) Principle of drug action. Harper and Row, New York.

Goodman, L. S., and Gilman, A. (1970) The pharmacological basis of therapeutics, 4th ed. The Macmillan Co., London, Toronto, p. 83.

Gopinath, C., and Ford, E. J. H. (1975) The role of microsomal hydroxylase in the modification of chloroform hepatotoxicity in rats. Br. J. Exp. Path. 56:412-422.

Goujon, E. M., Nebert, D. W., and Gielen, J. E. (1972) Genetic expression of aryl hydrocarbon hydroxylase induction. IV. Interaction of various compounds with different forms of cytochrome P-450 and the effect on benzo(a)pyrene metabolism *in vitro*. Mol. Pharmacol. 8:667-680.

Haugen, D. A., Coon, M. J., and Nebert, D. W. (1976) Induction of multiple forms of mouse liver cytochrome P-450. Evidence for genetically controlled de novo protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. J. Biol. Chem. 251:1817-1827.

Hewitt, H. B. (1956) Renal necrosis in mice after accidental exposure to chloroform. Brit. J. Exp. Path. 37:32-39.

Hewitt, W. R., Mizajima, H., Cote, M. G., and Plaa, G. L. (1979) Acute alteration of chloroform-induced hepato- and nephrotoxicity by mirex and kepone. Toxicol. Appl. Pharmacol. 48:509-527.

Hill, R. W., Clemens, T. L., Liu, D. K., Vesell, E. S., and Johnson, W. D. (1975) Genetic control of chloroform toxicity in mice. Science 190:159-161.

Hirsch, G. H. (1976) Differential effect of nephrotoxic agents on renal transport and metabolism by use of techniques. Environ. Hlth. Perspect. 15:88-99.

Hook, G. E. R., Haseman, K., and Lucien, G. W. (1975) Induction of suppression of hepatic and extrahepatic microsomal foreign compound-metabolizing enzyme system by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chem. Biol. Interact. 10:199-214.

Hook, J. B., McCormack, K. M., and Kluwe, W. M. (1978) Renal effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. In Pentachlorophenol, ed. by K. R. Rao. Plenum Publishing Corp., New York, p. 384.

Hoover, J. E. (1970) Remington's pharmaceutical science, 14th ed. Mack Publishing Co., Easton, Pennsylvania, p. 1056.

Ichihara, K., Kusunose, E., and Kusunose, M. (1969) Some properties of distribution of the β-hydroxylation system of medium charge fatty acids. Biochem. Biophys. Acta 176:704-712.

Ichiara, K., Kusunose, E., and Kusunose, M. (1971) A fatty acid β-hydroxylation system solubilized from porcine kidney cortex microsomes. Biochem. Biophys. Acta 239:178-189.

Ichikawa, Y. (1975) Distribution of NAD(P)H-dependent cytochrome P-450 mixed function oxidase system in the brush border membrane of rabbit kidney cortex. Biochem. Biophys. Acta 394:406-415.

Ilett, K. F., Reid, W. D., Sipes, I. G., and Krishnai, G. (1973) Chloroform toxicity in mice: correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecule. Exp. Mol. Path. 19:215-229.

Jacobsen, L., Anderson, E. K., and Thorberg, T. O. (1964) Accidental chloroform nephrosis in mice. Acta Path. Microb. Scand. 61:503-513.

Jakobsson, S. V., and Cinti, D. L. (1973) Studies on the cytochrome P-450 containing monooxygenase system in human kidney cortex microsomes. J. Pharmacol. Exp. Ther. 185:226-234.

Jakobsson, S. V., Thor, H., and Orrenius. (1970) Fatty acid inducible cytochrome P-450 of rat kidney cortex microsomes. Biochem. Biophys. Res. Commun. 39:1073-1080.

Jakoby, W. B., and Keen, J. H. (1977) A triple-treat in detoxification: the glutathione s-transferase. Trends Biochemical Sci. 2:229.

Johnson, M. K. (1965) The influence of some aliphatic compounds on rat liver glutathione levels. Biochem. Pharmacol. 14:1383-1385.

Johnstone, G. J., Ecobichon, D. J., and Hutzinger, G. (1974) The influence of pure polychlorinated biphenyl compounds on hepatic function in rat. Toxicol. Appl. Pharmacol. 28:66-81.

Kay, S. (1970) Handbook of emergency toxicology, 3rd ed. Charles C. Thomas, Publisher, Springfield, Illinois, p. 206.

Kluwe, W. M., and Hook, J. B. (1980) Metabolic activation of nephrotoxic haloalkanes. Fed. Proc. 39:3129-3133.

Kluwe, W. M., McCormack, K. M., and Hook, J. B. (1978) Selective modification of the renal and hepatic toxicities of chloroform by induction of drug-metabolizing enzyme systems in kidney and liver. J. Pharmacol. Exp. Ther. 207:566-573.

Kluwe, W. M., and Hook, J. B. (1978) Polybrominated biphenyl-induced potentiation of chloroform toxicity. Toxicol. Appl. Pharmacol. 45:861-869.

Kochakian, C. D., and Harrison, D. G. (1962) Regulation of nucleic acid synthesis by androgen. Endocrinology 70:99-118.

Koths, K. E., Godchaun, W., Doeg, L. H., and Doeg, K. A. (1971) The effect of testosterone on the synthesis of mouse kidney mitochondrial and microsomal protein *in vivo* and *in vitro*. Endocrinology 91:125-134.

Krus, S., Storzynski, S., Zaleska-Rutezynska, Z., and Nacizek-Wieniawaska, A. (1974) The role of testosterone in developing chloroform-induced renal tubular necrosis in mice. Nephron 12:275-280.

Kuntzman, R. (1969) Drug and enzyme induction. Ann. Rev. Pharmacol. 9:21-36.

Levis, G. M. (1970) 2-Hydroxy fatty acid oxydase of rat kidney. Biochem. Biophys. Res. Commun. 38:470-477.

Litterst, C. L., Mimnaugh, E. G., Reagan, R. L., and Gram, T. E. (1975) Comparison of *in vitro* drug metabolism by lung, liver and kidney of several common laboratory species. Drug Metab. Dispos. 3:254-265.

Lowry, O. H., Rosebrough, N. J., Fair, A. G., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mansuy, D., Beaune, P., Crestell, T., Lange, G., and Leroux, J. P. (1977) Evidence for phosgen formation during liver microsomal oxidation of chloroform. Biochem. Biophys. Res. Commun. 79:513-517.

McClean, A. E. M. (1970) The effect of protein deficiency and microsomal enzyme induction by DDT and phenobarbitone on the acute toxicity of chloroform and a pyroolizidine alkaloid, retrosine. Br. J. Exp. Path. 51:317-321.

McCormack, K. M., Kluwe, W. M., Sanger, V. L., and Hook, J. B. (1978) Effect of polybrominated biphenyls on kidney function and activity of renal microsomal enzyme. Environ. Health Perspect. 23:153-157.

McCormack, K. M., Melrose, P., Rickert, D. E., Dent, J. G., Gibson, J. E., and Hook, J. B. (1979) Contaminant dietary exposure to polychlorinated biphenyls and polybrominated biphenyls: tissue distribution and arylhydrocarbon hydroxylase activity in lactating rats. Toxicol. Appl. Pharmacol. 47:95-104.

McCormack, K. M., Braselton, W. E., Sanger, V. L., and Hook, J. B. (1980) Residual effect of polybrominated biphenyls following perinatal exposure in rats. Toxicol. Appl. Pharmacol. 53:108-115.

Melvin, D. R. (1979) Carcinogenicity of chloroform. Environmental Health Perspective 33:171-182.

Nebert, D. W., and Gelboin, H. V. (1969) The *in vivo* and *in vitro* induction tissues, strains and developmental and hormonal states. Arch. Biochem. Biophys. 134: 76-89.

Nebert, D. W., Gielen, J. E., and Goujon, E. M. (1972) Genetic expression of arylhydrocarbon hydroxylase induction. III. Changes in the binding of n-octylamine to cytochrome P-450. Mol. Pharmacol. 8:651-666.

Nebert, D. W., and Gelboin, H. V. (1968) Substrate-inducible microsomal arylhydrocarbon hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. J. Biol. Chem. 243:6242-6249.

Nebert, D. W., Leuitt, R. C., Orlando, M. M., and Felton, J. S. (1977) Effect of environmental chemical on the genetic regulation of the microsomal enzyme system. Clin. Pharmacol. Ther. 22:640-658.

Parke, D. V. (1968) The biochemistry of foreign compounds. Pergamon Press, Oxford, p. 269.

Paul, B. B., and Rubinstein, D. (1963) Metabolism of carbon tetrachloride and chloroform by the rat. J. Pharmacol. Exp. Ther. 141:141-148.

Peets, E. A., Henson, M. F., and Neri, R. (1974) On the mechanism of the antiandrogenic action of flutamide in the rat. Endocrinology 94:532-554.

Pohl, L. R., Bhooshan, B., Whittaker, N. F., and Krishna, G. (1977) Phosgen, a metabolic of chloroform. Biochem. Biophys. Res. Commun. 79:684-691.

Pohl, L. R., and Krishna, G. (1978) Deuterium isotope effect in bioactivation and hepatotoxicity of chloroform to phosgen. Biochem. Pharmacol. 28:561-563.

Pohl, L. R., George, J. W., Martin, J. L., and Krishna, G. (1979) Deuterium isotope effect *in vivo* bioactivation of chloroform to phosgen. Biochem. Pharmacol. 28: 561-563.

Pfeiffer, C. A., Emmel, V. M., and Gardner. (1940) Renal hypertrophy in mice receiving estrogen and androgen. Yale J. Biol. and Mol. 12:493-501.

Reitman, S., and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Path. 28:56-63.

Reuber, M. D. (1979) Carcinogenicity of chloroform. Environ. Health Perspect. 31:171-182.

Reynolds, E. S., and Yee, A. G. (1967) Liver parenchymal cell injury. V. Relationships between patterns of chlromethane C_{14} incorporation into constituents of liver *in vivo* and cellular injury. Lab. Invest. 16:591-603.

Scholler, K. L. (1970) Modification of the effect of chloroform on the rat liver. Br. J. Ana. 42:603-605.

Selye, H. (1939) The effect of testosterone on the kidney. J. Urol. 42:637-641.

Shire, J. G. M., and Bartke, A. (1972) Strain differences in testicular weight and spermatogenesis with special references to C57, BL/1.5 and DBA/2J mice. J. Endocr. 55:163-171.

Shubic and Ritchie, A. C. (1953) Sensitivity of male DBA mice to the toxicity of chloroform as a laboratory hazard. Science 117:285.

Sipes, I. G., Krishna, G., and Gillette, J. R. (1977) Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: role of cytochrome P-450. Life Science 20:1541-1548.

Smith, H. W., Finkelstein, W., Aliminosa, L., Crawford, B., and Graber, M. (1945) The renal clearance of substituted hippuric acid derivations and other aromatic acid in dogs and man. J. Clin. Invest. 24:388-404.

Steel, R. G. D., and Torrie, J. H. (1965) Principles and producers of statistics. McGraw-Hill, New York.

Taylor, D. C., Brown, D. M., Keeble, R., and Langley, P. F. (1974) Metabolism of chloroform. II. A sex difference in the metabolism of (14C) chloroform in mice. Xenobiotica 4:165-174.

Uehleke, H., and Werner, T. (1975) A comparative study on the irreversible binding of labeled halothane, trichlorofluromethane, chloroform and carbon tetrachloride to hepatic protein and lipids *in vitro* and *in vivo*. Arch. Toxicol. 34:289-308.

Uehleke, H., Werner, T., Greim, H., and Kramer, M. (1977) Metabolic activation of halokanes and tests *in vitro* for mutagenicity. Xenobiotica 7:393-400.

Von Oettingen, W. F. (1964) The halogenated hydrocarbons of industrial and toxicological importance. Elsevier, Amsterdam, New York, pp. 77-108.

Wade, N. (1977) Drinking water: health hazards still not resolved. Science 196:1421-1422.

Wartrous, W. M., and Plaa, G. L. (1972) Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Toxicol. Appl. Pharmacol. 22:528-543.

Wiebel, F. J., Leutz, J. C., and Gelboin, H. V. (1973) Arylhydrocarbon (benzo[a]pyrene) hydroxylase-inducible in extrahepatic tissue of mouse strains not inducible in liver. Arch. Biochem. Biophys. 154:292-294.

Wiebel, F. J., Leutz, J. C., Diamond, L., and Gelboin, H. V. (1971) Arylhydrocarbon (benzo[a]pyrene) hydroxylase in microsomes from rat tissue: differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biphys. 144:78-86.

Winslow, S. G., and Gerstner, H. B. (1978) Health aspects of chloroform - a review. Drug Chem. Toxicol. 1: 259-275.

Wilson, J. D. (1972) Recent studies on the mechanism of action of testosterone. N. Eng. J. Med. 287:1284-1291.

Yashikodama and Bock, F. G. (1970) Benzo a pyrene-metabolizing enzyme activity in liver and various strains of mice. Cancer Res. 30:1846-1849.

Zaleska-Rutczynska, Z., and Krus, S. (1972) Effect of chloroform on the mouse kidney. II. Resistance of the F_1 generation of a susceptible C3H/He strain and a resistant C57BL/6JN strain. Pathol. Pol. 23: 185-188.

Zaleska-Rutczynska, Z., and Krus, S. (1973) Genetic aspects of the high and low sensitivity to chloroform of male mice of various inbred strains and their crosses. Pol. Med. Sci. Hist. Bull. Abst. 1:245-250.