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FACTORS INFLUENCING HALOTHANE HEPATOTOXICITY IN THE RAT
HYPOXIC MODEL.

THE UNIVERSITY OF ARIZONA,

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FACTORS INFLUENCING HALOTHANE HEPATOTOXICITY
IN THE RAT HYPOXIC MODEL

by

Richard Chen-Main Jee

A Thesis Submitted to the Faculty of the
COMMITTEE ON TOXICOLOGY (GRADUATE)
In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

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STATEMENT BY AUTHOR

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ABSTRACT

Halothane, an inhalational anesthetic, was hepatotoxic in phenobarbital pretreated male Sprague Dawley rats when administered under hypoxic conditions ($FI_{O_2}=0.14$). The degree of hepatotoxicity, determined from morphology and SGPT levels, correlated well with hepatic cytochrome P_{450} content and the inspired halothane concentration. Maximal lesion intensity developed within 12 to 24 hours after exposure to 1% halothane ($FI_{O_2}=0.14$). Preanesthetic treatment of rats with possible antidotal agents, such as diethyldithiocarbamate (DDC) reduced halothane metabolite formation (blood levels) and prevented halothane mediated liver injury.

The results of this animal study support the hypothesis that reductive biotransformation of halothane results in the production of toxic intermediates which initiate halothane-induced liver injury.

INTRODUCTION

It is well documented that the hepatic mixed function oxidase system is involved in the metabolism of endogenous compounds, drugs and other xenobiotics. Biotransformation generally results in the production of metabolites that are less toxic than the parent molecule. However, occasionally drugs and chemicals are not detoxified, but rather are bioactivated to chemically reactive metabolites. Bioactive or reactive metabolites are so called because they readily react with cellular components and, in many cases, covalently bind to these substituents. Slater (1966), Brodie (1967) and others have suggested that covalent binding of these metabolites to tissue macromolecules is responsible for producing cellular injury. It was proposed that toxicity is the result of the covalent interaction between reactive metabolites and tissue macromolecule. If this hypothesis is true, then the site of cellular injury should be the site of covalent binding and the degree of tissue damage should closely parallel the extent of this binding (Gillette, 1974).

Since the liver is the primary site of drug metabolism, it would be expected that some reactive metabolites would first bind to liver tissues and produce hepatic toxicity as a direct result. This appears to be the case for several known hepatotoxins.

Several chemically inert aromatic hydrocarbons are converted by the hepatic mixed function oxidase system to alkylating agents that react with tissue macromolecules and produce centrilobular necrosis.

One example from this class of compounds is bromobenzene, which is chemically unreactive and cannot covalently bind to tissue components. However, in vivo metabolism results in the production of a chemically reactive metabolite (an arene oxide) that covalently binds in the liver. Brodie (1971) observed by autoradiography of liver that ^{14}C from radiolabeled bromobenzene became covalently bound to centrilobular sites. These same sites had previously been identified as the primary site of bromobenzene toxicity (Koch-Weser, 1952). Pretreatment of rats (Brodie, 1971) and mice (Reid, 1971) with phenobarbital, an inducer of hepatic drug metabolizing activity, increased the amount of covalently bound ^{14}C from bromobenzene, as well as the rate of bromobenzene metabolism. In all studies, increased metabolism due to phenobarbital pretreatment paralleled the increased severity of liver necrosis. Additionally, the rate of metabolism of bromobenzene in various animal species has corresponded to the incidence of hepatic toxicity in these species (Mitchell, 1971). Other halogenated benzene compounds (chlorobenzene, iodobenzene, and o-dichlorobenzene) that bind covalently to liver tissue have been observed to produce hepatocellular necrosis. However, compounds that do not appreciably bind to liver macromolecules, such as fluorobenzene and p-dichlorobenzene, do not result in liver injury (Reid, 1973).

Similarly, centrilobular necrosis due to toxic doses of acetaminophen has been shown to be the result of a chemically reactive metabolite (Mitchell, 1973a; Jollow, 1973; Potter, 1973; Mitchell, 1973b). Above a critical threshold dose of acetaminophen (300 mg/kg in mice), the

severity of hepatic necrosis paralleled the extent of covalent binding of radiolabeled acetaminophen. Both hepatotoxicity and covalent binding mediated by acetaminophen were increased in phenobarbital pretreated animals (Mitchell, 1973a; Jollow, 1973).

Carbontetrachloride (CCl_4), long used as a household and industrial solvent, can produce hepatotoxicity following either acute or chronic exposures. The ease with which CCl_4 can produce fatty liver and liver necrosis has made it useful as an experimental tool for the study of liver function and liver toxicity. An extensive volume of literature has accumulated on its hepatotoxicity in various species. As early as 1951, and again in 1964, CCl_4 was shown to be metabolized to CO_2 in experimental animals (McCollister et al., 1951; Rubinstein and Kanics, 1964). Butler (1961) demonstrated in vitro that rat liver was responsible for the conversion of CCl_4 to CHCl_3 . He suggested that CCl_4 became chemically reactive by the replacement of chlorine by hydrogen producing free radicals that might react with enzymes or other tissue components. Recknagel (1967) further supported the free radical concept and suggested that these reactive intermediates of CCl_4 account for the major, early occurring morphologic alterations of the endoplasmic reticulum (Recknagel, 1967) and the decrease in protein synthesis (Smuckler, Iseri, and Beditt, 1962). Several lines of evidence point to the homolytic cleavage of CCl_4 to the trichloromethyl free radical ($\cdot\text{CCl}_3$) (Slater, 1972), including the detection of CHCl_3 (Butler, 1961; Fowler, 1969) and hexachloroethane (CCl_3CCl_3) (Fowler, 1969; Fowler and Alexander, 1969). The relationship between metabolism

and toxicity is further confirmed by studies where prior treatment of rats with phenobarbital increased CCl_4 toxicity (Gardner and McLean, 1969). Other studies have demonstrated that treatments that alter the severity of the hepatic lesion caused by CCl_4 administration also parallel changes in the levels of covalent binding of radiolabeled CCl_4 to liver protein and phospholipid (Traiger and Plaa, 1971; Maling et al., 1974). From their studies of CCl_4 , Sipes et al. (1977) concluded that cytochrome P-450 was the site of activation of CCl_4 and that metabolism proceeds along a cytochrome P-450 dependent reductive pathway.

As previously stated, one of the metabolites of CCl_4 is chloroform (CHCl_3) (Butler, 1961; Paul and Rubinstein, 1963) which has been recognized as a hepatotoxin since 1848 (Defalque, 1968). Van Dyke and Gandolfi (1964) showed that CHCl_3 was metabolized in some part by the liver to CO_2 and Cl^- . In 1969 Cohen and Hood observed, although they did not identify, two metabolites by thin layer chromatography, in addition to CO_2 and Cl^- . D. M. Brown et al. (1974) observed that as much as 80% of administered $^{14}\text{CHCl}_3$ was recovered as $^{14}\text{CO}_2$ in mice, and speculated that the intermediate was trichloromethanol, which would decompose to phosgene (COCl_2). In other studies, the administration of radiolabeled CHCl_3 resulted in ^{14}C covalent binding to cellular components including protein, in liver (Illett et al., 1973; Brown et al., 1974) and in kidney (Illett et al., 1973). The degree of binding paralleled the severity of hepatic and renal necrosis in untreated and phenobarbital pretreated animals exposed to CHCl_3 . Other studies with

inducers of the mixed function oxidase system (phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-p-dioxin) of liver and kidney have further demonstrated the cause and effect relationship of metabolism and tissue toxicity (Kluwe and Hook, 1978; Kluwe, McCormack, and Hook, 1978). Data from these studies lend further support to the concept that reactive, toxic metabolites are generated at sites close to the sites of tissue injury. Studies with inhibitors of drug metabolism piperonyl butoxide (Illett et al., 1973), disulfiram (Scholler, 1970) and SKF-525A (Sipes et al., 1977) further supported the concept of bioactivation of CHCl_3 by the hepatic mixed function oxidase system. Furthermore, Sipes et al. (1977) showed that CHCl_3 , unlike CCl_4 , proceeded along a cytochrome P-450 dependent oxidative pathway for metabolism.

Most inhalational anesthetic agents are halogenated hydrocarbons and in view of the existing information on two members of this class, carbon tetrachloride and chloroform, there is some concern as to the possible role of bioactivation in the production of toxicity. Isolated clinical cases of hepatotoxicity have been reported following anesthesia with several of these compounds: enflurane (Sadove and Kim, 1974; Denlinger, Lecky, and Nahrvoold, 1974), fluoroxene (Reynolds, Brown, and Vandam, 1972), methoxyfluorene (Joshi and Conn, 1974; Lindenbaum and Leifer, 1963) and halothane (Brody and Sweet, 1963; Bunker and Blumenfeld, 1963; Lindenbaum and Leifer, 1963). But evidence for the agent specific production of hepatotoxicity is equivocal at best. Evidence for the bioactivation of chemically inert

drugs to alkylating, arylating, or acetylating agents that covalently bind to liver macromolecules is largely indirect. The evidence consists first in the demonstration of some degree of metabolism and second in the correlation of the extent of covalent binding to the severity of cellular injury. Halothane is the only anesthetic that has been studied to any extent in this regard.

In 1947, halogenated hydrocarbons were introduced as anesthetics because of their reduced flammability as compared to organic ether. Halothane, 1,1,1 trifluoro-2-bromo-2-chloroethane, is one such synthetic fluorinated compound accepted for clinical use in the United States in 1957. Its pharmacological properties of smooth anesthetic induction and anesthetic potency quickly made halothane one of the most widely used general anesthetics. Presently, approximately 10 million halothane anesthetics are administered in this country each year.

Its popularity has been somewhat diminished because of the occasional report of sporadic and unpredictable post-anesthetic hepatitis. Hepatic necrosis attributed to halothane exposure has been described as a progressive liver injury indistinguishable from viral hepatitis by common clinical laboratory tests (Brown and Sipes, 1974). Although the etiology of this disease is unknown, the pathogenesis often results in hepatic coma or mortality, secondary to liver failure. The association between halothane and hepatitis prompted the National Halothane Study in 1962. This survey retrospectively examined 856,515 recorded anesthetic administrations over a three year period (1959-1962) in an attempt to correlate clinical factors that might be predictive of halothane initiated liver injury. Age, obesity, sex and prior exposure

to halothane were equivocally related to the occurrence of post-anesthetic hepatitis. More recently, studies have indicated that the incidence of liver damage following halothane anesthesia ranges from one in 7000 to one in 35,000 clinical cases (Inman and Mushin, 1974; Bottinger, Daline, and Mullen, 1976). The conditions predisposing surgical patients to halothane caused liver injury are still questionable. The lack of adequate animal models to provide a controlled means of studying the liver injury and, thus, clearly associating hepatic damage with a definite mechanism of toxicity has contributed to this equivocal state.

At present there is considerable evidence to support the causal relationship of halothane metabolism and the development of liver toxicity. Van Dyke and co-workers (Van Dyke, Chenoweth, and Van Posnak, 1964) demonstrated halothane biotransformation in vivo and in vitro which was increased following phenobarbital treatment. Subcapsular hepatic necrosis has been observed following the administration of halothane to phenobarbital pretreated rats by Reynolds and Moslen (1977).

Although halothane may be metabolized by both oxidative and reductive routes (Van Dyke and Chenoweth, 1965; Van Dyke and Gandolfi, 1976), its reductive biotransformation appears to lead to bioactivation rather than to detoxification. Uehleke, Hellmer, and Tabarelli-Poplawski (1973) and Van Dyke and Gandolfi (1974) found that covalent binding of ^{14}C halothane metabolites was enhanced after anerobic incubation of the radiolabeled anesthetic with isolated hepatic

microsomes. Similarly, isolated rat liver perfused with blood, low in dissolved oxygen tension, yielded higher levels of halothane metabolites covalently bound to subcellular macromolecules and an increased production of reductive metabolites (Van Dyke and Wood, 1973; Nastainczyk, Ullrich, and Sies, 1978). In vivo studies with rats exposed to halothane under a hypoxic atmosphere have also exhibited an increased reductive defluorination of halothane and elevated levels of halothane intermediates bound to hepatic subcellular components (Widger, Gandolfi, and Van Dyke, 1976; Sipes et al., 1977).

Since some hepatotoxic compounds such as carbon tetrachloride are reported to cause a more intense liver necrosis when metabolism is enhanced by pretreatment with phenobarbital, a somewhat similar role for halothane reductive biotransformation may be indicated in the mediation of this post-anesthetic liver injury.

Animal Models

Recently hepatic necrosis due to halothane anesthesia has been observed in rats pretreated with polychlorinated biphenyls (Aroclor 1254). Sipes and Brown (1976) found that a single intraperitoneal injection of Aroclor 1254 five days before halothane exposure (1% for 2 hours in 99% O₂) produced extensive centrilobular necrosis and elevated SGPT levels 24 hours after anesthesia. This first animal model of halothane hepatotoxicity, involving the potent biotransformation enzyme inducer Aroclor 1254 (PCB) required an extensive increase in a broad spectrum of hepatic biotransformation enzymes (Sipes and Brown, 1976; Reynolds and Moslen, 1977). Such an alteration by Aroclor 1254

in the reductive or non-oxygen dependent pathway of halothane metabolism might produce reactive intermediates that may cause liver injury; possibly by reacting with and becoming covalently bound to hepatic sub-cellular components (Van Dyke and Gandolfi, 1974; Van Dyke and Wood, 1975; Sipes and Brown, 1976). However, two major drawbacks to this model are that the spectrum of biotransformation enzymes elevated by Aroclor 1254 is not what would be expected in the normal human liver system; and that Aroclor 1254 may produce its own alterations in liver morphology, independent of the effects of halothane. Thus, the Aroclor 1254- or PCB- model of halothane induced liver injury may represent the additive or synergistic effect of two weak hepatotoxins.

In 1977, Brown and Sipes reported preliminary studies with a new animal model for halothane induced liver injury. The critical difference in this model was that the rats were exposed to anesthetic concentrations of halothane at mildly hypoxic conditions (14% O₂: 85% N₂). Subsequent studies by this group (McLain, Sipes, and Brown, 1979) and others have established the hypoxic model of halothane hepatotoxicity. This model is based on the premise that the reductive, hepatotoxic pathway of halothane biotransformation will be enhanced by exposure at hypoxic conditions. To further enhance this pathway, the animals were pretreated for several days with phenobarbital. treated for several days with phenobarbital.

The pertinence of low oxygen exposure in the hypoxic model may directly relate to reports of decreased hepatic arterial blood flow due to clinical halothane administration (Gelman, 1976; Benumof et al., 1976).

Since patients are frequently premedicated prior to anesthesia with agents which increase the biotransformation of anesthetics (Van Dyke and Chenoweth, 1965; Rice, Sbordore, and Masse, 1978), this hypoxic model of halothane hepatotoxicity may more realistically represent the conditions present during clinical anesthesia than previous animal models.

STATEMENT OF PROBLEM

This study was designed to characterize the halothane hypoxic model and the factors which modify the halothane induced liver injury. Particular emphasis was placed on determining critical levels of microsomal enzyme induction, inspiration of oxygen and halothane for the production of liver injury. The time course for lesion development and repair were investigated with and without the administration of potential inhibitors of lesion progression. Since male and female differences in biotransformation have proven important in the toxic response to various other compounds, the sex difference in response to halothane and hypoxia was tested for the dependence of liver injury due to discrete sex differences in the biotransformation of this volatile anesthetic.

MATERIALS AND METHODS

Chemicals

The following reagent grade chemicals were purchased from the Sigma Chemical Company (St. Louis, Mo.): Cystamine dihydrochloride, Lpcysteine hydrochloride, N-acetylcysteine hydrochloride, actinomycin D, 3-methylchloanthrene, cycloheximide, tetsosterone proprionate, and chloamphenicol. Skf 525-A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) and Aroclor 1254 were kindly supplied by Smith Kline and French Laboratories and Monsanto Inc., respectively. Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) and vinblastine were obtained from the Aldrich Chemical Co. (Milwaukee, Wis.) and the Eli Lilly Co. (Indianapolis, Ind.). Halothane (Fluothane^R) was purchased from Ayerst Laboratories, Inc.

Experimental Animals

Male and female Sprague-Dawley rats (175-225 gm) were obtained from Hilltop Laboratories (Chatsworth, Ca.) for use in the experiments. The animals were housed in temperature controlled, isolated rooms (free from environmental contamination and regulated for diurnal light cycles). Prior to experimentation the animals were fed laboratory chow (Wayne Lab Blox) and water ad libitum. Unless specifically noted, all the rats received 0.1% phenobarbital in their drinking water for 10 days to maximally induce the liver microsomal drug metabolizing enzymes (Daly, 1970).

Animal Exposures

Animal exposures to halothane were conducted in a Lexan chamber (180 liter) using a Vernitrol Anesthesia vaporizer set at a flow rate of 6 liters/min. Internal chamber concentrations of halothane were quantified by using gas-tight syringe air samples, which were analyzed on a Varian Model #1440 gas chromatograph, equipped with thermoconductivity detectors and gas sampling valves (250 μ liter loops). The desired chamber oxygen concentrations were obtained by the mixing of pure oxygen and nitrogen gases and were measured with an Ohio membrane-oxygen analyzer. The internal chamber temperature was controlled at 27 $\pm$ 0.5 degrees centigrade by a thermostatic heat exchanger. Rapid equilibration of the chamber atmosphere was achieved by an AC internal muffin fan.

Animal Sacrifice and Tissue Preparation

At specified times following halothane exposure, the rats were sacrificed by cervical dislocation, so blood samples could be taken from the inferior vena cava for serum glutamic pyruvic transaminase (SGPT) analysis. The animal livers were quickly removed, blotted and dissected to obtain 2 transverse sections (3-4 mm width) from the superior right hepatic lobe. These tissue specimens were immersed in 10% phosphate buffered formalin (pH 7.4) for 48 hours. Paraffin embedded samples were sectioned into 5 micron slices with an AO microtome, and stained with standard Hemoxyl-in-eosin.

Histologic Scoring

Morphologic examination of each stained liver slice was performed in a single-blind fashion with a Nikon binocular microscope at 110 and 220X. Ten randomly selected microscopic fields across each slide section were evaluated and assigned histology scores. Each field received a histology value which was based on the presence of cell disruption or cell necrosis, and the extent of microscopic field area involved with the cell injury at 110X (percent area of the field of vision). The ten subsequent scores per slide were then averaged for the assignment of an overall mean histology score for each slide preparation. Thus, the degree of liver injury or morphologic alteration was descriptive of both the microscopic field of view and the overall liver slide section preparation. The following scale was used to score all liver sections and fields.

<u>Histology Score</u>	<u>Definitions</u>
0	Normal
1	Mild cell disruption (less than 25% tissue area)
2	Moderate cell disruption, appearance of balloon cells, (less than 50% tissue area)
3	Extensive cell disruption with numerous balloon cells (more than 50% tissue area)
4	Extensive cell disruption with occasional hepatocellular necrosis
5	Extensive cell disruption and hepatocellular necrosis covering more than 25% of plane of view

According to this scale, cell disruption was defined as any aberration in all architecture without the presence of hepatocellular necrosis. These changes in cell morphology were characterized by a dissociation of hepatic cell chords, cytoplasmic enlargement, intracellular fatty accumulation, or the infiltration of reticuloendothelial cells. Cellular necrosis was identified by layers of cells containing karyolytic or pyknotic nuclei. The focal points of these necrotic areas were predominantly in sharply defined perimeter areas surrounding the hepatic central veins.

Difluorochloroethylene (F_2),
Trifluorochloroethane (F_3) Assay

Immediately following halothane exposure, 0.2 ml blood samples were removed from the rats, via the tail vein, and transferred by heparinized syringe to 1 ml septum vials kept on ice.

The blood samples were equilibrated to room temperature, vortexed for 20 seconds, and placed on a block heater at 37° C for 20 minutes.

With the use of a gas-tight syringe, the entire head space (1 ml), in equilibrium with the weighed blood sample, was injected into a Varian #1440 gas chromatograph. Chromatographic separation was attained with Poropak Q, 100-120 mesh with the following temperature and flow settings:

Injection port	180° C
Column	150° C
Detector	240° C

Nitrogen carrier gas	30 ml/min
Compressed air	300 ml/min
Hydrogen gas	30 ml/min

A flame ionization detector (FID) and Varian CDS 101 integrator were used for sample detection and quantification. Standards were prepared with blank blood samples containing known concentrations of CDE and CTE.

Trifluoroacetic Acid (TFA) Analysis

Aliquots of urine (0.1 ml) or plasma (0.2 ml) were pipetted in 1 ml gas-tight, septum vials (Supelco) containing 500 µl concentrated sulfuric acid. The vials and the acid should be ICE-CHILLED before the samples are added, then capped, with septum in place.

The vials were then vortexed (10 sec) and 0.1 ml dimethyl-sulfate (DMS) was added and the vials were resealed. Next the vials were heated at 60° C (for 20 minutes) to promote the methyl-esterification of the TFA; then allowed to equilibrate at 37° C for 20 minutes (minimum). The head space of each vial was injected on a Varian (Model 1440) gas chromatograph equipped with a Poropak Q column and flame ionization detector. (A 0.25 ml gas sampling valve was present on the G.C.) The following channel settings were used for instrument calibration and sample assays:

FID ---- 150° C
Injection port ---- 130° C
Column ---- 110° C
Carrier gas flow ---- 30 ml/min

After the initial air peak, two separate peaks are resolved. The first peak is methylated TFA and the second peak is methyl bromide.

Animal Groups

To perform these studies, the animals were divided into groups as follows:

Group A. Rats in this group were exposed to 1% halothane for 2 hours at forced inspired oxygen concentrations of 0.10, 0.14, 0.21, or 0.99 percent. The controls for this group received no halothane at their assigned oxygen concentrations. The group receiving 10% oxygen ($F_{I}O_2 = 0.10$) were exposed only to 0.5% halothane due to the acute lethality of 1% halothane at this oxygen tension. All animals were sacrificed at 24 hr post anesthesia.

Group B. Animals in this group were divided into subgroups (n=6) for a 2 hr exposure to halothane at concentrations of either 0.1%, 0.3%, 0.5%, 1%, or 1.3%. During halothane exposure, these animals were maintained at $F_{I}O_2 = 0.21$ or 0.14. The animals were sacrificed 24 hr post exposure.

Group C. To achieve different levels of liver microsomal enzyme induction, rats were pretreated with a single intraperitoneal injection of various doses of 3-methylcholanthrene (3 MC), Aroclor 1254 (PCB), or Phenobarbital (PB). Varying degrees of induction of the drug metabolizing enzymes were achieved by administration of four dose levels of each compound to groups of rats. PCB (15, 100, 300, 500 mg/kg) and PB (10, 30, 100 mg/kg) were administered 48 hr prior to exposure

conditions of the hypoxia model (1% halothane, 14% oxygen) while 3-MC (5, 10, 30, 100 mg/kg) was given 24 hr preanesthesia.

These resultant grade enzyme inductions were compared to the standard phenobarbital pretreatment of 10 days as described above. Animals were sacrificed 24 hr following halothane exposure for comparison of their histological scores. Identically pretreated but non-halothane-exposed animals were sacrificed at the time of anesthesia, the livers removed and microsomal fractions were obtained by differential centrifugation. The microsomal cytochrome P-450 content was determined by the method of Omura and Sato (1964). Cytochrome P-450 contents were then correlated to liver injury.

Group D. Rats of this group were exposed to 1% halothane at $F_{I}O_2 = 0.14$ for either 3 min, 15 min, 30 min, 1 hr, or 2 hr and then sacrificed at 24 hr post anesthesia for comparison of liver histologies. All of these time intervals were measured from the time of loss of physical motion, which occurred at approximately 5 min after onset of exposure.

Group E. Animals of group E were sacrificed at 3 hr, 6 hr, 12 hr, 2 days, 3 days, 4 days, 5 days, or 10 days after exposure to 1% halothane at $F_{I}O_2 = 0.14$ for 2 hr.

Group F. Two inhibitors of drug metabolism, SKF-525-A (50 mg/kg ip) or metyrapon (250 mg/kg ip) and three compounds containing free sulfhydryl groups, cysteine (1.9 gm/kg po), cystamine (1.0 gm/kg po) or N-acetylcysteine (1.0 gm/kg po) were administered as a single dose to rats at various times before or after halothane exposure for 2 hr at $F_{I}O_2 = 0.14$. Control animals received the appropriate blocking agent

but were subjected to hypoxia ($F_{I}O_2 = 0.14$) only. All animals in this group were sacrificed 24 hr post exposure to halothane or hypoxia.

Group G. Rats of this group were pretreated with DDC (diethyldithiocarbamate) at a daily dose of 300 mg/kg, ip for four days prior to halothane-hypoxic exposure. Following halothane exposure part of the DDC treated animals were sacrificed for measurements of volatile halothane metabolites (blood trifluoroacetic acid, difluorochloroethylene, and trifluorochloroethane). The remainder of the DDC treated animals were sacrificed 24 hr after halothane exposure so that comparisons (histology, SGPT, urinary fluoride, and urinary trifluoroacetic acid) could be made with non-DDC pretreated animals receiving halothane at $F_{I}O_2 = 0.14$.

Group H. Animals were exposed to hypoxic model conditions and were maintained on treatment regimens of cycloheximide, actinomycin D, vinblastine, or chloramphenical until time of sacrifice. Each rat received either intraperitoneal injections of cycloheximide (1.25 mg/kg) or actinomycin D (0.15 mg/kg) every 12 hours; or oral administrations of vinblastine (0.15 mg/kg) or chloramphenical (100 mg/kg) every 24 hours. Animals of each drug treatment group were sacrificed at 2, 4, 7, or 10 days after halothane exposure for comparison of hepatic morphological changes.

Group I. PB-pretreated female rats were exposed to 1% halothane at $F_{I}O_2 = 0.14$ or 0.21 for 2 hr and sacrificed at 24 hr postanesthesia. Sections of liver and blood samples were obtained for comparison histologic rating and SGPT values, respectively.

Group J. Female rats were pretreated with testosterone proprionate (one subcutaneous injection of 2.5 mg/kg, every 4th day for 12 days) for comparison with non-masculinized female rats and male animals exposed to halothane under hypoxic model conditions. At the time of sacrifice (24 hrs post halothane hypoxic exposure) liver tissue, blood and 24 hr urine samples were obtained for measurements of known halothane metabolites (free inorganic fluoride, bifluorochloroethane, difluorochloroethylene, and trifluoroacetic acid), histology scores, and SGPT.

Statistics

The values are reported as the mean $\pm$ standard error of the mean. Unless specifically noted, all animal groups contained at least 6 rats. Statistical significance of the data was determined by the Student's "t" test.

RESULTS

The typical hepatic morphological alterations due to halothane exposure of phenobarbital at $F_{I}O_2 = 0.14$ are illustrated in Figure 1. This section was obtained from a liver removed at 24 hr after the end of a 2 hr anesthesia with 1% halothane. This liver section was assigned a histology score of 4 since multiple but sharply circumscribed zones of cellular degeneration were observed in the centrilobular regions. Although the general liver architecture was maintained, it may be seen that injured centrilobular cells are extremely swollen and vacuolized. Under closer examination (220X), many of these hepatocytes contain strands of degeneration cytoplasm with pyknotic or eccentric nuclei. These areas of cell injury and coagulative necrosis are present while cells in the periportal region of the lobule appear undamaged. In assessing factors which may affect this cell injury, exposure of phenobarbital pretreated male rats (Group A) to halothane resulted in liver damage only when the halothane was administered at hypoxic concentrations of oxygen (Table 1). Halothane exposure at atmospheric oxygen concentrations or in a pure oxygen atmosphere did not lead to hepatic damage except for some mild cellular disruption in rats exposed to 21% oxygen. No histological alterations were evident without the presence of halothane, thus hypoxia alone cannot account for the observed liver damage. As shown in Figure 2, a dose response relationship was observed between the inspired concentrations of halothane

Figure 1. Photomicrograph: Liver Section from the Halothane-Hypoxic Model.

Table 1. Effects of Inspired Oxygen on Halothane-Induced Hepatotoxicity -- All values are mean $\pm$ SE. Phenobarbital-induced male rats were exposed to halothane for 2 hr and sacrificed 24 hr after anesthesia. Criteria for assignment of histology score is described in Methods.

Inspired O ₂ Concentration	N	Histology Score with 1% Halothane	N	Histology Score without Halothane
10%	10	4.8 $\pm$ 0.3 ^{a,b}	6	0.5 $\pm$ 0.3
14%	135	4.2 $\pm$ 0.01 ^b	22	0.5 $\pm$ 0.1
21%	20	1.0 $\pm$ 0.4	20	0.5 $\pm$ 0.2
99%	13	0.3 $\pm$ 0.2	10	0.3 $\pm$ 0.2

^a 0.5% halothane

^b p < 0.05 when compared to 21% or 99% oxygen and when compared to histology score in the absence of halothane.

Figure 2. Effects of Halothane Concentration on the Degree of Halothane Induced Liver Injury.

Phenobarbital-pretreated male rats were exposed to halothane for 2 hr at inspired oxygen concentrations of 14% or 21%. The values represent the mean $\pm$ SE for liver morphological alterations (histology score) and SGPT units 24 hr after exposure. $N \geq 6$. The histology score and SGPT values for non-halothane exposed rats were 0 and 25 ± 2 , respectively.

(Group B) and subsequent liver damage, only when halothane was delivered under hypoxic (14%) concentration of oxygen. Under the conditions of these experiments lesion intensity was maximal at 0.5% halothane.

However, 1% halothane was used in the subsequent experiments because it can be related to previous studies and because it produces the desired pharmacological effect of anesthesia. Rats were also exposed to 1.3% halothane, but many died of acute respiratory failure during anesthesia.

Pretreatment with the microsomal enzyme inducers phenobarbital or Aroclor 1254 (PCB) was prerequisite for lesion development. The level of microsomal cytochrome P-450 in animals pretreated with those agents (Group C) correlated well with the ability of halothane to induce liver injury (Figure 3). Liver lesions did not develop in animals pretreated with 3-MC, an agent that preferentially induces cytochrome P-448. A single injection of phenobarbital two days prior to using the rats in the halothane hypoxic model system produced liver lesions of a similar frequency and intensity to those observed in rats treated extensively for 10 days to phenobarbital water.

Maximal lesion intensity was noted following a 30 minute exposure of rats (Group D) in the halothane hypoxic model (Figure 4). There was no noticeable difference in morphological change between the 30 minute and normal two exposures, whereas the SGPT values increased linearly with length of exposure. Two shorter exposure (3 and 15 minutes) periods produced mild to moderate cellular disruption, but no necrosis or elevated SGPT values. In a separate experiment (data not shown) rats (N = 4) exposed to 0.3% halothane for four to six hours in a hypoxic atmosphere produced only minimal lesions (histology score =

Figure 3. Comparison of Microsomal Cytochrome P-450 with Lesion Intensity after Halothane Exposure in Male Rats Pretreated with 3-methylcholanthrene (5,10,30,100 mg/kg ip), Phenobarbital (10,30,100 mg/kg ip, or 0.1% in Drinking Water for 10 Days), or Aroclor 1254 (15,100,300,500 mg/kg ip).

Animals were sacrificed 24 hr. after exposure to 1% halothane and 14% oxygen for 2 hr. Cytochrome P-450 concentrations were measured in rats receiving pretreatment but no halothane. $N \geq 6$.

Figure 4. Effects of Length of Exposure to Halothane on Halothane-Induced Liver Injury.

Male phenobarbital-treated rats were exposed to 1% halothane at 14% oxygen for the selected time and sacrificed 24 hr post anesthesia. Values represent mean $\pm$ SE for histology score and SGPT. $N \geq 6$.

$1.25 \pm .25$; SGPT = 25 ± 6). Thus, concentration of halothane appears to be more important than length of exposure in the halothane hypoxic model.

The cellular changes produced in the halothane hypoxia model plateaued by 12 hours (Figure 5) following exposure (Group E). Multiple to confluent centrilobular necrosis was observed in all livers obtained at 12, 24, or 48 hours. A rapid, almost symmetrical, recovery occurred within four days following the exposure. Interestingly, by two hours after exposure subcapsular changes in the liver were noted and by 6 hours mild cell disruption was observed. Both SGPT (Figure 5) and ICDH levels (data not presented) were progressively elevated following exposure with maximal levels attained at two days post exposure. These enzyme levels quickly returned to normal values by the third day after exposure.

When rats were pretreated with the biotransformation inhibitors, SKF-525A or metyrapone, prior to exposure in the halothane hypoxia conditions, there was an extensive reduction in the intensity of the liver lesion (Table 2). Marginal inhibition was observed when these inhibitors were administered 4 hours after the end of exposure. By 8 hours after exposure, these agents were ineffective in preventing lesion development. However, three sulfhydryl containing compounds, cysteine, cystamine, and N-antylcysteine, were effective at inhibiting lesion development when administered 4 to 8 hours post anesthesia (Table 2). Cystamine was the most effective since it produced nearly complete protection when given at 8 hours post anesthesia (Figure 6,7) while other agents had only marginal effect at this time.

Figure 5. Time Course for the Development and Repair of Halothane-Induced Liver Injury.

Phenobarbital-pretreated male rats were exposed to 1% halothane at 14% oxygen for 2 hr and then sacrificed at various times after end of exposure. Values were plotted as mean $\pm$ SE for $N \geq 6$.

Table 2. Effects of Drug Metabolism Inhibitors and Certain Free Sulfhydryl Compounds on Halothane-Induced Liver Injury: Comparison of Pre or Post Halothane Treatment -- All phenobarbital-pretreated rats were sacrificed 24 hr after end of a 2 hr exposure to 1% halothane, delivered in 14% oxygen. Values represent histology score SE for $\bar{n} \geq 6$ rats per group.

Time of Admin. of Putative Inhibitor	Histology Score					
	Cystamine 1.0 g/kg po	Cysteine 1.9 g/kg po	N-acetylcysteine 1.0 g/kg po	SKF-525A 50 mg/kg ip	Metyrapone 250 mg/kg ip	Halothane hypoxic model: no inhibitor treatments
2 hr pre anesthesia	4.4 ± 0.2	4.0 ± 0.2	3.7 ± 0.1	2.1 ± 0.4 ^a	1.4 ± 0.5 ^a	4.5 ± 0.3
4 hr post anesthesia	1.5 ± 0.1 ^a	1.3 ± 0.1 ^a	1.3 ± 0.1 ^a	3.0 ± 0.2	2.6 ± 0.3 ^a	
8 hr post anesthesia	1.5 ± 0.2 ^a	2.6 ± 0.5 ^a	2.7 ± 0.2 ^a	3.1 ± 0.2	3.6 ± 0.2	
12 hr post anesthesia	3.1 ± 0.3	3.8 ± 1.0	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	

^a p < 0.05 when compared to halothane hypoxic model (no inhibitor treatments).

Figure 6. Photomicrograph. Liver Section 24 hrs after Hypoxic Model Conditions.

Figure 7. Photomicrograph: Liver Section 24 hrs after Cystamine Post-treatment.

DDC, diethyldithiocarbamate, was found to protect against hepatotoxicity produced under conditions of the halothane-hypoxic model (See Methods, Animals and Exposures), only when administered in a dosage regimen of one daily dose (300 mg/kg, ip) for 4 days prior to halothane exposure (Figure 8). This 4-day dosage schedule of DDC was used since previous studies presented findings indicative of hepatic cellular changes related to the administration of a single dose of DDC in phenobarbital pretreated rats (Figure 9). The morphology changes found 24 hr after a single dose of DDC (300 mg/kg, ip) are evidenced by extensive cell vacuolization in the centrilobular regions of the animal livers. Hydropic degeneration and ballooning of cells was present in these defined areas, but without the loss of any cell nuclei. However, when phenobarbital pretreated rats received DDC for 4 days rat liver morphologies appeared normal and indistinguishable from non-DDC treated control histologies (Figure 10). Under this extensive administration schedule of phenobarbital pretreatment and the 4-day DDC pretreatment of animals prior to halothane and hypoxia, the DDC treated rats produced lower levels of halothane metabolites than non-DDC pretreated animals. The DDC pretreatment significantly reduced concentrations of urinary trifluoroacetic acid, blood trifluoroacetic acid, and urinary inorganic fluoride ion (F^-) in these animals (Table 3).

Although DDC administration only modestly decreased the blood levels of halothane metabolites, trifluorochloroethane (F_3) and difluorochloroethylene (F_2) (Table 3) no presence of liver injury or SGPT elevation was apparent in the DDC pretreated animals following halothant (Figure 8).

Figure 8. Photomicrograph: Liver Section 24 hrs after Hypoxic Model Conditions and DDC Pre-treatment.

Figure 9. Photomicrograph: Liver Section 24 hours after 1 Dose DDC in the Phenobarbital Pretreated Rat.

Table 3. The Effects of DDC on the Halothane Hypoxic Model.

Trifluoroacetic Acid			
	$\mu\text{m/ml}$ blood	$\mu\text{m/ml}$ urine	
HH	$.89 \pm .29$ (SD)	9.95 (pooled values)	
DDC HH	$.30 \pm .17$ (SD)	.568	
	$R = .998$ $p < .05$	$R = .999$ $p < .05$	

Hepatic Cytochrome			
	P450 $\mu\text{m/mg}$ liver pr	SGPT $\mu\text{m/ml}$	
HH	$.95 \pm .13$ (SD)	43.3 ± 8.7 (SD)	
DDC HH	$1.02 \pm .37$	108.3 ± 20.98 (SD)	
Control n=2	.76	23 ± 2.8	

	Fluoride μmol urine	Net Fluoride ion μm blood	Histology
HH	$7.09 \pm .39$ (SD)	5.47	$4.1 \pm .2$
DDC	$3.46 \pm .04$ (SD)	1.84	$.3 \pm .3$
Control	$1.62 \pm .08$ (SD)	-	-

	Difluorochloroethylene nm/gm blood	Trifluorochloroethane nm/gm blood	
HH	$2.63 \pm .8$	5.13 ± 3.1	
DDC	$1.62 \pm .17$	$3.0 \pm .75$

Figure 10. Photomicrograph: Liver Section after 4 Doses DDC in the Phenobarbital Pretreated Rat.

Agents known to interfere with protein or nucleic acid synthesis prolonged the presence of liver injury after halothane hypoxic exposure. Rats treated with chloramphenicol, cycloheximide, actinomycin-D, or vincristine were found to have elevated liver histology scores on the 4th day after halothane exposure on day 10 (Figure 11). The histology scores in animals treated with chloramphenicol or cycloheximide remained elevated on day 10 following halothane, whereas liver morphologies from animals receiving vincristine or actinomycin-D were similar to control livers. The histology scores from animals which received one of these agents but no halothane were indistinguishable from control rat livers.

When female Sprague-Dawley rats (Group I) were used in the halothane hypoxia model, only minimal cellular disruption was noted at 24 hours after exposure (Table 4, Figure 12). In addition, SGPT values in treated female rats were never elevated above control levels, whereas concurrently exposed male rats produced the typical histological score and elevated SGPT values (Table 4). To determine if female rats developed halothane hepatotoxicity more slowly than males, livers were removed from females periodically to ten days after exposure. At no time did liver lesions develop subsequent to halothane exposure.

In contrast to Group I, female rats pretreated with testosterone (Group J) produced histology scores similar to male animals exposed to hypoxic model conditions (Figure 13). The masculinized female animals exhibited only small elevations in SGPT even though intermittent areas of centrilobular injury were present in the liver sections (Figure 14).

Figure 11. Effects of Protein and Nucleic Acid Synthesis Inhibitors on Halothane Liver Injury.

Table 4. Failure of Female Rats to Develop Halothane-Induced Liver Injury in the Hypoxic Model -- All rats were pretreated with phenobarbital and exposed to 1% halothane for 2 hr at the designated inspired oxygen concentration. SGPT values and liver histology scores were determined in samples obtained at 24 hr after anesthesia.

Condition	Sex	N	SGPT Units	Histology Score
1% Halothane 14% Oxygen	F	22	30.4 $\pm$ 7.9	0.4 $\pm$ 0.3
1% Halothane 21% Oxygen	F	12	35.2 $\pm$ 6.2	0.2 $\pm$ 0.2
Control	F	10	29.5 $\pm$ 7.3	0.2 $\pm$ 0.2

1% Halothane 14% Oxygen	M	12	122 $\pm$ 28 ^a	4.2 $\pm$ 0.1 ^a

^a p < 0.05 when compared to female rats at 14% oxygen.

Figure 12. Photomicrograph: Liver Section of Female Sprague Dawley after Halothane-Hypoxic Exposure.

Figure 13. Effects of Testosterone and Cystamine on Female Rats under Hypoxic Model Conditions.

Tes = testosterone treated. Cysta = cystamine treated. All animals were examined for histology score and SGPT levels following hypoxic model conditions.

Figure 14. Photomicrograph: Liver Section of Female Rat after Testosterone Pretreatment and Hypoxic Model Conditions.

Post-anesthetic treatment with cystamine (4 hr post) inhibited the liver morphology changes in testosterone-treated females and concurrently exposed male animals of this group (Figure 13).

Female rats pretreated with polychlorinated biphenyls (PCB) exhibited moderate increases in histology scores and SGPT levels (Figure 13). The histological changes produced in the livers of female rats pretreated with PCB alone and PCB pretreated rats anesthetized with halothane under hypoxic conditions are represented by Figures 15 and 16. These morphology changes were representative of intermediate liver injury, rather than the confluent necrosis present in the PCB treated males. To further demonstrate the presence of a sex difference in halothane biotransformation, halothane metabolites were measured in male, female, testosterone-pretreated female, and castrated male rats after hypoxic model conditions. Female and castrated-male animals exhibited decreased levels of urinary inorganic fluoride, serum fluoride, difluorochloroethylene, and trifluorochloroethane with correspondingly decreased histology and SGPT levels (Table 5). The testosterone pretreated female rats displayed histology, urinary fluoride and trifluorochloroethane values similar to the halothane exposed male animals.

Figure 15. Photomicrograph: Liver Section of Female Rat after PCB Pretreatment.

Figure 16. Photomicrograph: Liver Section of Female Rat after PCB Treatment and Hypoxic Model Conditions.

Table 5. Halothane Metabolites in Male, Female, Testosterone-Female, and Castrated-Male Rats.

	Male	Female	Testosterone-Female	Castrated-Male
Urinary F ⁻ (24 hr sample) Moles	6.80 ± 1.0	2.40 ± 0.9*	5.12 ± 0.1	3.90 ± 0.6*
Serum F ⁻ Moles	1.78 ± 0.2	0.29 ± 0.04*	0.37 ± 0.1*	0.42 ± 0.07*
Difluorochloroethylene nm/gm bld	1.70 ± 0.1	1.25 ± 0.2*	1.73 ± 0.1	0.8 ± 0.1
Triflurochloroethane nm/gm bld	5.25 ± 3.2	2.53 ± 0.6	7.01 ± 1.7	1.89 ± 0.9*

* p <.05 vs male

DISCUSSION

This study, which is a characterization of the halothane hypoxia hepatotoxicity model (McLain et al., 1979), yields insight into the factors that promote halothane hepatotoxicity. It also demonstrates plausible antidotal agents that may reduce the severity of the halothane-induced liver lesion.

It is apparent that elevation of the content of hepatic microsomal cytochrome P-450 is required for the expression of halothane-initiated liver damage in the hypoxic model (Figure 3). Previous studies have shown that this increase in cytochrome P-450 results in enhanced biotransformation of halothane (McLain et al., 1979). It is of interest that a single, large dose of PB will promote halothane liver damage in our hypoxic model, although the degree of damage was only two-thirds of that attained by continuous PB administration. PCB pretreatment can also promote halothane induced hepatotoxicity. Induction of hepatic biotransformation enzymes with 3-MC fails to promote halothane-initiated liver lesions in the hypoxia model. This lack of effect may be due to the low level of cytochrome P-448 present after 3-MC pretreatment or to the fact cytochrome P-448 does not mediate the reductive bioactivation of halothane. Previous studies by Van Dyke and Gandolfi (1976) support the latter reason. They demonstrated that the reductive metabolism of halothane is enhanced by pretreatment with phenobarbital but not 3-MC.

The data presented in Figure 2 are the first to demonstrate a dose-response curve for halothane-related hepatotoxicity. Thus, under appropriate conditions, halothane is a true hepatotoxin and does not produce toxicity by a hypersensitivity reaction. The presence of some liver toxicity at 0.1% halothane in the hypoxic model is not alarming since anesthetics are believed to be at saturating biotransformation enzyme levels at one-tenth to one-thirtieth their anesthetic concentrations (Hitt et al., 1977).

A hypoxic inspired oxygen concentration ($F_{I}O_2$ less than 0.21) is an absolute necessity for the production of extensive liver necrosis with halothane in PB-treated rats (Table 1). Contrarily, in PCB pre-treated rats liver lesions develop following halothane anesthesia at hyperoxic, atmospheric or hypoxic levels of inspired oxygen (Sipes and Brown, 1976; Reynolds and Moslen, 1977). The reason for this difference is not known. However, PCBs, being potent and wide spectrum inducers, may induce the reductive pathway to such a degree that a decrease in oxygen is not necessary to promote the reductive metabolism of halothane. Evidence for this was reported by Sipes and Brown (1976), who found a five-fold increase in in vitro covalent binding of (^{14}C -)halothane equivalents to lipids of microsomes from PCB-treated rats when incubations were performed in air. Since lipid binding occurs subsequent to a reductive or nonoxygen-dependent bioactivation of halothane (Widger et al., 1976), it appears that PCBs are potent inducers of that pathway. However, it should also be stressed that the lesion produced in PCB-treated rats is more severe if the halothane is delivered under hypoxic conditions.

An interesting discovery in the characterization of the hypoxic model for hepatotoxicity is the appearance of liver damage following a short exposure to the halothane-low oxygen conditions. This rather swift toxic response may be explained by the rapid saturation (approximately 8 min) of the liver with the highly lipophilic halothane (Lowe et al., 1974). Apparently the damage incurred by halothane under hypoxic conditions is very rapid and subsequent longer exposure to halothane has little additional effect on liver cell architecture. Sawyer et al. (1971), has reported that high levels of anesthetics inhibit their own biotransformation. Thus, the same may be the case when toxicity is related to metabolism.

The rapid repair of the halothane lesion in our rodent model is not surprising. Rat liver has a very rapid regeneration rate (Haug et al., 1973) which may explain its ability to repair extensive damage induced by several hepatotoxins (Smuckler, Koplitz, and Sell, 1976). This passive nature of the halothane-initiated liver lesion may reflect the moderate hepatotoxicity of halothane (compared to CCl_4), the lack of total inhibition of critical cell processes in a large number of liver cells, or the remarkable rapid repair of the halothane-damaged cellular apparatus by the rat liver system.

The fact that SGPT levels were significantly, but not markedly elevated underscores that halothane is a weak hepatotoxin. Large areas of coagulative necrosis were not observed in the majority of cases. Most lesions were rated with histological scores of 3 or 4, which are described as extensive cell disruption and vacuolization, but only occasional necrosis.

When various agents were used to intensify the severity of hepatocellular injury the administration of cycloheximide, chloramphenicol, vinblastine, or actinomycin-D only extended the presence of liver injury after exposure to halothane and hypoxia, but did not allow the complete degeneration of the liver morphology. These results may indicate an incomplete inhibition of cellular repair processes in rat liver since these agents were administered at such non-toxic low dose levels. However, the effectiveness of cycloheximide and chloramphenicol in extending the presence of the liver injury may represent a requirement for protein synthesis as an initial event for hepatic repair following this type of hepatocellular insult.

The human condition involving halothane-initiated liver damage is normally progressive and often fatal (Carney and Van Dyke, 1972). The difference between our animal model and the human condition is unknown, but may relate to differences in species susceptibility and to the fact that the human cases observed by the clinician are only the worst cases.

Another interesting difference between the human situation and this animal model is that female rats did not develop halothane-induced lesions. Clinical reports indicate that in humans the female is more likely to develop halothane hepatitis (Zimmerman, 1978). For this reason the resistance of female rats to halothane and hypoxia was probably due in part to a decrease in halothane biotransformation. This difference between male and female rats in the metabolism may be reversed, since testosterone pretreatment of female animals increased the

concentration of halothane metabolites and produce liver injury. The inductive effect of testosterone upon various hepatic mixed-function oxidase reactions in animals has been reported. Similarly, castrated male rats, which have lower rates of drug metabolism than non-castrated males, neither developed high levels of halothane metabolites nor morphological evidence of liver injury. The sex difference in halothane associated liver injury is probably dependent upon sex related differences in drug biotransformation and, thus, differences in halothane metabolism.

The drug metabolism inhibitors, SKF-525A and metyrapone, inhibited halothane-induced liver injury most effectively when administered prior to anesthesia. We could not demonstrate any significant inhibition of lesion development when these agents were administered at times later than 4 hr post halothane exposure. This would not be surprising, except that de Ferreyra et al. (1977) have reported that SKF-525A reduced CCl_4 -induced hepatotoxicity when administered 12 hr after CCl_4 . However, the pharmacokinetic profile of these agents may differ because of their physical properties and different routes of administration. The data do provide additional evidence that the halothane metabolism related to its hepatotoxicity is mediated by a cytochrome P-450 system.

Another possible mechanism used in other studies to reduce hepatotoxicity as a result of reactive intermediates is the administration of free sulfhydryl containing compounds. One such sulfhydryl containing compound effective against carbon tetrachloride experimental

hepatotoxicity is DDC (den Tonkelaar and van Logten, 1974; Popp, Shinozuka, and Farber, 1978; Siegers, Filser, and Bolt, 1978). Since this compound is a metabolite of a clinically used drug (Disulfram^R), it was considered as a possible antidotal candidate for our animal model. However, the pre-exposure and post-exposure administration of a comparable single DDC dose (300 mg/kg, ip) used in other studies (Popp et al., 1978) produced equivocal morphological results in the hypoxic model (hydropic degeneration and balloon cells (Figure 8). These results were probably the result of DDC toxicity since DDC may be metabolized to carbon disulphide, a moderately hepatotoxic compound and enhanced DDC metabolism may have been present in our phenobarbital pretreated animals. Later studies showed that by varying the dosage regimen of DDC no morphology damage was present in the livers of the phenobarbital pretreated rats after 4 days of DDC treatment (Figure 9). When this 4 day treatment regimen was used in phenobarbital pretreated rats, and these DDC-phenobarbital pretreated animals were exposed to halothane ($FI_{O_2} = 0.14$), there was protection from halothane hepatotoxicity. At the same time, this protection of liver toxicity coincided with decreases in the level of halothane metabolites (Table 3). This observation lends further support to the role of halothane biotransformation in the pathogenesis of hepatotoxicity due to halothane. The absolute decrease in metabolites formed and the relative change in metabolites produced was probably responsible for protection from halothane hepatotoxicity in our animal model. It is of interest that DDC

significantly decreased levels of urinary F^- while only marginally decreasing serum levels of F_2 and F_3 . Since F_2 , F_3 , and inorganic F^- are all products of halothane reductive metabolism (Cohen, 1978) it appears that measurements of any one metabolite of halothane biotransformation could be misleading and not indicative of total reductive halothane metabolism. In this case, the "point in time measurements" of serum F_2 and F_3 were only slightly decreased while the urinary F^- level (based on a 12 hr collection) was significantly reduced and more informative.

Other, less toxic, sulfhydryl-containing compounds are known to be effective when administered several hours after a variety of hepatotoxins (de Ferreyra, et al., 1977, 1979). Of the three compounds reported here, cystamine was the most effective, since it afforded protection when administered at 8 hr after anesthesia. It is too early to speculate if these agents will be useful in the human situation. Until sensitive indicators of halothane-induced liver injury are available, diagnosis of such injury will be delayed until overt signs and symptoms develop. This is usually 3 days or longer after clinical anesthesia. It is unlikely that reactive intermediates of halothane would still be present after 3 days and that sulfhydryl compounds would provide nucleophilic sites for these intermediates. It is more likely that the sulfhydryl compounds protect by other mechanisms. They may stabilize both injured and intact cell membranes and components and prevent their irreversible damage (Castro et al., 1973). Also, they may stimulate repair processes that enhance cell regeneration. If one

or both of these latter mechanisms are shown to occur, then sulfhydryl therapy may prove useful in clinical halothane hepatitis.

The hypoxic model of halothane induced hepatotoxicity represents many of the cellular and subcapsular changes associated with its clinical counterpart of centrilobular damage. This animal model is based on the reductive biotransformation of halothane and thus, requires induction of hepatic drug metabolizing enzymes; while proper dose concentrations of halothane under hypoxic conditions appear to effect necrosis more significantly than duration of halothane exposure. The Sprague-Dawley rat responds to this halothane model with sex related differences which are dependent in part to the level of halothane biotransformation and reversible by testosterone pretreatment of female animals. Various agents which decrease the halothane mediated injury appear to act by inhibiting halothane metabolism, scavenging of reactive intermediates, and/or enhancing hepatic repair.

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