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CHARACTERIZATION OF HYPERTHYROIDISM ENHANCEMENT OF HALOTHANE-INDUCED HEPATOTOXICITY*

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Abstract—Administration of anesthetic doses of halothane to hyperthyroid male rats results in the development of hepatic necrosis. The severity of the hepatic lesion was dependent on the dose of triiodothyronine (T_3) and the length of time it was administered. Pretreatment of rats with iodinated metabolites of thyroxin which do not induce hyperthyroidism did not result in any signs of hepatotoxicity after halothane exposure. The administration of halothane to hyperthyroid female rats or mice of either sex did not result in the development of any overt hepatotoxicity. Likewise, hyperthyroidism did not enhance the hepatotoxicity of another hepatotoxin bromobenzene. The *in vitro* enzymatic activities associated with cytochrome P-450-dependent metabolism and glutathione *S*-transferase conjugation activity were markedly altered in hyperthyroid rats. Cytochrome P-450 levels, aminopyrine *N*-demethylase activity, glutathione levels and glutathione *S*-transferase activity were all significantly lower in hyperthyroid rats. However, other enzyme activities were stimulated by T_3 pretreatment; aniline hydroxylase activity was increased by 45% and cytochrome *c* reductase activity was increased by 54% in hyperthyroid rats. Glutathione levels were also reduced significantly in hyperthyroid male rats. Maximal changes in both the cytochrome P-450 system and in the glutathione detoxification system were required before halothane demonstrated its hepatotoxic effects. Thus, a new balance between cytochrome P-450-dependent bioactivation and glutathione conjugation of halothane may be necessary for the exaggerated hepatotoxicity of halothane seen in hyperthyroid male rats.

The hyperthyroid state has been shown to potentiate the liver injury caused by a number of different hepatotoxins. Carbon tetrachloride [1], 1,1-dichloroethylene [2], chloroform [3] and acetaminophen [4] are all hepatotoxins whose toxicity is markedly potentiated if administered to hyperthyroid rats. Each of these hepatotoxic agents is bioactivated by cytochrome P-450-dependent pathways [5]. Since hyperthyroidism has been shown to alter the *in vitro* and *in vivo* activity of cytochrome P-450 [6], changes in the metabolism of these hepatotoxic agents in hyperthyroid animals probably results in an increased production of a toxic metabolite leading to an increase in the amount of cellular injury and death. For example, acetaminophen causes severe hepatic necrosis in hyperthyroid rats, and there is quantitatively more reactive metabolite (as measured by a glutathione adduct) and less unchanged parent drug and nontoxic metabolites of acetaminophen produced in these animals [5]. This indicates that changes in cytochrome P-450-dependent metabolism may contribute to potentiation of acetaminophen hepatotoxicity even though the level of cytochrome P-450 is decreased.

Halothane also produces overt hepatic necrosis in hyperthyroid rats [7]. Hepatic centrilobular necrosis develops in rats pretreated with triiodothyronine to induce hyperthyroidism and then anesthetized with 1% halothane for 2 hr in an atmosphere of 21% oxygen. Other anesthetics such as enflurane and pentobarbital did not cause hepatotoxicity in hyperthyroid rats. It was proposed that perhaps an intracellular hypoxia induced by hyperthyroidism causes an increase in the reductive biotransformation of halothane to a reactive metabolite which produces cell death and hepatic necrosis [7]. However, increasing the oxygen concentration to 99% only marginally decreased the severity of the hepatic necrosis. Therefore, the purpose of this study was to further characterise the halothane-induced hepatotoxicity in hyperthyroid animals by attempting to correlate the observed hepatotoxicity with the biochemical changes associated with hyperthyroidism.

METHODS

Animals. Outbred strains of male and female Sprague–Dawley rats, Hap:SD(BR), or Swiss-origin mice (Hap:ICR) (Harlan Industries, Indianapolis, IN) were used throughout these studies. The rats (100–200 g) were housed in wire cages, and the mice (25–40 g) were housed in plastic cages. Both were allowed food (Wayne Lab-Blox, Allied Mills, Chi-

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cago, IL) and water *ad lib*. Temperature, 24°, and a 12-hr light/dark cycle in the animal facility were maintained automatically.

Drugs and chemicals. The following drugs and chemicals were obtained in the highest purity available (greater than 98%) and were used in these studies. Halothane was a gift of the Ayerst Laboratories, Inc., New York NY. Streptozotocin, triiodothyronine, thyroxin, diiodothyronine, reverse triiodothyronine, glutathione (reduced form), nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate, glucose-6-phosphate dehydrogenase (Torule enzyme), cytochrome *c* (from horse heart muscle), semicarbazide (hydrochloride salt) and aniline (hydrochloride salt) were obtained from the Sigma Chemical Co., St. Louis, MO. Aminopyrine and *p*-aminophenol were obtained from the Aldrich Chemical Co., Milwaukee, WI. Bromobenzene, 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were obtained from the Eastman Kodak Co., Rochester, NY.

Treatments. Water soluble drugs were dissolved in 0.9% NaCl (saline) before injection. Triiodothyronine (T_3), thyroxin (T_4), diiodothyronine (T_2) and reverse triiodothyronine (rT_3) were dissolved in 0.75 N NaOH before saline was added. The pH of the final solution was between 8.5 and 9.5. Food was withheld from fasted or starved animals for a period of 48 hr. Diabetes was produced by administering streptozotocin to male rats. Streptozotocin was dissolved in 0.1 M citrate buffer (pH 4.5) and administered intravenously under light ether anesthesia. Water insoluble drugs were dissolved in corn oil. All the drugs were administered by intraperitoneal injection unless noted otherwise.

Halothane was administered via inhalation at a concentration of 1% halothane in a 21% oxygen atmosphere. The halothane was vaporized using a calibrated Fluotec vaporizer (Cyprane, North America Inc., Tonowanda, NY), and the halothane concentration of the inspired gas mixture was monitored by gas chromatography (Varian model 3700, Varian Instruments, Palo Alto, CA). The oxygen concentration was monitored with a polarographic oxygen analyzer (Ohio 200 Oxygen Monitor, Ohio Medical Products, Madison, WI).

Histopathology. Rats were decapitated, and livers were perfused *in situ* with ice-cold saline. A single lobe of the liver was removed and fixed in 10% buffered formalin. Tissues were then imbedded in paraffin, sectioned, and stained with hemotoxylin and eosin.

Assays. Reduced hepatic glutathione [8] and serum glutamate-pyruvate transaminase (SGPT) [9] were determined as previously described. Blood was collected from animals following decapitation.

Microsomes and 105,000 *g* supernatant fraction (cytosol) were isolated using the following procedure. Rats were decapitated, and the livers were perfused *in situ* with cold saline. A 33% homogenate (w/v) was made with 0.25 M sucrose using a Potter–Elvehjem homogenizer with a motor-driven teflon pestle. The homogenate was centrifuged at 9,000 *g* for 15 min. The supernatant fraction was removed and centrifuged at 18,000 *g* for 15 min. Microsomes were isolated when the 18,000 *g* supernatant fraction was centrifuged at 105,000 *g* for 60 min. The cytosol was removed, and the microsomes were homogenized in 1.15% KCl and centrifuged again at 105,000 *g* for 45 min. The supernatant fraction was discarded, and the microsomes were rehomogenized in 0.1 M phosphate buffer (pH 7.4). Protein determinations were done following the method described by Lowry *et al.* [10] using bovine serum albumin as a standard. Microsomal protein was adjusted to 20 mg/ml, the cytosolic protein was adjusted to 10 mg/ml with the appropriate buffer. The microsomal protein concentration was adjusted to 1 mg/ml with 0.1 M phosphate buffer (pH 7.4) for measurement of cytochrome P-450. Cytochrome P-450 content was determined as previously described [11]. NADPH–cytochrome *c* reductase activity was determined using the method of Masters *et al.* [12].

Aniline hydroxylase activity in isolated microsomes was determined by the method described by Imai *et al.* [13]. The reaction was initiated by the addition of 2 mg of microsomal protein and lasted for 20 min at 37°. The amount of *p*-aminophenol formed was determined after the protein was precipitated by the addition of 0.5 ml of 10% trichloroacetic acid. Purified *p*-aminophenol was used to establish a standard curve.

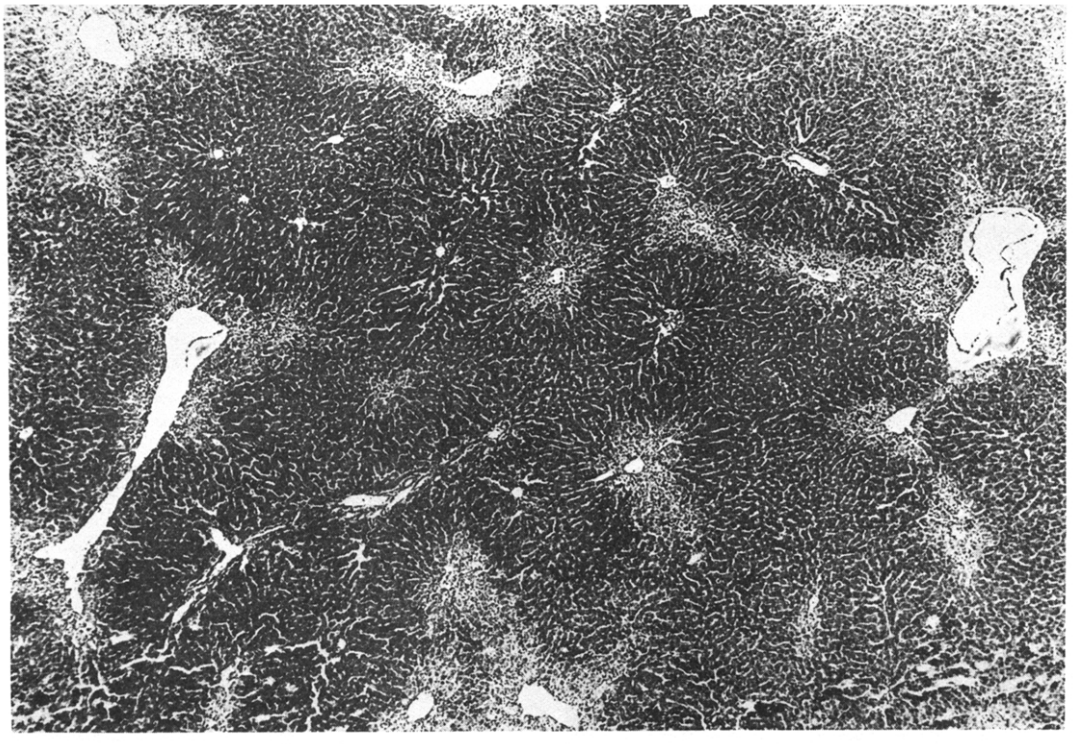
Aminopyrine *N*-demethylase activity was determined according to the method of Cochin and Axelrod [14]. The reaction was initiated with the addition of 0.2 mg of microsomal protein and continued for 30 min at 37°. The reaction was terminated by the addition of 1.0 ml of 15% trichloroacetic acid. The amount of formaldehyde formed during the incubation was determined using the method described by Nash [15]. Known concentrations of formaldehyde were used to establish a standard curve.

Glutathione *S*-transferase activity of the 105,000 *g* supernatant fraction was estimated by determining the rate of glutathione conjugation to two halogenated benzenes [16]. 1,2-Dichloro-4-nitrobenzene conjugation by glutathione *S*-transferase was determined in an incubation solution consisting of 0.1 M phosphate buffer (pH 7.5) with 3 μmoles of dichloronitrobenzene and 0.025 mg of cytosolic protein. The reaction was initiated by the addition of 15 μmoles of reduced glutathione and was monitored at 344 nm. An extinction coefficient of 8.5 mM^{-1} cm^{-1} was used to determine the rate of glutathione conjugation. 1-Chloro-2,4-dinitrobenzene conjugation with glutathione was assayed in 0.1 M phosphate buffer (pH 6.5) containing 3 μmoles of chlorodinitrobenzene and 0.015 mg of cytosolic protein. The reaction was monitored at 340 nm and was initiated by the addition of 3 μmoles reduced glutathione. An extinction coefficient of 9.6 mM^{-1} cm^{-1} was used.

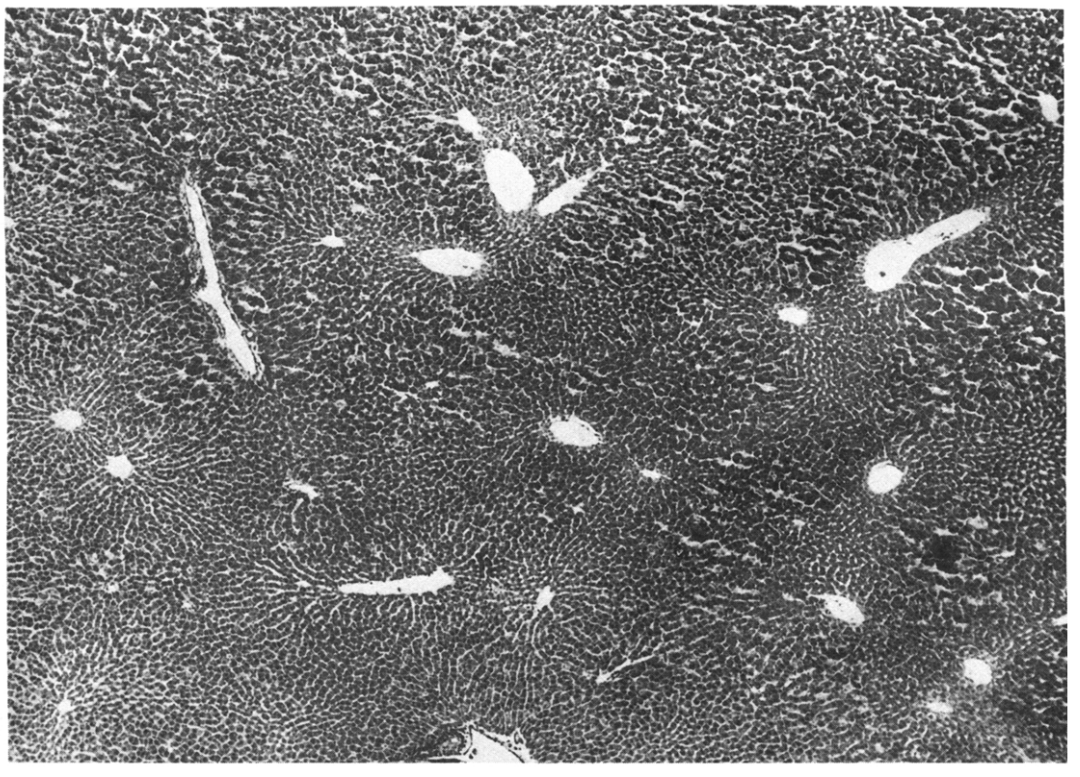
Statistics. All results are reported as the mean ± S.E.M. Differences between means were determined using Student's *t*-test. Changes were considered significantly different when the P value was less than 0.05.

RESULTS

Histopathology. The administration of anesthetic doses of halothane to hyperthyroid rats results in the



(A)



(B)

Fig. 1. (A) Halothane-induced hepatic histopathology in hyperthyroid rats. Male rats were pretreated with 3 mg/kg triiodothyronine for 6 days to induce hyperthyroidism. On day 7, 1% halothane was administered for 2 hr. Shown is a representative section of a liver taken 4 hr after halothane exposure. Hematoxylin-eosin stain, 2.5× magnification. (B) Repair: Male rats were pretreated with 3 mg/kg triiodothyronine for 6 days and were exposed to 1% halothane for 2 hr on day 7. Shown is a representative liver section taken 24 hr after halothane exposure. Hematoxylin-eosin stain, 2.5× magnification.

development of hepatic necrosis. Euthyroid control rats exposed to halothane under the same experimental conditions do not develop any hepatic lesions. Male rats were pretreated with 3 mg/kg triiodothyronine (T_3) for 6 days to induce hyperthyroidism. During the pretreatment period, the rats exhibited clinical signs of hyperthyroidism, such as loss of body weight and increased body temperature. Twenty-four hours after the last dose of triiodothyronine, the rats were exposed to 1% halothane for 2 hr via inhalation in a 21% oxygen atmosphere. Four to twelve hours after halothane administration, frank hepatic necrosis developed (Fig. 1A). This hepatic necrosis was primarily located in the centrilobular area of the hepatic lobule with lesions radiating from the centrilobular towards the periportal area of the hepatic lobule. The lesion, however, was rarely lethal, and the area of hepatic damage was readily repaired. Approximately 24 hr after halothane administration, repair mechanisms had almost completely replaced the necrotic centrilobular area (Fig. 1B), and only a few necrotic cells and lymphocytes were still evident.

The severity of the hepatic lesion was dependent on the dose of T_3 and the length of time it was administered. Histopathological examination of livers from rats pretreated for 1, 2 or 3 days with T_3 did not reveal any overt hepatic necrosis after halothane exposure. At least 4 days of T_3 administration (3 mg per kg per day) was required before overt hepatic necrosis developed after halothane exposure (Table1). Maximal lesion development occurred about 4 hr following halothane administration, and repair mechanisms quickly replaced the necrotic areas. If the dose of T_3 was lowered to 100 μg per kg per day and administered for 10 days, then maximal lesion development did not occur until 12–24 hr after the halothane exposure, and repair processes were not completed until 48 hr post-exposure.

Elevation of serum liver enzyme levels. Serum glutamate-pyruvate transaminase (SGPT) levels were elevated in hyperthyroid rats exposed to 1% halothane for 2 hr in a 21% oxygen atmosphere (Fig. 2). This elevation of SGPT levels was time dependent. Significant elevations occurred immediately after halothane inhalation was terminated, and SGPT activity continued to rise for 4 hr after discontinuing halothane exposure. Maximal elevations

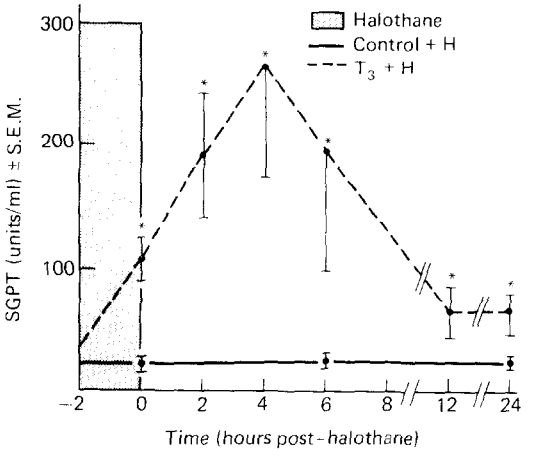


Fig. 2. Halothane-induced elevation of serum glutamate-pyruvate transaminase (SGPT) activity. Halothane (1% for 2 hr) was administered to hyperthyroid or euthyroid rats. The rats were killed at the indicated times before or after halothane administration. Data represent the mean ± S.E.M. of five to twelve rats. An asterisk indicates a significant difference from control.

of SGPT levels were approximately 11-fold greater than SGPT levels of euthyroid rats exposed to halothane or hyperthyroid rats not exposed to halothane. The maximal elevation of SGPT levels in hyperthyroid rats exposed to halothane preceded the appearance of the maximal hepatic lesion, as shown by histopathology. The return of SGPT levels toward basal levels occurred about 12–24 hr after halothane exposure.

Analogues of triiodothyronine. Male rats were pretreated with equimolar doses of T_3, reverse T_3, diiodothyronine (T_2), and (+)- and (−)-thyroxin (T_4) for 6 days. On day 7, the rats were anesthesized with 1% halothane for 2 hr. The hepatotoxicity of halothane after each of these pretreatment regimens was determined by monitoring SGPT activity and assessing histopathological changes. Pretreatment of rats with iodinated metabolites of thyroxin which do not induce hyperthyroidism did not result in any signs of hepatotoxicity after halothane exposure. However, treatment with equimolar concentrations (4.6 μmoles/kg) of (−)-T_4 produced marginal elev-

Table 1. Effect of T_3 dose and pretreatment period on halothane-induced hepatotoxicity*

Length of T_3 pretreatment (days)	dose (mg/kg)	Time of sacrifice† (hr)	Livers with necrosis‡ (%)	SGPT (units/ml)
4	3.0	2	42	118 ± 26§
6	3.0	4	78	225 ± 64§
10	0.1	24	80	ND‖

* Male rats were pretreated with the indicated dose of triiodothyronine (T_3) for the indicated length of time. Twenty-four hours after the last T_3 injection, the rats were exposed to 1% halothane for 2 hr. Groups of seven to fifteen rats were used.

† Time after halothane exposure.

‡ Percentage of rats that developed overt hepatic necrosis after halothane.

§ Significantly different from corresponding control (euthyroid).

‖ Not determined.

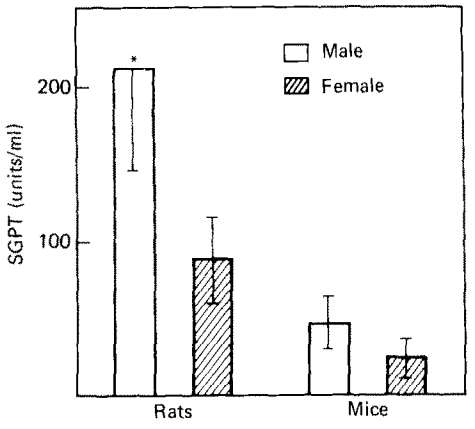


Fig. 3. Effect of halothane on male and female hyperthyroid rats and mice. Hyperthyroid male and female Sprague–Dawley rats and Swiss-origin mice were exposed to 1% halothane for 2 hr. The animals were killed 4 hr after halothane exposure. Values represent the mean ± S.E.M. of six to ten animals. An asterisk indicates a significant difference from female of same species.

ations of SGPT activity and a low level of overt hepatic necrosis ofter halothane exposure. If equipotent doses (23.0 μmoles/kg) of (−)-T_4 were administered, then the elevations in SGPT activity and amount of hepatic necrosis which developed were similar to the changes which occurred in T_3-pretreated rats exposed to halothane. The hepatic necrosis that developed after halothane administration in (−)-T_4-pretreated rats was also similar to the lesion in T_3-pretreated rats exposed to halothane. A centrilobular necrosis developed which involved three to six cell layers.

Species and sex comparison. The administration of halothane to hyperthyroid female rats did not result in the development of an overt hepatotoxicity, as evidenced by minimal elevations of SGPT activity (Fig. 3) and a low incidence (2–5%) of detectable hepatic damage. Male and female mice rendered hyperthyroid by the administration of T_3 also did not display any signs of hepatotoxicity after halothane exposure. There were no elevations of SGPT activity nor was there any histopathological evidence of hepatic injury.

Biochemical changes related to biotransformation activity. The *in vitro* enzymatic activities associated with cytochrome P-450-dependent metabolism and glutathione *S*-transferase conjugation activity were markedly altered in hyperthyroid rats (Table 2). Cytochrome P-450 levels and aminopyrine *N*-demethylase activity were depressed by 45% and 30%, respectively, of euthyroid animal levels. Other enzyme activities were stimulated by T_3 pretreatment. Aniline hydroxylase activity was increased by 45% and cytochrome *c* reductase activity was increased by 54% in hyperthyroid rats.

Conjugation activity is also altered in hyperthyroid rats. Dichloronitrobenzene and chlorodinitrobenzene conjugation with glutathione is catalyzed by glutathione *S*-transferase, and measurement with these two substrates accounts for approximately 90% of the total glutathione *S*-transferase activity found in rat hepatocytes [16]. The conjugation activity of glutathione *S*-transferase, as measured by both of these substrates, was markedly depressed in hyperthyroid rats. Dichloronitrobenzene conjugation was lowered by 39% and chlorodinitrobenzene conjugation was lowered by 30% in hyperthyroid rats.

Glutathione levels were also reduced significantly in hyperthyroid rats (Fig. 4). Prior to halothane administration, the glutathione levels were 60% of the euthyroid values. These levels stayed low throughout the halothane exposure, began to rebound 2 hr post-exposure, and approached control levels 24 hr after the exposure.

Temporal study. A temporal study (Fig. 5) was conducted which correlated T_3 pretreatment with the appearance of the halothane-induced hepatic necrosis and changes in the glutathione and glutathione *S*-transferase system and in mixed function oxidase activity, i.e. cytochrome P-450 levels and aniline hydroxylase activity. Glutathione levels fell after 1

Table 2. Oxidative and conjugative metabolism in hyperthyroid rats*

Route of metabolism	Hyperthyroid	Euthyroid	% Control
Cytochrome P-450 (nmoles P-450/mg protein)	0.45 ± 0.02†	0.81 ± 0.04	55
Aminopyrine *N*-demethylation (nmoles H_2CO/min/mg protein)	1.60 ± 0.04†	2.27 ± 0.14	70
Aniline hydroxylation (nmoles *p*-aminophenol/min/mg protein)	1.60 ± 0.16†	1.1 ± 0.2	145
NADPH–cytochrome *c* reductase (nmoles NADP†/min/mg protein)	97.6 ± 4.0†	62.7 ± 4.0	154
Glutathione *S*-transferase conjugation (nmoles/min/mg protein)			
1,2-Dichloro-4-nitrobenzene (nmoles conjugate/mg protein/min)	32.7 ± 1.6†	53.7 ± 2.1	61
1-Chloro-2,4-dinitrobenzene (nmoles conjugate/mg protein/min)	690 ± 49	990 ± 60	70

* Male rats were pretreated with 3 mg/kg/day T_3 (hyperthyroid) or saline (euthyroid) for 4 days. Animals were killed 24 hr after the last T_3 injection. Data are expressed as mean ± S.D. of six to ten microsomal or cytosolic preparations.

† The enzyme activity or level is significantly different ($P < 0.01$, Student's *t*-test) from euthyroid levels.

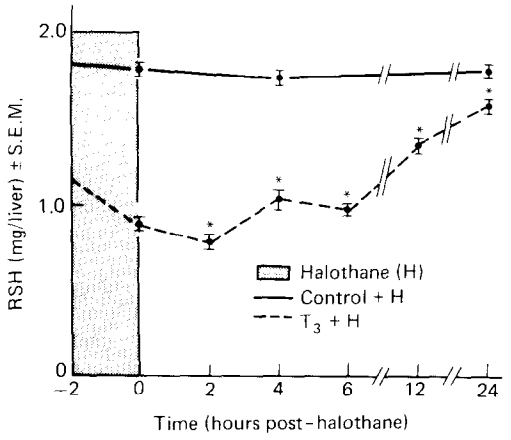


Fig. 4. Effect of halothane on hepatic glutathione levels (RSH) in hyperthyroid and euthyroid rats. Male rats were treated with 3 mg/kg triiodothyronine (T_3) for 6 days or saline (control) before exposure to 1% halothane (H) for 2 hr. The rats were killed at the indicated times before or after halothane administration. Data represent the mean ± S.E.M. of four to twelve rats. An asterisk indicates a significant difference from control.

day of T_3 pretreatment and remained low throughout the pretreatment period. Glutathione *S*-transferase activity began to fall after 2 days of pretreatment and reached maximal depression after 3 days of pretreatment. Cytochrome P-450 levels decreased after 2 days of pretreatment and remained depressed. Aniline hydroxylase activity did not begin to increase until after 3 days of T_3 pretreatment and reached peak levels after 4 days of T_3 administration. Changes in both cytochrome P-450-associated function and in glutathione and glutathione *S*-transferase activity appeared to be necessary before halothane induced any hepatotoxicity.

Effects of starvation and diabetes. Glutathione levels were decreased to the same level in starved rats and in hyperthyroid rats (Fig. 6). However, there

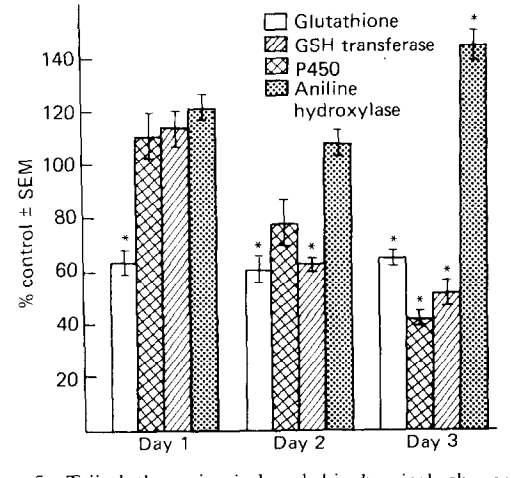


Fig. 5. Triiodothyronine-induced biochemical changes. Male rats were pretreated with 3 mg/kg triiodothyronine (T_3) or saline for 1, 2 or 3 days. The rats were killed 24 hr after the last T_3 injection. Data represent the mean percent control ± S.E.M. of six rats. An asterisk indicates that the mean is significantly different from control values.

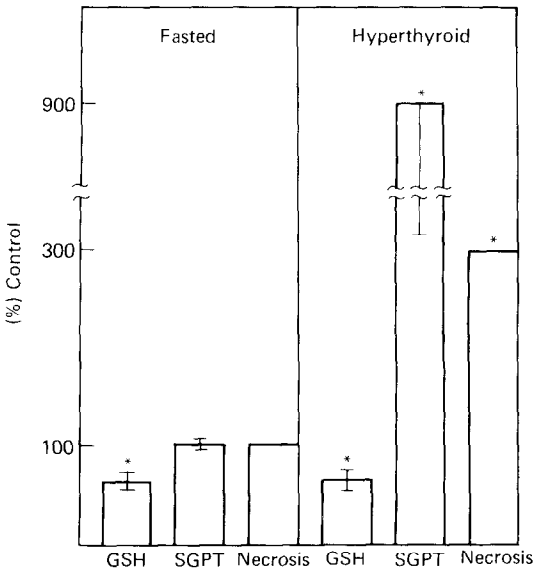


Fig. 6. Comparison of the effect of halothane on hyperthyroid and fasted rats. Male rats were either fasted for 48 hr or treated with 3 mg/kg triiodothyronine for 6 days (hyperthyroid). The rats were then exposed to 1% halothane for 2 hr. They were killed 4 hr after halothane exposure and hepatic glutathione (GSH), SGPT activity and hepatic necrosis were determined. Data represent the mean percent control ± S.E.M. of five to twelve determinations. An asterisk indicates a significant difference from control.

was no evidence of hepatotoxicity when starved rats were exposed to halothane. There was no increase of SGPT activity and no detectable morphological changes in hepatic structure, whereas hyperthyroid rats had marked elevations of SGPT activity and developed a severe centrilobular necrosis. These data indicate that the depletion of glutathione was not the only prerequisite for the development of halothane-induced hepatic necrosis. Streptozotocin-induced diabetes also did not alter halothane-induced hepatotoxicity. Cytochrome P-450 associated enzyme activities were altered in the diabetic state and were similar to the alterations which occur in hyperthyroidism. However, normal hapatic structure was seen 4, 24, and 48 hr after halothane exposure to diabetic rats. Neither were there any elevations in SGPT activity.

Bromobenzene. Bromobenzene is a potent hepatotoxin whose toxicity is dependent on cellular metabolism. Cytochrome P-450 has been shown to metabolize bromobenzene to an epoxide intermediate which binds to glutathione and other cellular macromolecules [5]. Bromobenzene was toxic in euthyroid rats, and a dose-dependent increase in SGPT levels occurred after bromobenzene administration (Fig. 7). However, hyperthyroid rats did not show any signs of hepatotoxicity, as measured by elevations of SGPT activity, after bromobenzene administration for a wide range of bromobenzene doses. The elevation of SGPT activity in euthyroid rats was also time dependent. Euthyroid rats displayed high levels of SGPT activity for several days while hyperthyroid rats showed no elevations in SGPT activity over a 3-day period (Fig. 8).

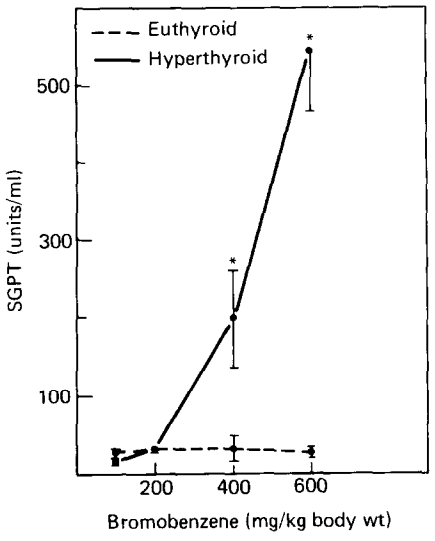


Fig. 7. Dose response of bromobenzene-induced hepatotoxicity in hyperthyroid and euthyroid rats. Male rats were treated with 3 mg/kg triiodothyronine (hyperthyroid) or saline (euthyroid). The indicated dose of bromobenzene was injected, and serum samples were collected 48 hr later. Values represent the mean ± S.E.M. of six to eight determinations. An asterisk indicates a significant difference from hyperthyroid.

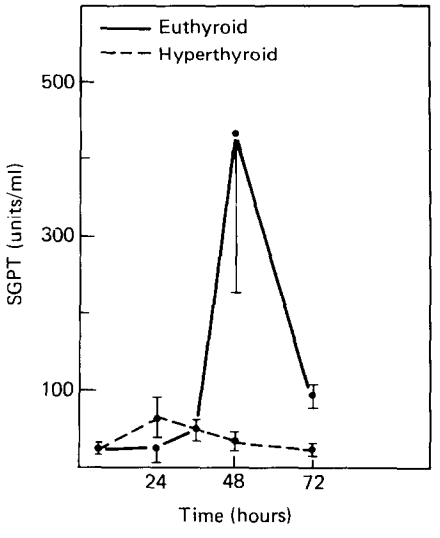


Fig. 8. Time course of bromobenzene-induced hepatotoxicity in hyperthyroid and euthyroid rats. Male rats were treated with 3 mg/kg triiodothyronine (hyperthyroid) or saline (euthyroid). Serum samples were collected at the indicated times after a 600 mg/kg dose of bromobenzene. Values represent the mean ± S.E.M. of five to six determinations. An asterisk indicates a significant difference from hyperthyroid.

DISCUSSION

Halothane toxicity is a rare and sporadic syndrome in humans. However, it has been determined recently that hyperthyroid animals are susceptible to halothane-induced liver injury [7]. A severe centrilobular hepatic necrosis developed in hyperthyroid rats after halothane exposure, which was insensitive to hypoxic or hyperoxic atmospheres but was dependent upon the degree of hyperthyroidism (dose and length of time triiodothyronine is administered) and the length of halothane exposure.

Halothane is metabolized by a cytochrome P-450-dependent biotransformation system [17, 18]. This metabolism, however, accounts for only a small amount of the total biotransformation. Most of the halothane is exhaled unchanged, while about 20% is metabolized almost entirely by cytochrome P-450-dependent enzymes. There is both oxidative and reductive cytochrome P-450-dependent metabolism of halothane. The primary oxidative metabolism, which accounts for 90% of the total metabolism of halothane, results in the production of inorganic bromide, inorganic chloride and trifluoroacetic acid [19]. Reductive metabolism of halothane results in the generation of inorganic fluoride. Two aliphatic reductive metabolites of halothane have also been described—chlorotrifluoroethane and chlorodifluoroethylene [20, 21].

The cytochrome P-450 system has been linked previously to halothane-induced hepatotoxicity using phenobarbital-pretreated rats as an animal model [22, 23]. However, to demonstrate overt hepatic necrosis in phenobarbital-pretreated rats, halothane must be administered in a low oxygen containing atmosphere [22, 24]. In this experimental model, reductive metabolism of halothane is increased markedly in phenobarbital-treated rats as measured by inorganic fluoride, chlorotrifluorethane and chlorodifluoroethylene generation [25–27]. This evidence of reductive metabolism being linked to hepatotoxicity *in vivo* was supported by *in vitro* covalent binding studies. Halothane has been shown to bind to microsomal protein and lipids primarily under anaerobic conditions. This anaerobic binding of halothane metabolites is dependent on cytochrome P-450 function.

Since the cytochrome P-450 system was implicated to be responsible for the bioactivation of halothane in the phenobarbital-hypoxia model, it was of interest to determine if this biotransformation system was responsible for halothane toxicity in hyperthyroid rats. Cytochrome P-450 function is altered significantly in hyperthyroid rats when compared to euthyroid controls. Cytochrome P-450 levels are depressed along with a variety of demethylation and hydroxylation enzyme activities both *in vivo* and *in vitro*, while other hydroxylation and reduction enzyme activities are markedly stimulated in hyperthyroid rats. It is of interest that cytochrome P-450 associated reductase activities were stimulated in hyperthyroid rats, since reduction reactions were associated with the bioactivation of halothane in the pheno-barbital-hypoxia model of toxicity. These changes in oxidative or reductive enzyme activities may be responsible for the hepatotoxicity of halothane in hyperthyroid rats.

Glutathione *S*-transferase is responsible for catalyzing the conjugation of glutathione with electrophilic molecules. This activity is depressed in hyperthyroid rats. This led to the speculation that perhaps

halothane-induced hepatic necrosis in hyperthyroid rats was due to both a depletion of glutathione and depressed glutathione *S*-transferase activity. However, the temporal study that correlated halothane toxicity to changes in glutathione and glutathione *S*-transferase demonstrated that just changes in glutathione and glutathione *S*-transferase activity did not result in the development of hepatic necrosis after halothane administration. Changes in the cytochrome P-450 system were also required before halothane caused overt hepatotoxicity in hyperthyroid rats. Thus, a new balance between the bioactivation of halothane by cytochrome P-450-dependent metabolism and the depression of detoxification mechanisms appears to be necessary for the exaggerated hepatotoxicity of halothane in hyperthyroid rats.

Other characteristics of this model of halothane toxicity also suggest that metabolism may be the cause of this toxicity. Hyperthyroid female rats are not as sensitive to halothane-induced hepatotoxicity as hyperthyroid male rats and both hyperthyroid male and female mice are insensitive to halothane-induced hepatotoxicity. Sex and species differences in biotransformation are well known, and this explanation is probably the most likely for the differences we have observed in the hyperthyroid inducible halothane toxicity.

The mechanisms by which phenobarbital-hypoxia and hyperthyroidism increase the susceptibility to halothane-induced hepatotoxicity appear to be different, as the former model is dependent upon hypoxic conditions. However, it is clear that clinical hyperthyroidism is a prerequisite for the latter. Support for this claim comes from the observation that reverse triiodothyronine, diiodothyronine and *d*-thyroxine, all of which are devoid of or have little hormonal activity, did not lead to an increased susceptibility to halothane-induced hepatotoxicity. Furthermore, equipotent doses of (−)-thyroxin, which is much less active than T_3, led to an injury very similar to that which was seen when T_3 was used. Additionally, increased susceptibility to halothane-induced hepatotoxicity did not occur with 3 days or less of T_3 pretreatment. Therefore, some condition produced by the hyperthyroid state is responsible for the increased susceptibility to toxicity rather than some direct enzymatic interaction between the thyroid hormones and halothane itself.

Other pathophysiological changes are associated with changes in oxidative and conjugative metabolism [6] similar to those reported for hyperthyroidism. For example, the starvation of rats for 48 hr has been associated with an increase in cytochrome P-450 function and a depletion of glutathione stores [28]. Several hepatotoxins have been shown to be more toxic in starved animals, presumably due to either a depletion of glutathione or an increased bioactivation by cytochrome P-450 [29–31] as both of these events are likely to result in an increased amount of reactive metabolite available to produce cell damage. Starvation, however, did not exacerbate the halothane-induced hepatotoxicity.

Diabetes also produces a complex pattern of metabolic alterations [32, 33]. Streptozotocin administered to rats induces a clinical diabetes and increases cytochrome P-450 levels by approximately 30%. Several enzyme activities associated with cytochrome P-450, which are altered in the diabetic state, reflect the alterations which occur in hyperthyroidism. Aminopyrine *N*-demethylase activity is decreased, while aniline hydroxylase and cytochrome *c* reductase activities are increased [33]. However, glutathione levels and glutathione *S*-transferase levels do not change. Diabetes also has been shown to enhance the hepatotoxicity of carbon tetrachloride, chloroform and trichloroethane [34, 35]. Diabetes, however, did not alter halothane-induced hepatotoxicity. Thus, these studies indicate that an increase in cytochrome P-450 mediated metabolism without a depression of glutathione is not sufficient to induce halothane hepatotoxicity. Therefore, if altered metabolism is the mechanism, it is a change specifically induced by the hyperthyroid state.

Hyperthyroidism is known to enhance the liver injury produced by other hepatotoxins [1–4]. However, these chemicals are all inherently hepatotoxic in the absence of hyperthyroidism. Paradoxically, bromobenzene which is hepatotoxic in euthyroid animals loses much or all of its hepatotoxicity in hyperthyroid animals. Thus, the potentiation of halothane-induced hepatotoxicity is not a generalized phenomenon applicable to all hepatototoxic halogenated hydrocarbons. In fact, the changes in biotransformation produced by hyperthyroidism can in some cases antagonize halogenated hydrocarbon-induced hepatotoxicity.

In conclusion, the hyperthyroid rat is sensitive to halothane-induced hepatotoxicity. Halothane-induced hepatotoxicity appears to be dependent on the metabolism of this volatile anesthetic. However, if the cytochrome P-450 system is responsible for the bioactivation of halothane in hyperthyroid rats, its role is markedly different in this model when compared to the phenobarbital-hypoxia model. The susceptibility of hyperthyroid rats to halothane-induced hepatotoxicity is dependent, at least in part, on perturbations of hepatocellular glutathione and glutathione *S*-transferase activity. A new balance between cytochrome P-450-dependent bioactivation and glutathione conjugation of halothane may be necessary for the potentiation of halothane-induced hepatotoxicity in hyperthyroid male rats.

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